University of Alberta

Field Physiology and Growth of Select Poplar Clones

by

Kelsey Ayton

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Abstract

In this study, growth of seven hybrid poplar clones and one pure poplar clone was investigated in relation to leaf, shoot, and root physiology under field conditions. Clones included: Walker (24 - *P. deltoides* x *P. xpetrowskyana*), Assiniboine (25 - open pollinated Walker cv. Assiniboine), Northwest (27 - *P. balsamifera* x *P. deltoides*), Berlin (42 - *P. laurifolia* x *P. nigra*), Okanese (2403 - Walker x *P. xpetrowskyana*), P38P38 (*P. simonii* x *P. balsamifera*), Balsam (1004 - *P. balsamifera*) and hybrid aspen (2782 - *P. tremuloides* x *P. tremula*). It was found that Berlin and Okanese had the greatest above ground biomass production, low stomatal conductance, high root conductance and high loss in branch conductance, while hybrid aspen, balsam, and P38P38 had average above ground biomass production, stomatal conductance, root conductance and loss in branch conductance, and Walker, Assiniboine, and Northwest had low above ground biomass production, high stomatal conductance, low root conductance and average loss in branch conductance.

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H	Height
C	Caliper
S_m	Shoot dry weight
R_m	Root dry weight
R_m:S_m	Root to shoot ratio
A	Net Photosynthesis
gs	Stomatal conductance
E	Transpiration
WUE	Water use efficiency
Ψ_p	Leaf pre-dawn water potential
Chl	Chlorophyll concentration
L_r	Root hydraulic conductivity
K_r	Root hydraulic conductance
K_b	Percent loss in branch conductance

Drought Tolerant – Plants with growth and survival that is generally not impacted by the affects of drought, as compared to plants that are drought susceptible, through either drought resistance or avoidance mechanisms.

Drought Resistant – Plants that utilize physiological strategies such as osmotic adjustment to maintain leaf turgor and thickened xylem walls to prevent cavitation, to resisting the negative impacts of drought and excessive water loss.

Drought Avoidance – Plants that utilize physiological strategies such as closing stomata to prevent was loss through leaves, to avoid losing water during drought conditions.

Drought Susceptible – Plants whose growth and survival are negatively impacted by drought conditions and do not express drought resistance or avoidance mechanisms.

1. Introduction and Literature Review

Climate fluctuations are a growing concern and changing the way in which industries that depend upon natural resources are conducting their operations. In the forest industry, where plantation forestry is being undertaken, drought is a climate variable that has a direct influence on the intensity of tree farming. *Populus* species and hybrids are short rotation, fast growing trees that can be selected for drought resistance traits to ensure a level of confidence in performance well suited for increasing productivity in Canada's forest industry (Stettler et al. 1996, Bradshaw et al. 2000, Dillen et al. 2010, Schreiber et al. 2011). The poplar farming program at Alberta-Pacific Forest Industries Inc. (Al-Pac) has been designed to supply 8-12% of the mill's fibre needs starting in 2025 and engages in many research trials to develop the most suitable poplars for farming purposes in conjunction with the best silvicultural practices.

However, there are also some concerns with poplar farming since the trees are known to be water and nutrient demanding (Boyer 1982, Lambs et al. 2006). This is especially important in Alberta where the average annual precipitation in most of the province is below 550 mm (Boyer 1982, Alberta Environment 2005, Lambs et al. 2006). Selection of trees that produce the most biomass with the greatest water and nutrient efficiency could be among the most effective ways to address environmental concerns and increase yield without a large amount of investment.

Earlier laboratory studies have identified different drought resistance strategies among the studied poplar clones (Arango-Velez et al. 2011). Of the

examined clones, P38P38 (*Populus simonii* x *P. balsamifera*), Walker (*P. deltoides* x *P. xpetrowskyana*), and Okanese (Walker x *P. xpetrowskyana*) showed high dependence on drought avoidance. The plants of these clones reduced water loss and maintained high leaf water potentials by closing their stomata in response to drought stress (Arango-Velez et al. 2011). These clones were also prone to xylem cavitation (Arango-Velez et al. 2011). Other clones, including a balsam poplar (*P. balsamifera*) and Northwest (*P. balsamifera* x *P. deltoides*) were able to maintain their stomatal conductance, had high root-to-shoot ratios, and were relatively resistant to xylem cavitation under drought conditions thus demonstrating drought tolerance mechanisms (Arango-Velez et al. 2011).

A key component in developing poplar breeding programs is identifying which traits and growth strategies of highly-productive poplar clones contribute to their high growth rates. Drought tolerance traits, such as resistance to xylem cavitation, prevent the effects of drought from occurring despite water loss, while drought avoidance traits, such as stomatal closure, prevent the effects of drought from occurring by avoiding water loss. Both tolerance and avoidance traits contribute to drought resistance whereby trees sustain a high level of productivity under drought conditions. If these traits can be identified, they can be used in tree improvement programs to assist in optimizing performance. The same concept applies for developing clones with drought resistance. Once the traits and growth strategies that contribute to drought resistance have been distinguished, they can be used to select for clones that will be productive in drought-prone climates. In general, however, clones that produce the greatest biomass under field conditions

are the clones which are considered most desirable for farming, whether or not they are susceptible to drought. For example, a clone that reduces its biomass production under drought by only a small amount and still outperforms a clone which shows no susceptibility to drought is the more desirable clone. By identifying the traits which contribute to high biomass and those that contribute to drought resistance, clones with the best traits of both high biomass production and drought resistance can be used for breeding.

Earlier studies have shown that growth responses to drought widely varied between the poplar clones used in the present study (DesRochers et al. 2007, Arango-Velez et al. 2011). An additional irrigation field experiment could help determine to what extent water availability is limiting poplar growth in Northern Alberta. If water availability limits growth, clones given an additional water supply should show increased growth compared to trees that were not given any additional water. If it is found that growth is not limited by water availability, the physiological strategies employed by the poplar clones may be determined through extensive measurements under field conditions. The "best" physiological traits and strategies can then be identified based on the traits associated with the highest growth yields.

1.1 The Genus *Populus*

Forests provide a plethora of benefits such as the use of wood for structural material, pulp and paper, and fuel, as well as environmental benefits such as sources of watershed protection, windbreaks for erosion control, habitat,

and carbon sequestration (Bradshaw et al. 2000). Trees of the genus *Populus*, commonly referred to as poplars, are members of the kingdom Plantae, order Malipighiales, and family Salicaceae, together with the very closely related genus *Salix* (the willows) (Eckenwalder 1996, Bradshaw et al. 2000). The genus *Populus* contains 29 recognized species under six sections: *Abasco, Turanga, Leucoides, Aigeiros, Tacamahaca,* and *Populus,* most of which have extensive distribution ranges (Eckenwalder 1996, Bradshaw et al. 2000). My study includes five hybrids between the *Tacamahaca* and *Aigeiros* sections: Walker (*P. deltoides* x *P. xpetrowskyana*), Assiniboine (open pollinated Walker cv. Assiniboine) Northwest (*P. balsamifera* x *P. deltoides*), Berlin (*P. laurifolia* x *P. nigra*) and Okanese (Walker x *P. xpetrowskyana*), a hybrid from the section *Tacamahaca*, P38P38 (*P. simonii* x *P. balsamifera*), a pure poplar from the section Tacamahaca, balsam (*P. balsamifera*), and hybrid aspen from the section *Populus* (*P. tremuloides* x *P. tremula*) (Eckenwalder 1996, Huybregts et al. 2007).

The aspens of the *Populus* section are upland "drier" habitat species. The hybrid aspen in my study was a cross between *P. tremuloides* and *P. tremula*. P. *tremuloides* (Quaking aspen) is widely distributed across 111° of longitude and 48° of latitude across Canada and northwestern Alaska, the western United States (the mountains of Washington to California, southern Arizona, Trans-Pecos Texas, northern Nebraska, Iowa and eastern Missouri it ranges east to west Virginia, western Virginia, Pennsylvania, and New Jersey), and the mountains of Mexico (Perala 1990). Annual precipitation and temperature ranges in the home range of quaking aspen are highly variable (Perala 1990). Quaking aspen grows

best on soils with silt that has a high proportion of clay although it grows on a variety of soils such as shallow and rocky soil or deep loamy sands where water is available and aerated, typically found in cool and moist areas (Perala 1990). Eurasian aspen (*Populus tremula*) is typically found in cool temperate and boreal regions across the British Isles to Iceland, and north of the Arctic Circle (Scandinavia and Russia) to central Spain, Turkey, North Korea, China and Japan. Eurasian aspen grows best in moist, non stagnant soil, which can range from coarse soils and loamy sands to clays (von Wühlisch, 2009). Overall, although *P. tremula* and *P. tremuloides* do not have overlapping native distributions, their preferred habitats are very similar.

While the riparian cottonwoods of the *Tacamahaca* and *Aigeiros* sections are more commonly found near river systems with *Tacamahaca* poplars occurring in cooler climates than *Aigeiros* (Braatne et al. 1996, Eckenwalder 1996, Cooke and Rood 2007). The native range of balsam poplar, the pure species in my study from the section *Tacamahaca* is from approximately 55° to 165° W longitude and 42° to 68° N in latitude across North America and is the northernmost growing American hardwood (Zasada and Phipps, 1990). Balsam are found in climates ranging from a maritime zone to continental (most of the home range) with average temperature ranges from -30° to -4° C in January and 12° to 24° C in July, and show their best growth on flood plains although they are also found on upland sites (Zasada and Phipps, 1990). Precipitation is variable throughout the home range of balsam poplar, however, extended periods of drought are uncommon (Zasada and Phipps, 1990). Clones P38P38 and northwest also derive

parentage from *P. balsamifera*. The other parent of clone P38P38, *P. simonii*, is also from the section *Tacamahaca* and native to northern China from Qinghai to the east coast (longitudinally) and from Heilongjiang River to the Yangtze River (latitudinal) growing under extreme temperatures and with typically drought conditions (Wang 2012). *P. laurifolia* is the last clone from the section *Tacamahaca* that contributes parentage to clones in this experiment, specifically Berlin (42), Walker (24), Okanese (2403) and Assiniboine. The native home range of *Populus laurifolia* is from west to east Siberia, Central Asia, Mongolia, Japan and northwest India with soils that are sandy or loamy clay soil that is moist to wet (Hortipedia 2011).

Clones Walker (24), Okanese (2403), Assiniboine (25), Northwest (27) and Berlin (42) all have parental contributions from clones of the section *Aigeiros*, specifically *P. deltoides*, a native poplar to the prairie region of Alberta, and *P. nigra*, a non-native poplar. The home range of eastern cottonwood (*P. deltoides* var deltoides) extends from 28 to 46° N latitude along the eastern coast (Van Haverbeke 1990). The western edge of its home range overlaps with that of the plains cottonwood (also *P. deltoides* var occidentalis) which extends from 92° to 115° W in longitude and 30° to 55° N in latitude (Cooper 1990, Van Haverbeke 1990). The eastern cottonwood grows best on moist well-drained sands or silts near streams, and although the plains cottonwood is also found on this soil type, it performs best on deep, rich, well-drained loams (Cooper 1990, Van Haverbeke 1990). The mean January temperature ranges from -10° C to 8° C for eastern cottonwood and from -15° C to 4° C for plains cottonwood (Cooper 1990, Van

Haverbeke 1990). Although eastern cottonwood poplar tend to occur in areas of higher rainfall than other poplar, in the driest areas of its range they draw water primarily from streams, where as plains cottonwood occur in areas frequented by drought (Cooper 1990, Van Haverbeke 1990). Black poplar (*P. nigra*) is found in southern, central and east Europe, north Africa and eastwards to central Asia the Black or Water Poplar is almost certainly native to lowland England (Encyclopedia or Life 2012). Black poplar grows best with medium or coarse textured soil; tend to have low to high drought tolerance which is cultivar dependant (USDA 1990). It can be assumed that the difference in habitat between where sections are found has lead to differences in adaptation, traits and phenologies between the groups.

Poplars are primarily dioecious (separate male and female trees), deciduous (or semi-evergreen), single trunked trees. They are typically wind-pollinated and produce very tiny seeds which are wind and water dispersed, but the trees can also reproduce asexually from root collars, branch cuttings, or (as for the section *Populus*) from root sucker shoots on horizontal roots (Eckenwalder 1996, Bradshaw et al. 2000, Dillen et al. 2010). Poplars are known to have rapid growth with diffuse-porous, light-weight wood (Bradshaw et al. 2000). The rapid growth of poplars and intolerance to shade have lead to their role as vegetative pioneers (Eckenwalder 1996, Bradshaw et al. 2000, Schreiber et al. 2011).

Poplar breeding programs in western Canada are a priority for wood and pulp manufacturers as breeding programs can be used to develop poplar clones which produce high-quality wood at the highest efficiency, and least amount of

time and resource input, while resisting the cold, dry climate (Dillen et al. 2010, Schreiber et al. 2011). Development of these programs often involves the use of native aspen and non-native hybrid poplar clones (Schreiber et al. 2011). Poplars in general are desirable trees for cultivation due to their efficient vegetative propagation, rapid juvenile growth, high biomass yields, coppice ability, and their ability to adapt to environmental changes (Dillen et al. 2010). The use of hybrid poplars for cultivation has a variety of advantages, such as the abundance of genetic variation that is available in natural-parent populations, ease and efficiency of propagation and cloning, ability to cross species within and between sections of the genus, and the fertile nature of resulting hybrids leading to the potential for backcrossing and further hybridization, as well as heterosis (Stettler et al. 1996, Bradshaw et al. 2000, Schreiber et al. 2011). Breeding programs which aim to increase yield can do so by either selecting directly for clones with the highest yields, or selecting individual traits that contribute to and are correlated with high yields, such as photosynthetic capacity and leaf traits (Ridge et al. 1986, Barigah et al. 1994, Marron and Ceulmans, 2006). Interest now exists in growing poplars on marginal agricultural lands in the Canadian Prairies as an appealing source of guaranteed income for farmers and a way to cultivate high performing poplar clones for wood and pulp production (Silim et al. 2009).

1.1.1 Hybridization

There are three main reasons why hybridization is ideal in poplar breeding programs. First, is the combination of desirable traits from different species to

achieve higher productivity from the possible additive or synergistic effects of those desirable traits (Stettler et al. 1996). Some examples of traits that are targeted to increase through hybridization are: rooting ability, stem growth, branching, disease resistance, seasonal phenology, and leaf traits such as larger leaves achieved by crossing a species with large leaf cells with another species with a greater number of leaves (Ridge et al. 1986, Stettler et al. 1996). Second, is to obtain hybrid vigour (Stettler et al. 1996). Heterosis or hybrid vigour generally refers to the phenomenon where first generation offspring have greater productivity than the parent generation (Stettler et al. 1996). For example, Marron and Ceulmans (2006) found that first generation hybrids had positive hybrid vigor for volume and volume growth rate of leaves for several crosses of poplar species. Hybrid vigor likely results when there is genetic dominance for one or more superior traits from two parent species that are passed on to the offspring, resulting in additive or synergistic effects of the superior traits (Dillen et al. 2010). The key component to hybrid vigor is the heritability of advantageous traits that lead to increased biomass production (Marron and Ceulmans, 2006). The third reason hybridization is ideal is to capture greater phenotypic plasticity in variable environments (Stettler et al. 1996). Within sections, natural hybridization occurs freely, and in some cases, such as between sections *Aigeiros* and *Tacamahaca*, natural hybridization will occur between sections where species' ranges overlap, or are sympatric (Braatne et al. 1996, Eckenwalder 1996).

1.2 Role of the Canopy and Leaves in Biomass Production

1.2.1 *Photosynthesis*

Growth and maintenance of a tree ultimately depends on the rate at which the canopy of the tree can assimilate carbon dioxide and light through the process of photosynthesis whereby light is used to power the conversion of carbon dioxide and water into carbohydrates the tree needs (Wolfe et al. 1998, Dillen et al. 2010). The photosynthetic potential of a canopy can depend on several physiological and morphological factors such as leaf area growth and morphology, individual leaf photosynthetic capacity, leaf orientation and distribution and size of leaves and branches (Dillen et al. 2010). Leaf traits normally associated with productivity include the leaf internal structure, growth and physiology of individual leaves, photosynthetic performance, and water use efficiency (Marron et al. 2005).

The rate of photosynthesis of an individual leaf is affected by both external factors such as light, temperature, and carbon dioxide concentration, and internal factors such as mesophyll cell number (the photosynthetic cells of a leaf), and chlorophyll content (the pigment required to undergo photosynthesis) (Emerson 1929, Wolfe et al. 1998, Marron et al. 2005). The mesophyll leaf tissue layer is responsible for photosynthesis and the higher the number of mesophyll cells per unit area, the greater the biomass production through greater rates of carbon dioxide assimilation (Wolfe et al. 1998, Marron et al. 2005). A wide range of photosynthetic rates for *Populus* clones have been reported. Ceulemans and Isebrands (1996) have synthesized from numerous studies a range of 1.3 - 25

μmol CO_2 m^{-2} s^{-1}. This is despite the belief that the average photosynthetic capacity of trees is low (3 - 6 μmol CO_2 m^{-2} s^{-1}) (Dillen et al. 2010).

Although it is accepted that the process of photosynthesis provides the carbohydrates needed for biomass production, the direct relationship is not clear as many studies have demonstrated positive correlations between photosynthesis and biomass production, while others have not shown a significant relationship (Dillen et al. 2010). One explanation for the poorly correlated relationship between biomass production and photosynthesis is the sensitivity of instantaneous photosynthesis rates to environmental variation (Dillen et al. 2010).

1.2.2 *Stomatal Conductance*

In order for the process of photosynthesis to occur, there needs to be gas exchange between the leaf and the surrounding atmosphere; specifically, water and oxygen are released from the leaf while carbon dioxide is taken in. This occurs through pores called stomata; the rate at which gas exchange occurs is known as stomatal conductance. Productivity and biomass production are regulated by stomata as the rate and duration of carbon dioxide assimilation directly affects the rate of photosynthesis (Silim et al. 2009). When leaf water potential hits a certain threshold, which varies among species, turgor pressure of the guard cells lining the stomatal opening drop and the stomata close to prevent gas exchange and water loss (Shulte and Hinckley 1987, Jones and Sutherland 1991, Tardieu and Simonneau 1998, Silim et al. 2009, Dillen et al. 2010). Stomatal closure is one way by which leaves can regulate short term water loss by

trees (Blake et al. 1984, Shulte and Hinckley 1987, Jones and Sutherland 1991, Tardieu and Simonneau 1998, Silim et al. 2009, Dillen et al. 2010). Stomatal traits, which are believed to be heritable (Pearce et al. 2005), such as the size and number of stomata, vary across *Populus* species leading to differences in stomatal control of regulation of water loss and gas exchange (Dunlap and Stettler, 2001, Al Afas et al. 2005, Dillen et al. 2010).

1.2.3 *Water Use Efficiency*

There are several ways in which water use efficiency is defined in relation to plant growth. Bonhomme et al. (2008) define water use efficiency as the ratio between net carbon dioxide assimilation and stomatal conductance. Marron et al. (2005) define water use efficiency as the ratio between biomass accumulation and transpiration. In general, water use efficiency is an index that can be compared between trees where a given unit of productivity is obtained for a given unit of water used in the process. The correlation between water use efficiency and leaf carbon isotope ratios is an accepted means of comparing water use efficiency over the lifespan of a leaf whereas the previously mentioned methods are instantaneous measurements of water use efficiency (Farquhar and Richards 1984). Water use efficiency is reported to be highly variable among poplars (Marron et al. 2005, Bonhomme et al. 2008). Both stomatal conductance and photosynthetic capacity have been found to influence water use efficiency with consequences for biomass production depending on which factor has the strongest influence on water use efficiency (Marron et al. 2005, Bonhomme et al. 2008). Recently, increased

emphasis has been placed on water use efficiency as water is becoming a more and more limited resource. Trees with higher water use efficiency are more likely to be productive under water limited conditions; however, biomass production and water use efficiency are not always closely correlated (Marron et al. 2005, Bonhomme et al. 2008). Tupker et al. (2003) found that increased ambient carbon dioxide conditions increase both growth and water use efficiency in some hybrid poplar clones. Bonhomme et al. (2008) suggest that the lack of relationship between water use efficiency and productivity leaves room for selection of genotypes with both high water use efficiency and high productivity.

1.2.4 *Leaf Water Potential*

Leaf water potential drives water flow through leaves as water moves from an area of high to low water potential (Sack and Holbrook 2006). Water flows into the leaf from branches, through the petiole and veins, to the living leaf tissue, and into airspaces where it evaporates through open stomata (Sack and Holbrook 2006). High leaf water content, or high water potential, is required to maintain open stomata (Sack and Holbrook 2006). At night, when there is no light by which photosynthesis can occur, transpiration or water loss is assumed to not occur and water potential gradients across a plant equilibrate with soil water potential; therefore, predawn water potential is assumed to be representative of soil moisture in the root zone (Améglio et al. 1999). Predawn water potential has been widely used as an indicator of water stress since, when soil water is not a limiting factor, leaves will saturate with water at night and leaf water potential

will be close to zero. When soil moisture content decreases, predawn water potential will decrease with increasing intensity of water deficit stress (Améglio et al. 1999, Intrigliolo and Castel 2006). During the day, when photosynthesis and transpiration are occurring, leaf water potential drops if soil moisture and water conductivity within the tree can not keep up with water demand; this can lead to stomatal closure (Améglio et al. 1999, Intrigliolo and Castel 2006). Stomatal closure, as previously mentioned, restricts photosynthesis and biomass production.

1.3 Role of the Stem in Biomass Production

The stem of a plant, particularly because of its woody properties and ease of harvest compared to the root system, is of high importance for timber and the wood-pulp industries (Chaffey 1999). The stem of a tree is primarily composed of outer bark tissues, phloem, xylem, and vascular cambium (meristematic tissue from which the secondary vascular tissues arise) (Chaffey 1999). Long distance transport in plants between the roots and leaves occurs through the vascular tissues: the xylem transports water and nutrients from the soil to leaves and the phloem transports photosynthates, amino acids and electrolytes from the leaves to other tissues in the plant (Windt et al. 2006).

1.3.1 Phloem

The phloem is the transport tissue in plants that moves sugars, organic compounds including signalling molecules and defence proteins, from the leaves

where they are synthesized (sources) to other tissues where they are required (sink tissues) (van Bel 2003, Dafoe et al. 2009). The recognition that the phloem transports signalling molecules suggests that phloem may play a critical role in a plant's ability to adapt to environmental changes (van Bel 2003, Windt et al. 2006, Dafoe et al. 2009). The movement of phloem sap down a plant occurs due to positive turgor pressure gradients between source and sink tissues (Dafoe et al. 2009). Solutes build up in source tissues creating a high concentration relative to sink tissues. Phloem loading and unloading then occurs to move the solutes to areas of low concentration in sink tissues where they are required (van Bel 2003, Windt et al. 2006). Phloem sap requires a certain amount of water content in order to transport solutes, which is obtained from the xylem (Windt et al. 2006).

1.3.2 *Xylem*

The primary function of the xylem of plants is to transport water and nutrients, such as nitrogen, phosphorous, potassium, calcium, magnesium, sulphur a many micronutrients from the roots, up the stem of a plant, to the leaves where it is used to produce organic compounds that are then transported throughout the tree in the phloem. Water is pulled up through the xylem via cohesion-tension: as water evaporates from the surface of a leaf during transpiration, the cohesive (and adhesive) properties of hydrogen bonded water molecules pull more water molecules up the xylem (Windt et al. 2006). The xylem replenishes water used in transpiration, growth, and in phloem sap (Windt et al. 2006). Shoot morphology and xylem structure have been shown to be dependent on soil nutrient content,

specifically nitrogen (Hacke et al. 2010). The properties of xylem in poplar species are especially important due to the high susceptibility of xylem vessels to cavitation (Fichot et al. 2010).

1.3.3 *Xylem Embolism and Cavitation*

In plant physiology, an embolism or cavitation occurs when there is a blockage of water flow in vessels or tracheids of plants caused by an air bubble (Sperry et al. 1988). Cavitation occurs when there is a break in the water column between the roots and leaves; embolism occurs when a vessel or tracheid becomes completely air filled (Tyree and Sperry 1989). Embolisms occur when air is forced out of solution during freezing, or when there is a breakage in the water stream during transpiration causing a micro-void between hydrogen bonded water molecules (Sperry et al. 1988; Tyree and Sperry 1989; Tyree and Ewers, 1991). When there is a break in the water column, the tracheid or vessel is usually filled with water vapour with a small amount of air, which is followed closely by air diffusing from surrounding tissue to fill the air vacuum that is created and referred to as "air seeding"; this creates a full embolism that can lead to cavitation (Sperry and Tyree 1988, Tyree and Sperry 1989). Embolism and cavitation can lead to a lack of water supply to the canopy of the tree, which eventually kills the whole tree (Sperry et al. 1988). As water is lost, xylem conduits are put under increasingly negative pressure, which can reach a tipping point where cohesion between water molecules is broken and vaporization of air out of solution with water can occur (Sperry and Tyree 1988). When an embolism cavitates a

tracheid, or vessel, there will be a loss of hydraulic conductivity which then causes a greater pressure gradients between the roots and branches making further embolism more likely to occur (Tyree and Ewers, 1991). Evolution has lead to an effective design to minimize the effect of embolisms on trees. Instead of a single xylem conduit which can be blocked by a single large bubble, xylem is composed of individual tracheid and vessel units with pit membranes that block the spread of embolism air pockets (Sperry et al. 1988).

In order for an embolism to be removed, the xylem must be under positive pressure (depending on the pressure of air at the air-water interface) (Tyree and Sperry 1989). Previous studies suggest that root pressure can generate enough pressure to help repair embolism, especially in the spring time when water is not being lost to transpiration of leaves (Sperry et al. 1988, Tyree and Sperry 1989).

Sperry et al. (1988) found that in sugar maple, high amounts of embolism occurred despite high average rainfall during the growing season and was higher in the trunk than in twigs despite previous assumptions that twigs were more vulnerable to embolism than tree trunks. The high amount of embolism was attributed to winter freezing and sublimation or evaporation of water during temporary thawing of the xylem. Normally, the bubbles formed during freezing are small enough to dissolve back into the xylem water solution during the spring thaw (Sperry et al. 1988). Embolisms have also been found to be pathogen induced or induced by herbivory (Tyree and Sperry 1989).

Roots are more vulnerable than stems to embolism, but also more reversible as root pressures are more likely to exceed atmospheric pressure when

soil moisture is returned and therefore may make less of a contribution to overall loss of plant hydraulic conductance due to cavitation (Alder et al. 1996, Huckin et al. 2005).

Poplars are highly susceptible to cavitation (Fichot et al. 2010, Schreiber et al. 2011); also, Schreiber et al. (2011) found that hybrid poplars (riparian species) were more vulnerable to cavitation than trembling aspen (non-riparian species). Stomatal closure is one way in which plants can regulate the risk of cavitation and usually there is a "safety margin" between the water potential which causes leaves to close their stomata and the water potential at which cavitation occurs (Huckin et al. 2005). However, both Fichot et al. (2010) and Schreiber et al. (2011) found that cavitation resistance was either weakly or not at all correlated to other physiological traits.

1.4 Role of Roots in Biomass Production

Due to their inability to move or relocate in response to stress, trees must have the ability to adapt to changes in their environment (Marjanović et al. 2005). Roots are one organ which enables trees to do this by reaching resource-rich areas. Roots are important, they take up nutrients and water, store carbon compounds and provide physical support, yet roots are under-researched (Brunner and Godbold 2007, Gou et al. 2010).

Roots are highly variable across species and can vary down to their cellular anatomy, even in a root system of an individual plant (Steudle and Peterson 1998). Early root growth of poplars tends to be under strong genetic

control (Al Afas et al. 2008, Dillen et al. 2010). Nutrient distribution in soil is heterogeneous, and as such roots tend to proliferate in patches which are high in nutrient content, but plants differ (genetically) in their ability to detect and respond to nutrient rich patches; therefore some genotypes and species are better able to take advantage of nutrient patches than others (Misra et al. 1998, Hodge et al. 1999, Woolfolk et al. 2003). The fact that roots vary in both morphology and physiology is widely accepted, yet the factors contributing to this variation and differences in function is less well understood (Friend et al. 1991, Brunner and Godbold 2007).

1.4.1 *Fine Roots*

Because of the obvious difficulty that is involved in studying root systems, there is far more known about the above-ground systems of plants than the below-ground systems (Sanford 1989, Friend et al. 1991, Misra et al. 1998, Resh et al. 2003, Block et al. 2006, Dillen et al. 2010). This is especially true for the fine root component of root systems as the fine roots are the most difficult part of the system to recover when excavating (Block et al. 2006). The most common definition of fine roots are roots under 2 mm in diameter which "explore" the area surrounding the root system and are especially important in the uptake of water and nutrients water used for carbon assimilation (Friend et al. 1991, Block et al. 2006, Brunner and Godbold 2007). Fine root production and mortality are quite dynamic, with noted periods of increased rates of biomass production (early spring and summer) and mortality (fall) leading to seasonal fluxes in biomass of

functioning fine roots (Steele et al. 1997, Block et al. 2006). These seasonal fluxes have been linked with favourable growing conditions and genetic mechanisms which determine carbon allocation patterns and occur notably alongside leaf processes such as expansion (during the period of increased root production) and leaf senescence (during the period of increased root mortality) (Joslin et al. 2001, Davis et al. 2004, Brock et al. 2006). Because of their rapid turnover rate, fine roots act as a large carbon sink on the whole plant carbon budget (Dillen et al. 2010). The dynamic cycling of fine roots is one way in which trees are adapted to dynamic soil environments with ever changing nutrient and water availability, and interacting resources (Brock et al. 2006). Vogt et al. (1996) have found that in several studies conducted in the 1980's, mean annual temperature, mean annual temperature-to-precipitation ratio, and soil nitrogen explained a great deal of the variation in fine root biomass or turnover.

Biomass values may not reflect changes to below ground systems or responses to disturbance likely because of the high functioning of fine roots and their disproportionately low contribution to biomass (Vogt et al. 1996). Fine root turnover has a low impact on overall root biomass (Friend et al. 1991).

1.4.2 *Coarse Roots*

The majority of below-ground biomass is found in the form of coarse roots which conduct nutrients and water, store carbon and nutrients, and provide structural support (Misra et al. 1998, Resh et al. 2003). Because of the constant rate of production and mortality, fine roots remain a relatively low proportion of

total root biomass, whereas coarse roots increase in their proportion of below-ground biomass as trees age (Sanford 1989, Misra et al. 1998, Resh et al. 2003). As above-ground biomass increases, the below-ground biomass of course roots must increase to ensure the proper anchorage of the tree (Resh et al. 2003).

1.4.3 *Biomass Allocation to Roots*

Biomass distribution between roots and shoots of poplar has been found to be highly variable between species and years of growth (Scarascia-Mugnozza et al. 1997). For example, Rhodenbaugh and Pallardy (1993) found that higher production of an early root system was associated with higher biomass productivity in poplar due to the ability to supply greater amounts of water to the leaves during times of reduced water availability and greater potential for expansion of tissues due to increased access to nutrients (Tschaplinstk and Blaket 1989a). Scarascia-Mugnozza et al. (1997) found that in the first year of establishment, roots play a very important roll and have higher biomass partitioning than in the following year. Tschaplinstk and Blake (1989a) found that high early root-to-shoot ratios corresponded with high biomass production, decreased moisture stress, and higher photosynthesis rates.

Overall, the distribution of photosynthates among plant structures is governed by both abiotic and biotic selection pressures (Brunner and Godbold 2007). The root-to-shoot ratio is under genetic control as well as a function of the water and nutrient balance of plants (Scarascia-Mugnozza et al. 1997). Cairns et al. (1997) found that root-to-shoot ratio was higher in coarse soils, although age,

soil texture index, tree type, temperature-to-precipitation ratio, and mean annual precipitation, did not significantly affect plant root-to-shoot ratios.

1.4.4 *Water and Solute Transport by Roots*

The water status of a tree is generally determined by three main processes: uptake of water and nutrients by the roots, the movement of water through the tree, and loss of water through transpiration; where transpiration is the driving force in the system (Marjanović et al. 2005). Transpiration creates a decrease in water potential in the leaves of plants; roots, therefore, passively take up water because there is a deficit across the plant. This water potential gradient drives water into the roots and up the plants to where it is required in the leaves (Steudle and Peterson 1998). Water uptake of tree roots occurs by radial transport of water through the outside of the root to the internal xylem transport vessels via apoplastic (through cell walls and intercellular spaces), symplastic (through plasmodesmata which are channels between cell walls) or transcellular pathways (through cells and across cell membranes). Radial transport in roots is considered to be the greatest source of resistance to water flow in plants (Steudle and Peterson 1998). This resistance is interpreted to be an indication that the tissue involved in the radial movement of water in roots is an important source of regulation of water flow in plants (Siemens and Zwiazek 2004). Solute movement occurs via the apoplastic pathway by diffusion and solvent-drag, and via the cell to cell pathway through passive or active movement across the plasma

membrane changing osmotic potential as it moves through the roots (Steudle and Peterson 1998).

1.5 Drought Stress

When plants are prevented from reaching their full genetic potential they are considered stressed, and it is often an environmentally occurring factor that is causing the limitation (Boyer 1982). Stresses which can reduce the growth potential of plants include disease, herbivory, competition (sometimes due to weeds), inappropriate soils, unfavourable climates, carbon dioxide or oxygen limitation, radiation levels, and water or nutrient limitation; where water limitation is often regarded as the most limiting factor (Boyer 1982). Future climate change puts the prairies of Alberta at risk of increasing drought; as such the success of poplar plantations in Alberta depends upon the selection of clones with the ability to produce high amounts of biomass under drought like conditions (Silim et al. 2009).

Water is required by trees for cell expansion, transport of solutes and photosynthates, cooling of leaves, and photosynthesis; as fast growing trees, poplar require a lot of water (Lambs et al. 2006). Drought can be defined as a soil and or atmospheric water deficit (Chavez et al. 2003). Poplars are regarded as being highly susceptible to drought stress, yet drought tolerance and adaptations vary greatly between species and clones (Dickmann et al. 1992, Tschaplinski et al. 1994; Tschaplinski et al. 1998, Monclus et al. 2006, Xiao et al. 2008). Indicators of drought stress include reductions in photosynthesis, stomatal closure reducing

stomatal conductance and transpiration, decreased growth rate, decreases in water use efficiency, reduced predawn leaf water potential, and leaf shedding (Dickmann et al. 1992, Xiao et al. 2008). From an economical standpoint, the greatest impact of drought on poplar is the limitation of tree survival and the reduction of tree biomass production. Some studies have shown that drought has a negative impact on shoot growth of some poplar species, while other studies have found that irrigation does not improve the stem growth of certain poplar species under field conditions (Voltas et al. 2006, Xiao et al. 2008). In a study of 29 different genotypes of *Populus deltoides* × *Populus nigra* hybrids, the biomass production of most clones, but not all, and not to the same extent, were negatively impacted by decreased soil water potential. The most highly effected genotypes (most drought susceptible) tended to be those that were the most productive under control (non water limited) conditions (Monclus et al. 2006). Xiao et al. (2008) found that individuals of the same species from different populations (one from a wet climate and one from a dry climate) had different growth rates where the wet climate population always had higher growth, but was more affected by drought stress while the dry climate population was less affected by drought stress but had lower growth under both dry and wet conditions. Drought stress adaptation occurs on many levels including morphological, physiological and genetic levels (Yang et al. 2010). Some clones have been shown to have increased drought tolerance when they are preconditioned to drought while others demonstrate that preconditioning does not have an effect (Silim et al. 2009). In general, poplar clones that are considered drought tolerant are those that maintain a growth rate

under drought stress that is equal to that of well-watered controls (Tschaplinski et al. 1994).

1.5.1 *Role of Leaves in Minimizing Drought Stress*

Carbon dioxide assimilation through photosynthesis is required for biomass production, which in turn requires gas exchange. The primary way in which plants control gas exchange is through regulation of their stomata. One of the first responses to drought is stomatal closure resulting in a reduction in gas exchange (Dickmann et al. 1992, Regier et al. 2009). Drought avoidance mechanisms allow plants to endure drought by avoiding dehydration through minimizing water loss, by the use of mechanisms such as stomatal closure (Jackson et al. 2000, Chavez et al. 2003). Trees have a threshold leaf water potential that when exceeded causes the closure of stomata and the reduction of stomatal conductance; when leaf water potential hits that certain critical limit, stomata close quickly to prevent water potential from dropping past that critical limit (Lambs et al. 2006). This closure is thought to be due to a loss of turgor in guard cells, which could possibly be due to signalling hormones (Chavez et al. 2003). Reduction in size or stomatal density can be a long term adaptive response to drought (Regier et al. 2009).

Stomatal responses to drought are not consistent for poplars; however, stomatal conductance tends to be lower in drought tolerant clones regardless of soil water potential, leading to higher water use efficiency (Silim et al. 2009). Water use efficiency is decreased by extended periods of drought in some

Populus species, and is variable between species (Dickmann et al. 1992). Water use efficiency is not always linked to high biomass production; under drought conditions, the species with the higher water use efficiency is not necessarily the clone with greater biomass production which may depend on the rate by which photosynthesis can occur and produce biomass given a certain stomatal conductance rate (Dickmann et al. 1992).

Trees which have the ability to maintain open stomata associated with maintaining high leaf water potential, have the potential to produce greater amounts of biomass under conditions where water is limited (Schulte et al. 1987, Silim et al. 2009). Reduced photosynthesis during drought is linked with lower plant height and stem diameter (Regier et al. 2009, Yang et al. 2010). In general, trees that are able to maintain open stomata under low leaf water potentials are considered to be drought tolerant as they are able to maintain photosynthesis and gas exchange under drought conditions (Jones and Sutherland 1991, Cochard et al. 2002, Silim et al. 2009). A study by Silim et al. (2009) found that clones of poplar that were more drought-tolerant had lower steady-state stomatal conductance, closed their stomata more gradually as soil dried, and had higher photosynthesis and stomatal conductance under moisture limited conditions than those that were drought-sensitive. Similar results were also found by Arango et al. (2011) detailed further in section 1.6.

Photosynthesis under drought can also be influenced by the effect that drought has on the chlorophyll in leaves. In one study, drought was found to decrease the chlorophyll content in leaves of poplar populations of *P. cathayana*

collected from a wet climate while it had no effect on the chlorophyll content in a population collected from a dry climate, *P. kangdingensis,* suggesting that *P. kangdingensis* is more adapted to drought conditions and more resistant to the impacts of drought on chlorophyll (Xiao et al. 2008).

Solute accumulation has been found to decrease the impact of drought on poplar growth, and improve plant recovery from drought stress (Schulte et al. 1987, Tschaplinski et al. 1994, Hare et al. 1998, Xiao et al. 2008). Solute accumulation allows plants to keep stomata open and cells to continue to elongate despite drought or water limited conditions (Hare et al. 1998, Xiao et al. 2008). In a study by Yang et al. (2010), stem height and leaf number were significantly reduced in one species of poplar, *P. cathayana* but not another, *P. kangdingensis* grown under drought conditions. In the same study, significant differences were found between solute accumulation levels between the two species where the more "drought resistant" *P. kangdingensis* had higher amounts of solutes in its leaves. This is consistent with other previous studies that have found that clones with faster growth under drought conditions tend to have higher solute concentrations in leaves, particularly in upper leaves which then tend to maintain turgor longer (Tschaplinstk and Blaket 1989b). Also, late-season accumulation of solutes occurs in some species of poplar as a way to adapt to short-term water deficits (Tschaplinstk and Blaket 1989).

1.5.2 *Cavitation During Drought Stress*

Stomatal closure in relation to a drop in leaf water potential is likely to avoid hydraulic cavitation (Cochard et al. 2002). One of the effects of drought that poplars are highly prone to is xylem cavitation (Tyree and Ewers 1991, Cochard et al. 2007, Fichot et al. 2010, Huckin et al. 2005). Some researchers have even credited xylem resistance to cavitation as the most important drought resistance trait (Tyree and Ewers 1991). Cochard et al. (2007) found that yield and xylem vulnerability to cavitation were correlated where more productive clones were more vulnerable to cavitation. The correlation found by Cochard et al. (2007) was possibly due to the carbon cost of allocating resources to resistance to cavitation, for example, thicker walled fibres, or producing a greater root-to-shoot ratio. However, few anatomical traits with the exception of xylem fibre wall thickness (a negative correlation) were correlated with xylem cavitation. This causes difficulty when trying to determine which traits make different poplar more susceptible to cavitation under drought conditions (Cochard et al. 2007). Although it has been hypothesized that an increase in resistance to drought induced cavitation comes at a cost to biomass production due to the cost of increased mechanical resistance, a recent study by Fichot et al. (2010) has shown that this assumption does not hold for all poplar species or genotypes. They did not find any trade-off between xylem safety and xylem transport efficiency, or that xylem resistance to cavitation was the result of increased mechanical strength at the cellular level (Fichot et al. 2010).

1.5.3 *Carbon Allocation to Roots During Drought Stress*

Low leaf water potential can be taken as a sign that trees are more water stressed than other trees with a higher leaf water potential (Dickmann et al. 1992). Dickmann et al. (1992) found that the cultivar 'Tristis 1' (*Populus tristis x p. balsamifera*) had higher leaf water potentials under drought conditions than the cultivar 'Eugenei' (*P. nigra x P. deltoides*) which was possibly due to its more extensive root system that would be able to "recharge" over night. One drought avoidance mechanism is to divert carbon resources to below-ground growth to capture a greater amount of the limited soil water, therefore increasing the likelihood of surviving drought conditions; however not all poplar species are adapted to behave this way (Tschaplinski et al. 1994, Tschaplinski et al. 1998, Jackson et al. 2000, Chavez et al. 2003).

1.6 Related Past Studies on Poplar Clones

A previous study by Arango-Velez et al. (2011) of the seven hybrid poplar clones used in this study found that the clones had different levels of drought resistance and exhibited different leaf physiological traits in response to drought. Overall, the balsam clone had the highest drought resistance (due to high resistance to xylem cavitation and stomatal conductance under drought stress) while Northwest, P38P38 and Walker clones were moderately drought resistant and Okanese, Berlin and Assiniboine clones had low drought resistance, based primarily on drought avoidance (stomatal physiology and leaf water potential) and drought tolerance (xylem resistance to cavitation) strategies. The previous study

also showed that Assiniboine had the most sensitive stomata to drought, followed by P38P38 and Berlin, then Okanese and Northwest, and finally the balsam and Walker clones had the least sensitive stomata to drought. Balsam was the only clone to not reduce its stomatal conductivity under severe drought stress. In order of decreased leaf water potential because of drought, Berlin had the highest decrease, followed by Northwest, then Balsam, Okanese and Assiniboine, and Walker and P38P38. Northwest, balsam and the P38P38 clones were all found to have low sensitivity to xylem cavitation while Walker and Assiniboine were moderately sensitive, and Okanese and Berlin were highly sensitive (Arango-Velez et al. 2011). Although they did not find any links with stomatal conductance, Northwest and balsam poplar clones where found to have larger stomata than the other clones. Overall, Arango-Velez et al. (2011) concluded that stomatal responses under mild drought stress could not explain growth rate differences in the studied clones and other factors may be causing the differences.

Arango-Velez et al. (2011) report, based on unpublished trials, that previous studies of Okanese (2403), P38P38 (33), and balsam (1004) clones had shown relatively high growth rates in these clones, while Berlin (42), Northwest (27) and Assiniboine (25) had shown low growth in unfavourable soils. Also, the study by Arango-Velez et al. (2011) found that Berlin (42) plants were sensitive to cavitation and had poor stomatal control which could result in stem dieback under drought conditions. In other studies clone P38P38 and Walker have been classified as a high performer whereas Berlin is known as an average performer (Schreiber et al. 2011). A past study of some of the clones in the current

experiment (P38P38, Berlin and Walker) and a native aspen (after 16 growing seasons) has shown that there is a difference between cavitation resistance, leaf water potential, and water use efficiency between hybrid poplar and hybrid aspen where hybrid poplar were more vulnerable to cavitation, had less negative leaf water potential, and had lower water use efficiency (Tupker et al. 2003, Schreiber et al. 2011).

Caution needs to be taken when comparing clones as clone history has been found to impact drought responses of some clone types. For example, Sherosha et al. (2011) found that clones of Okanese hybrids from Alberta and Saskatchewan did not differ in the number of days it took for stomatal conductance to be impacted by drought, but there was a two day difference in the time required for Alberta and Saskatchewan grown populations of Walker to show drought responses. Another study of the same clone with different origins and genotypes than those used in the current experiment had similar results then the past studies on the clones currently used in this experiment. In both experiments, Walker and Northwest were found to be moderately drought tolerant, and Assiniboine was found to have low drought tolerance. However, while the previous study of the Okanese clone used in this experiment found Okanese to have low drought tolerance, the other study on Okanese from Indian head, Saskatchewan found the clone to have high putative drought tolerance (Silim et al. 2009, Arango-Velez et al. 2011).

Additionally, the Northwest clone (27) has been shown to respond to fertilization (200 kg ha^{-1} nitrogen and 100 kg ha^{-1} phosphorous) when trees were

two years of age by increasing growth by about 15% (1 m^3 ha^{-1} $year^{-1}$) although it's growth was still lower than other available clones despite their lack of response to fertilization (van den Driessche et al. 2008). In a previous study, clones P38P38 (33) and Walker (24) have shown reduced growth with NPK fertilization at planting (while under high pH) possibly due to the reduced net assimilation rate and stomatal conduction under fertilization (DesRochers et al. 2006). The same study also found that Walker is drought sensitive (DesRochers et al. 2006). Studies have also been done under increased elevated carbon dioxide conditions which were found to increase growth of both hybrid aspen and some hybrid poplars (Tupker et al. 2003).

1.7 Study Objectives

In 2004, a controlled environment greenhouse study was conducted as the first step towards establishing drought screening protocols for poplar breeders (Arango-Velez et al. 2011). The present investigation was intended to carry through the results of this study to field conditions and identify the morphological and physiological attributes of drought resistant hybrid *Populus* clones that would make these clones suitable for plantations in water limited environments such as those in north-central Alberta. Previous genetic research trials at Al-Pac have revealed that under drought stress hybrid poplars respond with either: 1) tree mortality, 2) crown dieback with subsequent re-growth of leaders, 3) reduction in growth with no crown dieback, 4) reduction in growth, without crown dieback, with subsequent higher productivity after drought relief, and 5) maintenance of

relatively high growth rates despite periodic drought. It is highly likely that the variability in drought responses is not due to a single physiological trait, but more so the combination of both above ground (stem/branch hydraulic architecture, stomatal behaviour) and below ground (root form/morphology, root hydraulic properties, and root physiology) traits. The overall purpose of this experiment was to identify the combination of traits that contribute to hybrid poplar growth through the following objectives:

1. Identify whether water availability is limiting the growth of selected *Populus* clones grown under field conditions in north-central Alberta.
2. Identify the clones which are the least affected by water availability and the physiological processes that contribute to these responses. Identify the clones which have the highest productivity in terms of above ground biomass production under water limiting and non-limiting conditions and the physiological strategies that contribute to their productivity.

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2. Materials and Methods

2.1 Experimental Design

There were eight poplar clones used in this study: Walker (24 - *P. deltoides* x *P. xpetrowskyana*), Assiniboine (25 - open pollinated Walker cv. Assiniboine), Northwest (27 - *P. balsamifera* x *P. deltoides*), Berlin (42 - *P. laurifolia* x *P. nigra*), Okanese (2403 - Walker x *P. xpetrowskyana*), P38P38 (*P. simonii* x *P. balsamifera*), balsam (1004 - *P. balsamifera*) and hybrid aspen (2782 - *P. tremuloides* x *P. tremula*).

Three irrigation treatments were applied to each of the eight poplar clones. The irrigation treatments during the first season were: no additional water (control), approximately 10.8 – 11.5 L/tree in block 1 and 10.4 – 12.2 L/tree in block 2 (low additional water treatment), and approximately 17.2 – 20.95L/tree in block 1 and 19.35 - 23.05 L/tree in block 2 (high additional water treatment)which were applied over five irrigation periods. During the second irrigation season, due to high amounts of precipitation leading to visible soil saturation, additional water treatments were only applied once to avoid the potential effects of over-watering. The three irrigation treatments were therefore: approximately 1.75 – 2.25 L/tree in block 1 and 1.5 – 2.5 L/tree in block 2 (low additional water treatment), and approximately 4 – 4.25 L/tree in block 1 and 3.75 – 4.5 L/tree in block 2 (high additional water treatment).

All three irrigation treatments were applied to all eight clones. This created 24 different treatment combinations. The experiment had two blocks each containing five replicates of the 24 treatment combinations consistent with a

randomized block design. A random number generator was used to allocate each of the 24 different treatment combinations to rows within each replicate. In each row, nine individual trees of the appropriate clone were planted (1.0 meters apart). The nine individual trees each represented one subsample, the full row of nine subsamples made up one sample, and the 24 different treatment combinations (24 samples) represented one replicate. Border rows (of the aspen clone) were planted at the same spacing. A border tree was also planted four meters to the outward edge of each row on the first and last replicates. A general experimental design is depicted in Figure 2.1.

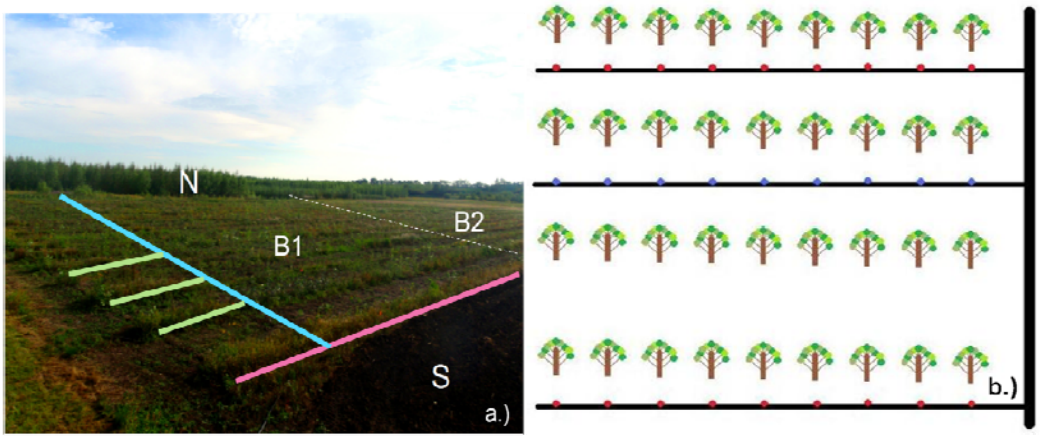

Figure 2.1: A visual depiction of the field plot. The field plot spanned 0.015 km^2. Figure a.) depicts the field plot with two blocks spanning the field space, a main irrigation line ran from the water tank West to East across the southern end of the plot, secondary irrigation lines ran South to North on the eastern edge of each replicate connecting to the main line, tertiary lines run East to West supplying water to sample rows. Control samples did not receive any supplementary water and therefore did not have tertiary irrigation lines. Figure b.) depicts one secondary irrigation line supplying three of the sixteen additional water samples. Tertiary lines were punched with either high or low flow emitters (depicted as blue low flow and red high flow emitters in Figure b.) to supply additional water to individual subsample trees. Each sample row within a rep was a different irrigation treatment and clone combination (3 irrigation treatments, and 8 clones for a total of 24 combinations).

2.2 Plant Material

The poplar trees were propagated from 10 cm stem cuttings collected in the winter of 2007/08 from stoolbeds at the Al-Pac mill site (54°49'N, 113°31'W), the hybrid aspen clone was propagated through micropropagation, and grown during summer 2008 at Bonnyville Forest Nursery (Schreiber et al. 2011). The trial was planted in spring 2009. A total of 270 individual trees were planted per clone. Trees were planted in 9-tree row plots with 3.0 meters between rows and 1.0 meter spacing within rows.

2.3 Field Site

The field study took place on a 1.5ha field plot containing well drained, medium-coarse textured Luvisolic soils based on the Tolman soil series as loam to sandy loam soil (DesRoches et al. 2006) at the Alberta-Pacific Forest Industries Inc. (Al-Pac) mill site located 50 kilometres northeast of Athabasca, Alberta (DesRoches et al. 2006). It was noted that at a depth of approximately 30 cm, however, the soil profile became dense clay.

2.4 Irrigation System

The additional water treatment irrigation system was first laid out in the summer of 2009 and completed in the spring of 2010 (all supplies were purchased from Consolidated turf in Edmonton and were Rain Bird equipment). Starting from the water tank, a main water valve controlled the flow of water through a 32 mm diameter (1 1/4") main line running west to east across the field, and a

connected pump supplied additional pressure to the system, allowing for equal pressure across the side lines. At the end of each replicate, a connector valve controlled the water supplied down each replicate via a 20 mm diameter (3/4") tube running north to south. Of the 24 treatment combinations in each replicate, the 16 rows designated as additional water treatments were connected to the side lines running down each replicate via a t-connector. Nine emitters were punched in the 15 mm diameter (1/2") tubing supplying each row, with 8 mm diameter (1/4") tubing connected to each emitter. Each of the 9 trees in the rows thus had an individual water supply. Black, one gallon per hour emitters were punched in the rows given the low water treatment and red, two gallons per hour emitters were punched in the rows given the high irrigation treatment. Within each replicate, an additional emitter was punched in the uppermost and lowest rows of both the low and high water treatments. The actual range of water being applied to each replicate could thus be quantified.

2.5 Biomass Measurements

A time line of measurements over the course of the study as well as precipitation and soil moisture measurements (season 2) is provided in Appendix 3, Figures 2.5.1 and 2.5.2.

2.5.1 *Height and Caliper*

For both seasons, height and caliper measurements were taken during the last week of September when total growth for the season was complete. Height

and caliper measurements were taken on the same individual trees throughout the study. Measurements were taken on two individual trees from the same row (irrigation x clone treatment) as subsamples and averaged as one sample. Five replicates for each of the two blocks were measured. Caliper measurements were taken at the very base of the shoot (typically ground level) and height measurements were taken from the shoot base to the bud tip of the tallest apical branch.

2.5.2 *Shoot Biomass Measurements*

For both seasons two individual trees from the same row (irrigation x clone treatment) were harvested as subsamples and averaged as one sample. Three replicates for each of the two blocks were measured. Shoots for the final mass for the 2010 and 2011 growing seasons were collected in the last two weeks August, both while taking HPFM measurements. Leaves were removed before drying and weighed separately. For the first growth season, shoots and leaves were dried at 70 ^{o}C for two days until the samples were completely dry. In the second growth season, the shoots and leaves were left in a greenhouse for approximately two weeks until both shoots and leaves were dry.

2.5.3 *Root Biomass Measurements*

First Season:

Roots were collected two weeks after the shoots were collected for both season one and two growth measurements. A reduced sample set was collected

for root weight as time restraints only allowed for the collection of roots of 3 reps within the two blocks and 10 water treatment x clone combinations. Roots of five of the eight clones, under the highest and control water treatments were collected. The four clones with the least similar parentage, as well as the two clones with the most similar parentage were sampled to ensure capturing the differences in conductivity between trees due to the greatest and least genetic differences, these clones included: Balsam, Berlin, hybrid aspen, Walker and Okanese (the two most genetically similar). Before drying, roots were washed thoroughly to remove excess soil and root volume was taken. The root volume was measured by placing roots in a beaker of water and measuring the mass of water displaced by the roots. The roots were dried at 70^{o}C for two days until the samples were completely dry.

Second Season:

Due to the size of the root systems by the end of the second season, and the available time, excavating the same number of roots at the end of the second season as those sampled during the first season was not possible. Instead, a representative sample of the clone populations were taken to derive formulas for predicting second season root biomass in combination with the below ground biomass from the first season and shoot mass.

From September 5th to 7th, two individual tree root systems were excavated of the selected clone and water treatment combinations in a single replicate. Growth parameters such as root mass, root volume, calliper, tree height and above ground biomass mass were taken. Using the samples from both the first and

second seasons, several models were created using stepwise multiple regression for predicting root biomass. The model with the highest R^2 value for each clone was selected to predict the root biomass for the remaining five replicates of samples. The "best fit" models tended to be the linear relationship between above shoot biomass and root biomass. Those models are included in Table 2.5.1.

Table 2.5.1: Models used to predict below ground biomass of Balsam (1004), Okanese (2403), Walker (24), Berlin (42) and hybrid aspen (2782) clones. "y" represents the predicted root biomass while "x" represents the known above ground biomass.

Clone	Formula	p-value	Adjusted R^2
Balsam (1004)	y = -9.15822 + 0.51533x	<.0001	0.9880
Berlin (42)	y = 1.37943 + 0.26841x	<.0001	0.9901
Okanese (2403)	y = 2.33571 + 0.26421x	<.0001	0.9296
Walker (24)	y = 4.97973 + 0.13465x	<.0001	0.7380
Hybrid Aspen (2782)	y = -0.19419 + 0.37635x	<.0001	0.9956

2.5.4 *Root Mass to Shoot Mass Ratios*

As root mass values were only available for a subset of the experimental clone and irrigation treatments, shoot mass to root mass ratios for only Balsam (1004), Okanese (2403), Walker (24), Berlin (42) and hybrid aspen (2782) clones of the highest and control irrigation treatments were calculated. For each replicate of the clone x irrigation treatments, the root masses were divided by the shoot masses to obtain index values. This provided 3 index value replicates for each of the 2 blocks.

2.6 Leaf Physiology Measurements

2.6.1 *Infra Red Gas Analyzer Measurements for Photosynthesis, Water Use Efficiency, Stomatal Conductance and Transpiration*

Season 1:

During the last three weeks in July 2010, stomatal conductance, photosynthesis, water use efficiency and transpiration measurements were all made using a PP Systems CIRAS-1 infrared gas analyzer, simultaneously. By July, the trees had all produced fully expanded, mature leaves. Measurements were made on the youngest set of fully expanded leaves (subsamples) of two trees in each treatment combination, within each replicate. The subsamples were averaged as one sample per replicate. The CIRAS-1 infrared gas analyzer was equipped with a broad leaf cuvette which measured 2.5 cm^2 segments of each leaf. The CIRAS-1 infrared gas analyzer measures stomatal conductance, photosynthesis and transpiration by determining the difference in carbon dioxide concentrations (within 1 ppm) and water concentrations (assimilation and transpiration, respectively) as a controlled concentration of carbon dioxide and determined concentration of water are passed through the leaf segment sealed in a closed chamber (broad leaf cuvette). Measurements were taken between 9 a.m. and 1 p.m. and one to two replicates (96 subsample leaves or 48 samples) were measured each day. Stomatal conductance, net photosynthesis and transpiration are determined from the differences in carbon dioxide and water concentrations based on the calculations shown below (performed automatically by the IRGA).

Water use efficiency was calculated as the ratio between the rate of productivity (net photosynthesis) and the rate of water use (transpiration).

Season 2:

During the first week of August, 2011, stomatal conductance, net photosynthesis, water use efficiency and transpiration measurements were all made using a ADC (Analytical Development Company Limited) LCA-4 infrared gas analyzer, simultaneously. Measurements were made on the youngest set of fully expanded leaves of two trees in each treatment combination, within each rep. The LCA-4 infrared gas analyzer was equipped with a leaf cuvette which measured leaf segments with an assumed 6.25cm^2 surface area of each leaf. The actual measured area of each leaf was marked, frozen, and measured in October 2011. Measurements were then corrected to represent the actual area measured. The LCA-4 infrared gas analyzer measures stomatal conductance, net photosynthesis and transpiration by determining the difference in carbon dioxide concentrations and water concentrations (assimilation and transpiration, respectively) as a measured concentration of carbon dioxide and determined concentration of water are passed through the leaf segment sealed in a closed chamber (leaf cuvette). Measurements were taken between 9 a.m. and 1 p.m. and two to three reps were measured each day. Stomatal conductance, net photosynthesis and transpiration are determined from the differences in carbon dioxide and water concentrations based on the calculations shown below (performed automatically by the IRGA). Water use efficiency was calculated as

the ratio between the rate of productivity (photosynthesis) and the rate of water use (transpiration).

Calculations:

Transpiration (E):

$$E = [(W \times (e_{out} - e_{in})] / (P - e_{out}) = \text{mmol } m^{-2} s^{-1}$$

Where W is the mass flow of air per unit leaf area entering the cuvette, e_{out} is the water vapor pressure of the air leaving the cuvette, e_{in} is the water vapor pressure of the air entering the cuvette and and P is the atmospheric pressure.

Stomatal Conductance (g_s):

$$g_s = 1/r_s = \text{mmol } m^{-2} s^{-1}$$

Where r_s is the stomatal resistance of the leaf calculated as:

$$r_s = [(e_{leaf} - e_{out}) / (E \times P)] - r_b$$

Where e_{leaf} is the saturated vapour pressure at leaf temperature, e_{out} is the water vapor pressure of the air leaving the cuvette, E is the transpiration rate, P is the atmospheric pressure and r_b is the boundary layer resistance to water vapour.

Photosynthesis (A):

$$A = C_{in} \times W - C_{out} \times (W + E) = \text{mmol } m^{-2} s^{-1}$$

Where C_{in} is the concentration of carbon dioxide entering the cuvette, W is the mass flow of air per unit leaf area entering the cuvette, C_{out} is the concentration of carbon dioxide leaving the cuvette and E is the transpiration rate.

Water use Effiency:

$$WUE = A/E = \text{mmol}_{CO2}\, \text{mmol}_{H2O}^{-1}$$

Where A is the rate of photosynthesis and E is the rate of transpiration.

2.6.2 *Chlorophyll Analysis*

By July 2010 and 2011, the trees had all produced fully expanded, mature leaves and during the last three weeks in July leaves were collected for chlorophyll analysis. Measurements were made on the youngest set of fully expanded leaves of two trees (subsamples) in each treatment combination within each replicate. Leaf samples were collected while performing predawn water potential measurements between 3 and 5 a.m. Leaf samples were collected in the dark, wrapped in aluminum foil to prevent light exposure and stored on ice for approximately 10 hours before being stored in a -80^{o}C freezer. Samples from the first season were analyzed in January, 2011, and samples from the second season were analyzed in October of 2011.

The frozen fresh samples were analyzed following the methods described by Tjoelker et al. (1995) and Barnes et al. (1992). Approximately 1.848cm^2 disks were punched from the frozen, fresh leaf samples, using a clean, rust-free hole punched and were weighed (two leaves for each treatment combination within each rep). The disks as well as 5 ml of dimethyl sulphoxide (DMSO) were placed in 15ml plastic centrifuge tubes and stored in an oven over night at 60^{o}C for 14 hours. Fresh DMSO (up to 7.5 ml) was then added. The chlorophyll content of the extracts was then analyzed spectrophotometrically at 665 nm and 648 nm. The concentrations of chlorophyll from the leaf extracts were calculated using the following formulas:

Total Chlorophyll Content (Chlorophylls a and b):

$$C_{a+b} = 7.49A_{665} + 20.34A_{648}$$

Where $A_{664.9}$ is the absorbency of a sample at the wavelength of 665nm and A_{648} is the absorbency of the same sample at the wavelength of 648nm. (Chlorophyll measurements were standardized to the milligram of chlorophyll (chloro) per milliliter of extractant and gram of fresh weight (fw) (mg_{chloro} / mg_{fw} mL).

2.6.3 *Pre-Dawn Leaf Water Potential Measurements*

Pre-dawn water potential measurements were taken using a Scholander pressure chamber according to the procedure outlined in Turner (1988). Measurements were taken between 3:00 a.m. and 5:30 a.m. Measurements were made on the youngest set of fully expanded leaves of two trees (subsamples which were averaged to obtain one sample) in each irrigation treatment and clone combination within each of the five replicates of the two blocks were measured; one to three replicates were measured per night. Individual leaves were fitted via rubber stopper adapters inside a pressure chamber so the leaf petioles would be visible from just outside the camber. Pressure was applied to the chamber until the point when the cut surface of the leaf petiole changed color signalling the presence of water on the cut surface. The positive pressure applied at the point where water is forced out of the petiole of the leaf is equal (but opposite) to the water pressure of the leaf. During season 1, measurements were taken during the first week of July. In season 2, measurements were taken in the third week of July.

2.7 Stem Hydraulic Conductance Measurements

During the second season, five of the eight clones, under the highest and control water treatments were collected. Time constraints did not allow for all water treatment and clone combinations to be sampled. The four clones with the least similar parentage, as well as the two clones with the most similar parentage were sampled to ensure capturing the differences in conductance between trees due to the greatest and least genetic differences. These clones included Balsam (1004), Walker (24), Okanese (2403), Berlin (42) and hybrid aspen (2782). Two subsamples (individual trees) were measured and averaged to obtain one sample from a replicate. This was repeated to obtain three replicates of the 10 treatment combinations per block. Conductance was measured under a gravity induced flow meter system (low pressure flow meter, LPFM) as described by Sperry et al. (1988). Between two and five (with a target of five, and most often five) two cm segments of each individual tree were measured for the initial conductance, flushed to remove any blockages from the segments (air embolisms), and re-measured for the maximum conductance of the stem segments. A 100 mmol KCl solution was used to flush the segments and measure the conductance. The percentage of the conductance that was blocked by embolisms in the stems was determined from the difference between the initial and maximum conductance.

Season 2:

LPFM measurements were performed in August in 2011. Each block was analyzed over the course of one week. One branch, of about 30cm in length, from each of the selected water treatment clone combinations, in each of the 3 reps,

was collected the first day of analysis early in the morning and again on the third day of analysis. All leaves were removed from the branches on site, the segments were then cut again under water to a length of about 20cm, and finally rolled in wet paper towel. The branches were kept in garbage bags containing water, and transported in a cooler until they could be stored in a 4^{o}C fridge. Analysis of the branches started approximately 4 hours after the beginning of branch collection and was completed within 2 days of the initial collection.

2.8 Root Hydraulic Conductance and Conductivity

2.8.1 *Root Hydraulic Conductance*

Five of the eight clones, under the highest irrigation treatment and control water were measured. Time constraints did not allow for all water treatment and clone combinations to be sampled. The four clones with the least similar parentage, as well as the two clones with the most similar parentage were sampled to ensure capturing the differences in conductance between trees due to the greatest and least genetic differences. These clones included Balsam (1004), Walker (24), Okanese (2403), Berlin (42) and hybrid aspen (2782). Two subsamples (individual trees) were measured and averaged to obtain one sample of a replicate. This was repeated to obtain three replicates of the 10 treatment combinations per block.

Root hydraulic conductance measurements were made using the high pressure flow meter (HFPM) as described by Tyree et al. (1993) and Tyree et al. (1994). Shoots of trees were cut 3 to 12 cm above the ground and the bark pealed

back with a razor blade. An adaptor was fitted to the exposed tissue. Degassed water was inserted into the adapter and all bubbles in the adaptor removed. The HPFM system was connected to the root system via a thin tube to the adapter. Degassed water was forced through the HPFM system, into the root system under increasing pressure. A calibrated volume of flow would pass through the connected systems under specific pressures. From the flow rates and pressure differences achieved over a measurement interval, a graph of pressure vs time was produced for each measurement. The slope of this graph represented the conductance of the root system. Two measurements of conductance were made on each root system and the second conductance measurement produced was used for analysis.

2.8.2 *Root Hydraulic Conductivity*

Hydraulic conductivity of the roots was derived by dividing the conductance of the whole root system by the mass of the root system. This was done by dividing the conductance replicate values described in section 2.8.1 by the root mass measurements described in section 2.5.4. This provided 3 replicate root conductivity values in each of the 2 blocks for each clone x treatment combination of the reduced sample set.

2.9 Statistical Analysis

2.9.1 *Analysis of Irrigation and Clone Treatments*

A two-way analysis of variance (ANOVA) using Proc Mixed in the statistical program SAS 9.2 (SAS, Toronto, Ontario, Canada) was used to determine if there were significant differences between treatments for all of the measured variables using the following model:

$$Y_{ijkl} = \mu + C_i + I_j + B_k + CI_{ij} + CB_{ik} + IB_{jk} + CIB_{ijk} + e_{ijkl}$$

Where Y_{ijk} is an observation on the lth tree sample of the ith clone under the jth irrigation treatment in the kth block, C_i is the effect due to the ith clone (1,...,8), I_j is the effect of the jth irrigation treatment (1,...,3), B_k is the effect due to the kth block (1,2), CI_{ij} is the interaction between the ith clone and jth irrigation treatment, CB_{ik} is the interaction between the ith clone and the kth block, IB_{jk} is the interaction between the jth irrigation treatment and the kth block, CIB_{ijk} is the interaction between the ith clone, the jth irrigation treatment and the kth block, and e_{ijkl} is the residual error. Clone and irrigation treatment are fixed effects while the block is a random effect.

Differences between means were considered significant at $p \leq 0.05$. The analyses of irrigation and clone treatments revealed that the irrigation treatments did not result in any significant differences in the biomass production of physiology of the clones. The irrigation treatments were therefore removed from the analysis. As such, the analysis of the results of this study will focus on comparing clones rather than the irrigation treatments.

2.9.2 *Analysis of the Clone Treatment*

A one-way analysis of variance (ANOVA) using Proc Mixed in the statistical program SAS 9.2 (SAS, Toronto, Ontario, Canada) was used to determine if there were significant differences between clones for all the measured variables using the following model:

$$Y_{ikl} = \mu + C_i + B_k + CB_{ik} + e_{ikl}$$

Where Y_{ikl} is an observation on the lth tree sample of the ith clone kth block, C_i is the effect due to the ith clone (1,...,8), B_k is the effect due to the kth block (1,2), CB_{ik} is the interaction between the ith clone and the kth block, and e_{ikl} is the residual error. Clone is a fixed effect while the block is a random effect.

Differences between means were considered significant at $p \leq 0.05$. Based on tests of normality (Shapiro-Wilk and Kolmogorov-Smirnov tests) and tests of homogeneity of variances (Bartlett's and Levene's tests) not all of the data fit the assumptions of the ANOVA and needed to be transformed. Transformations are provided in Table 2.10.1. Pair wise comparisons were made to determine the significant differences between clones using the least square means differences statements and were adjusted with the Tukey-Kramer adjustment to a total alpha value of $\alpha \leq 0.05$.

Table 2.10.1: Variables from the first and second seasons that violated the assumptions of ANOVA and needed to be transformed with the given transformations in order for the data to meet the assumptions of ANOVA.

	Variable	Transformation
Season 1 (2010)	Calliper (C)	$C' = \text{Log}(C)$
	Root Conductance (K_r)	$K_r' = (K_r + 0.000015)^{-1}$
	Shoot Mass (S_m)	$S_m' = \ln(S_m)$
	Root Conductivity (L_r)	$L_r' = (L_r + 0.0015)^{-1}$
	Pre-Dawn Water Potential (Ψ_p)	$\Psi_p' = (\Psi_p)^{-1}$
Season 2 (2011)	Percent loss in branch conductance (K_b)	$K_b' = (L_b + 2.825)^{-1}$
	Root Conductance (K_r)	$K_r' = (K_r + 0.001)^{-1}$
	Pre-Dawn Water Potential (Ψ_p)	$\Psi_p' = (\Psi_p + 4)^{-1}$

2.9.3 *Analysis of Correlations Between Biomass Measurements and Physiological Measurements*

Pearson correlation tests were performed in order to determine the relationships between growth variables and physiological variables for 5 of the 8 clones. Full data sets were only available for Balsam (1004), Okanese (2403), Walker (24), Berlin (42) and hybrid aspen (2782) clones, as such, correlations were only analysed for these clones. All of the data were tested for normality (Shapiro-Wilk and Kolmogorov-Smirnov tests) and those data sets that were not found to be normally distributed were transformed and the transformed data was used in the Pearson correlation analysis. These data transformations are provided in Table 2.10.2.

Table 2.10.2: Variables from the first and second seasons that violated the assumptions of the Pearson correlation analysis and needed to be transformed with the given transformations in order for the data to meet the assumptions. Transformations were applied to data of all clones except root conductivity (L_r) and root conductance (K_r) in season 1 for clone 1004 (data were normal), and the shoot mass (S_m) transformation for season 2 only applied to clone 2782.

	Variable	Transformation
Season 1 (2010)	Root Conductance (K_r)	$K_r' = (K_r + 0.000015)^{-1}$
	Root Conductivity (L_r)	$L_r' = (L_r + 0.0015)^{-1}$
	Pre-Dawn Water Potential (Ψ_p)	$\Psi_p' = (\Psi_p)^{-1}$
Season 2 (2011)	Shoot Mass (S_m)	$S_m' = \ln(S_m + 50)$
	Root Conductance (K_r)	$K_r' = (K_r + 0.001)^{-1}$
	Pre-Dawn Water Potential (Ψ_p)	$\Psi_p' = (\Psi_p + 4)^{-1}$

3. Results

3.1 Season 1 (2010):

3.1.1 *Morphological Parameters*

Significant differences between the eight clones were found for all morphological measurements including caliper, height, root dry weight, and shoot dry weight, but not for the root to shoot ratios which ranged in values from 2.4 (hybrid aspen (2782)) to 3.8 (Walker (24)) (Table 3.1.1, Figure 3.1.1). The Berlin (42) clone had the greatest height (139.7 cm) which was significantly different from all other clones, as well as the greatest caliper (18.03 mm) which was significantly different from all other clones except balsam (1004) poplar (Figure 3.1.1). Height followed the ranking of Berlin (42) > Okanese (2403) > P38P38

(33) > balsam (1004) poplar > hybrid aspen (2782) > Walker (24) > Assiniboine (25) (Figure 3.1.1). The three shortest clones (Assiniboine (25), Walker (24), and Northwest (27)) were not significantly different from one another in terms of height (Figure 3.1.1). These clones were not significantly different from one another in caliper, and had three of the four lowest calipers (Figure 3.1.1). Caliper rankings were Berlin (42) > balsam (1004) > Okanese (2403) > P38P38 (33) > Northwest (27) > hybrid aspen (2782) > Walker (24) > Assiniboine (25) (Figure 3.1.1). Balsam (1004) poplar, Berlin (42), and Okanese (2403) clones had the greatest shoot DW out of the eight clones (respectively), but did not have significantly different shoot DW's from each other (Figure 3.1.1). Even though clone Berlin (42) had the greatest height and caliper, balsam (1004) poplar had the greatest shoot DW. Shoot DW rankings were Berlin (42) > balsam (1004) > Okanese (2403) > P38P38 (33) > hybrid aspen (2782) > Northwest (27) > Walker (24) > Assiniboine (25) (Figure 3.1.1). Again, Northwest (27), Walker (24) and Assiniboine (25) clones had the lowest shoot DW values. Balsam (1004) poplar clone produced the greatest root DW, followed by Okanese (2403) > Berlin (42) > hybrid aspen (2782) > Walker (24) (Figure 3.1.1). The only significant differences between root DW's were between the clone with the highest root DW (balsam (1004)) and the two clones with the lowest root DW Walker (24) and hybrid aspen (2782)) (Figure 3.1.1).

Table 3.1.1 Category, trait, numerator degrees of freedom (Num DF), denominator degrees of freedom (Den DF), F value and P values from ANOVA's of morphological parameters for eight clones on three year old trees taken in the 2010 field season from July to September. Morphological parameters analyzed included: height (*H*), caliper (transformed data, *C*), shoot DW (transformed data, S_m), root DW (R_m), and root to shoot ratio (R_m:S_m); leaf physiological parameters analyzed included: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), and chlorophyll concentration (*Chl*); and root physiological parameters: root conductivity (transformed data, L_r), and root conductance (transformed data, K_r) where $\alpha \leq 0.05$. Only five clones were measured (hybrid aspen (2782), Okanese (2403), balsam (1004), Berlin (42), and Walker (24)) for root DW (R_m), root to shoot ratio (R_m:S_m), root conductivity (transformed data, L_r), and root conductance (transformed data, K_r).

Category	Trait	Num DF	Den DF	F Value	p-value
Mass	***H***	7	230	21.52	<.001
	C	7	230	30.83	<.001
	S_m	7	133	12.09	<.001
	R_m	4	53	3.99	0.007
	R_m:S_m	4	53	0.51	0.726
Leaf Physiology	***A***	7	213	2.11	0.044
	g_s	7	218	10.9	<.001
	E	7	218	2.62	0.013
	WUE	7	213	1.99	0.058
	Ψ_p	7	181	2.92	0.007
	Chl	7	230	2.34	0.027
Root Physiology	L_r	4	53	5.19	0.001
	K_r	4	55	11.81	<.001

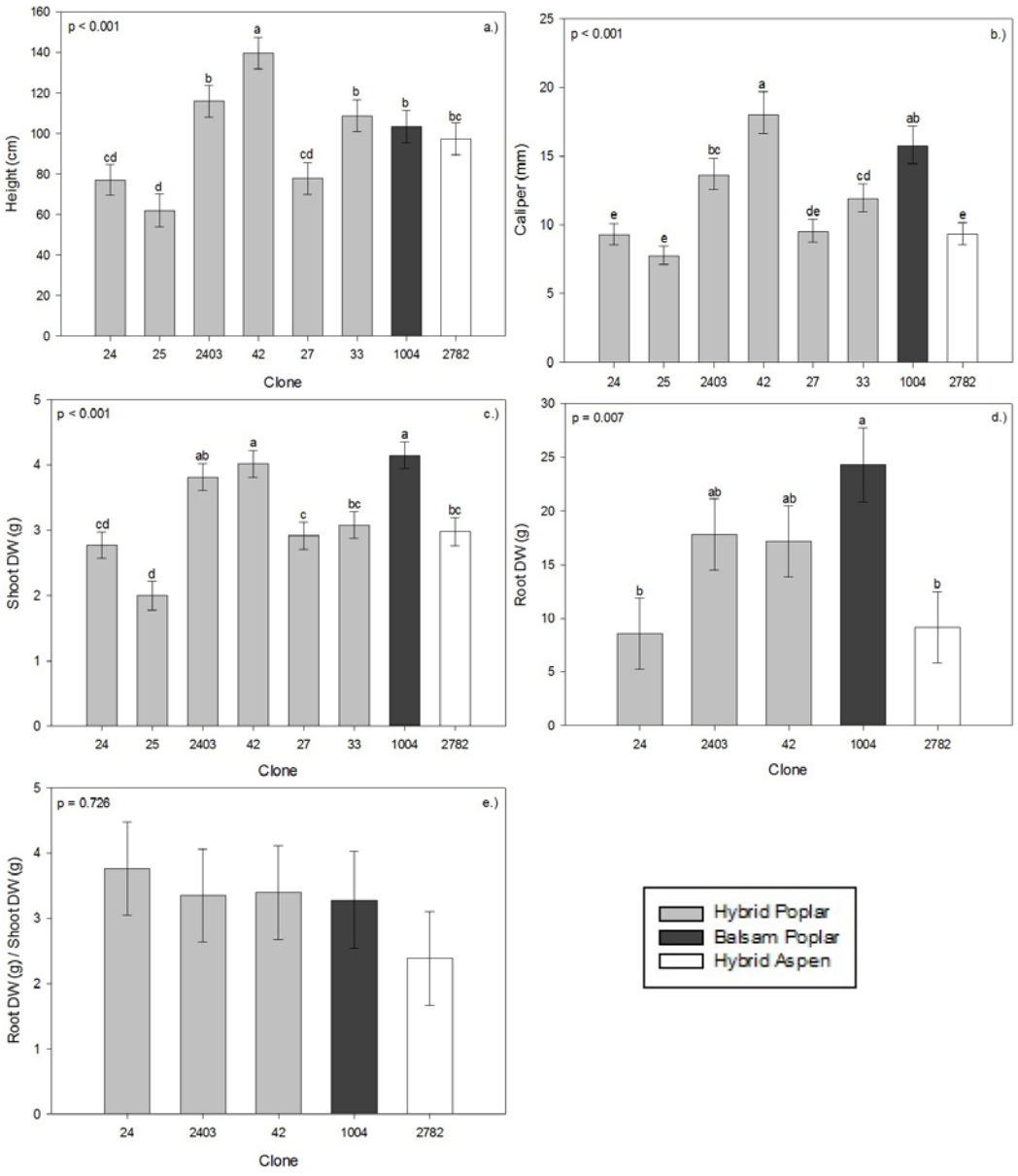

Figure 3.1.1: Means (±SE) of morphological parameters in different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (Hybrid aspen) for the 2010 field season taken in August when plants were in their third growing season: height (a), caliper (b), shoot DW (c), root DW (d), and root to shoot ratio (e). Significant differences between values of clones are represented by alphabetical groupings, where $\alpha \leq 0.05$ after a Tukey's adjustment was applied to p-values.

3.1.2 *Leaf Physiological Parameters*

Significant differences between clones were found for three of four gas exchange parameters including stomatal conductance (g_s), net photosynthesis (A), and transpiration (E), but not water use efficiency (Table 3.1.1, Figure 3.1.2). Significant differences between clones were also found for total chlorophyll concentrations and predawn water potential (Ψ_p) (Table 3.1.1, Figure 3.1.2). Differences between individual clones were not found for A; however, the three clones with the greatest shoot DW also had the greatest A (clones balsam (1004), Okanese (2403), and Berlin (42)) (Figure 3.1.2). The hybrid aspen (2782) clone had a g_s significantly lower than all other clones (0.11 $molm^{-2}s^{-1}$) followed by the three clones with the highest shoot DW: balsam (1004) = Berlin (42) < Okanese (2403) then clone P38P38 (33), and last the clones with the lowest shoot DW's: Northwest (27), Walker (24), and Assiniboine (25) (Figure 3.1.2). The only significant differences between individual clones for E rates were between clone Assiniboine (25) with the highest E rate and clone hybrid aspen (2782) with the lowest E rate (Figure 3.1.2). There were no significant differences between individual clones for chlorophyll concentration. Significant differences between individual clones were found in Ψ_p between balsam (1004) poplar (with a value nearest 0), and the clones with the lowest (most negative) water potential: Berlin (42) < hybrid aspen (2782) = Assiniboine (25) (Figure 3.1.2).

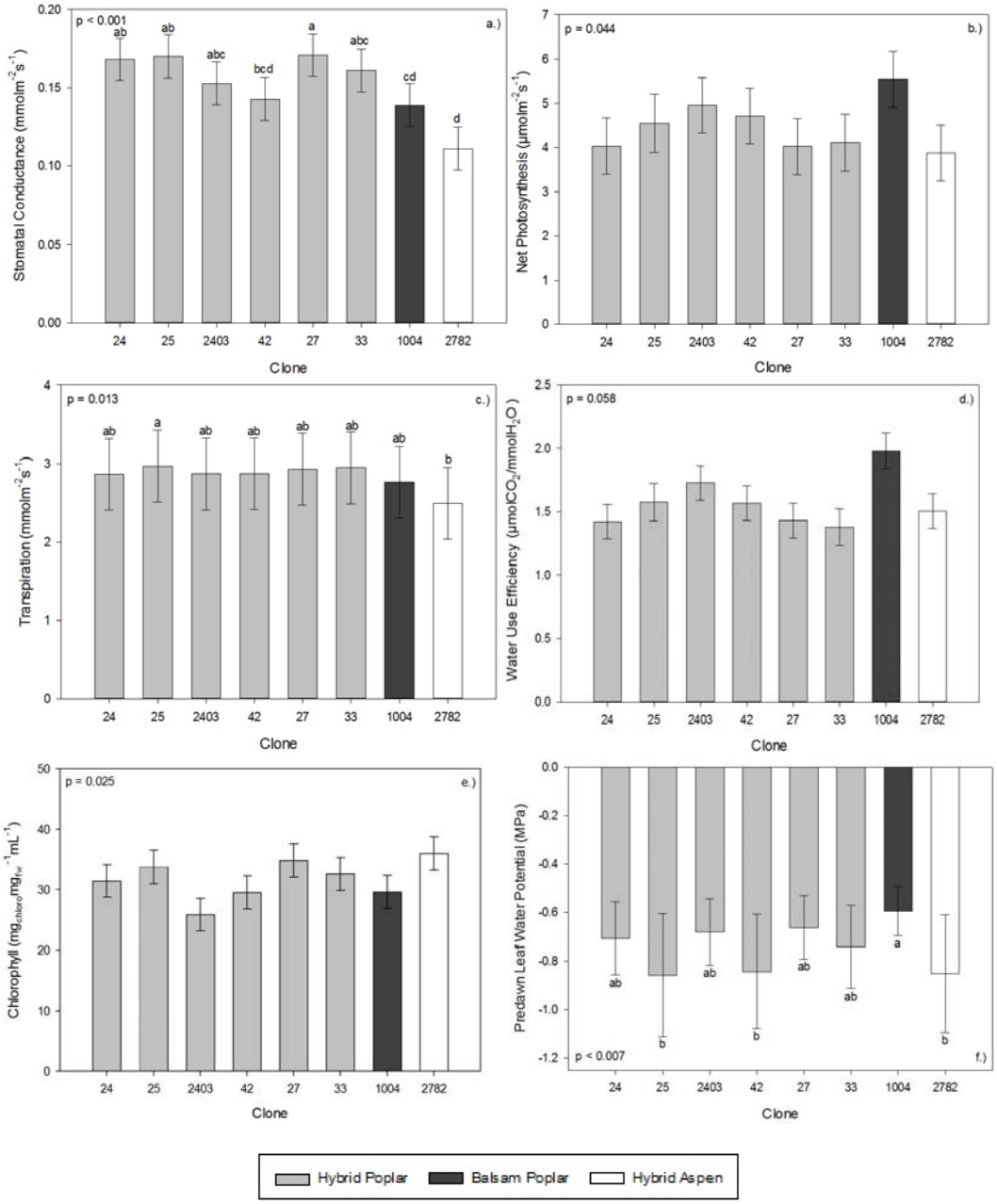

Figure 3.1.2: Means (±SE) of leaf physiological parameters in different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (hybrid aspen) for the 2010 field season measured in July 2010, when plants were in their third growing season: g_s (a), A (b), E (c), water use efficiency (d), chlorophyll concentration (e), and Ψ_p (f). Significant differences between values of clones are represented by alphabetical groupings.

3.1.3 *Root Hydraulic Conductance and Conductivity*

Significant differences between clones were found for root hydraulic conductance, and conductivity (Table 3.1.1, Figure 3.1.3). The three clones with the greatest shoot DW had the highest root hydraulic conductance: balsam (1004) > Berlin (42) > Okanese (2403), which were significantly different from the two other measured clones, but not significantly different from each other (Figure 3.1.3). Walker (24) (the clone with the lowest height and shoot DW) had the lowest root hydraulic conductance, but was not significantly different from the root hydraulic conductance of the hybrid aspen (2782) clone (Figure 3.1.3). The three clones with the greatest shoot DW also had the highest root hydraulic conductivity of the clones: Berlin (42) > balsam (1004) > Okanese (2403); however, only Berlin (42) with the greatest root conductance and significantly greatest height was significantly different from the two clones with the lowest root conductance (hybrid aspen (2782) and Walker (24)) (Figure 3.1.3). Walker (24) (the clone with the lowest height and shoot DW) had the lowest root hydraulic conductivity, but was not significantly different from the hydraulic conductance of hybrid aspen (2782) (Figure 3.1.3).

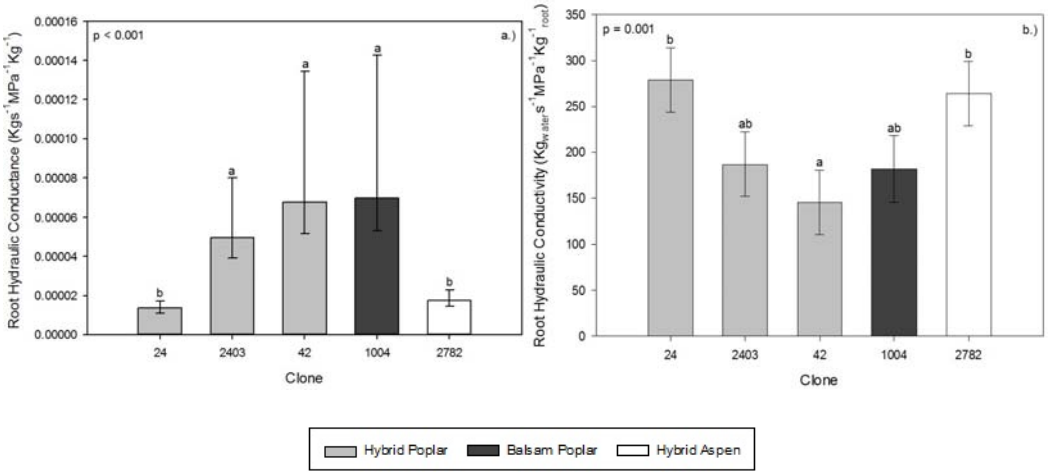

Figure 3.1.3: Means (±SE) of root hydraulic conductance and conductivity of different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (hybrid aspen) for the 2010 field season taken in August when plants were in their third growing season: root conductance (a) and root conductivity (b). Significant differences between values of clones are represented by alphabetical groupings, where $\alpha \leq 0.05$ after a Tukey's adjustment was applied to p-values.

3.1.4 *Correlations between Morphological Parameters and Physiological Measurements*

Pearson correlation analyses were performed to identify any correlations between measured morphological and physiological parameters. Because full sets of measurements were only available for five of the eight clones, correlations were performed on clone Walker (24) (Table 3.1.5 Appendix 1, Figure 3.1.4 Appendix 2), Okanese (2403) (Table 3.1.6 App. 1, Figure 3.1.5 App. 2), Berlin (42) (Table 3.1.7 App. 1, Figure 3.1.6 App. 3), balsam (1004) (Table 3.1.8 App. 1, Figure 3.1.7 App. 2), and hybrid aspen (2782) (Table 3.1.9 App. 1, Figure 3.1.8 App. 2).

Leaf Physiology

Three of the five analyzed clones had significant correlations between morphological and leaf physiology measurements: Okanese (2403), balsam (1004), and hybrid aspen (2782) (Table 3.1.6, 3.1.8, and 3.1.9, App. 1). Height and caliper of Okanese (2403) had a significant negative correlation with chlorophyll concentration (clone Okanese (2403), had the lowest chlorophyll concentration of the examined clones (Figure 3.1.2,Table 3.1.6, App. 1). Ψ_p of Okanese (2403) had a significant positive correlation with root to shoot ratio (Table 3.1.6, App. 1). The Ψ_p of hybrid aspen (2782) had a significant negative correlation with shoot and root DW's (Table 3.1.9, App. 1). Hybrid aspen (2782) had the lowest Ψ_p of all clones (Figure 3.1.2). Balsam (1004) poplar had three significant correlations between leaf physiology and morphological parameters: root DW was negatively correlated with leaf chlorophyll concentration and positively correlated with *A*, and water use efficiency was positively correlated with shoot DW (Table 3.1.8, App. 1).

Root Hydraulic Conductance and Conductivity

Root hydraulic conductance had significant positive correlations with root and shoot DW for four of the five analyzed clones: Walker (24), Berlin (42), balsam (1004) and hybrid aspen (2782) (Tables 3.1.5, 3.1.7, 3.1.8, and 3.1.9 App. 1). Walker (24) had the lowest root hydraulic conductance (Figure 3.1.3) as well as the lowest shoot and root DW (Figure 3.1.1), while clone balsam (1004) had the highest root hydraulic conductance (Figure 3.1.3) as well as the highest root

and shoot DW (Figure 3.1.1). Okanese (2403) was the only clone to not have a significant correlation between shoot and root DW and root hydraulic conductance, but had significant positive correlations between root hydraulic conductivity and both caliper and root to shoot ratio (Table 3.1.6, App. 1). Okanese (2403) also had a significant positive correlation between root to shoot ratio and root hydraulic conductivity (Table 3.1.6, App. 1).

3.2 Season 2 (2011):

3.2.1 *Morphological Measurements*

Significant differences between the eight clones were found for all morphological measurements including caliper, height, root DW, shoot DW, and the root to shoot ratios (Table 3.2.1, Figure 3.2.1). Berlin (42) was significantly taller than all other clones and Assiniboine (25) was significantly lower than all other clones (Figure 3.2.1.). The clones in order of height were: Berlin (42) > Okanese (2403) > P38P38 (33) > balsam (1004) > hybrid aspen (2782) > Walker (24) > Northwest (27) > Assiniboine (25) (Figure 3.2.1.). Caliper values followed a very similar ranking: Berlin (42) > Okanese (2403) > balsam (1004) > P38P38 (33) > hybrid aspen (2782) > Northwest (27) > Walker (24) > Assiniboine (25) (Figure 3.2.1.). Berlin (42) had the highest shoot DW, but was not significantly different from the shoot DW's for the clones with the next highest DW's, Okanese (2403) and balsam (1004), respectively (Figure 3.2.1.). The clones followed the shoot DW ranking of: Berlin (42) > Okanese (2403) > balsam (1004) > hybrid aspen (2782) > P38P38 (33) > Northwest (27) > Walker (24) >

Assiniboine (25) (Figure 3.2.1.). Balsam (1004) poplar had the highest root DW, followed by clone Berlin (42)>Okanese (2403)>hybrid aspen (2782)>Walker (24) (Figure 3.2.1.). The root DW of Walker (24) was significantly lower compared to all other clones (which did not have significant differences between one another) except for hybrid aspen (2782) (Figure 3.2.1.). Balsam (1004) poplar had the highest root to shoot ratio, followed by hybrid aspen (2782) > Okanese (2403) = Berlin (42) > Walker (24) (Figure 3.2.1.). All clones had significantly different root to shoot ratios from one another except for Berlin (42) and Okanese (2403), the clones with the greatest shoot DW (Figure 3.2.1.).

Table 3.2.1: Category, trait, numerator degrees of freedom (Num DF), denominator degrees of freedom (Den DF), F value and P values from ANOVA's of morphological parameters for eight clones on four year old trees taken in the 2011 field season from July to September. Morphological parameters analyzed included: height (*H*), caliper (transformed data, *C*), shoot DW (transformed data, S_m), root DW (R_m), and root to shoot ratio (R_m:S_m); leaf physiological parameters analyzed included: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), and chlorophyll concentration (*Chl*); and root physiological parameters: root conductivity (transformed data, L_r), and root conductance (transformed data, K_r), and shoot physiological parameters: percent loss in branch conductance (Transformed data, K_b) where $\alpha \leq 0.05$. Only five clones were measured (hybrid aspen (2782), Okanese (2403), balsam (1004), Berlin (42), and Walker (24)) for root DW (R_m), root to shoot ratio (R_m:S_m), root conductivity (transformed data, L_r), and root conductance (transformed data, K_r)

	Effect	Num DF	Den DF	F Value	Pr > F
Mass	***H***	7	227	44.6	<.001
	C	7	227	52.66	<.001
	S_m	7	133	13.53	<.001
	R_m	4	55	7.37	<.001
	Rm:Sm	4	55	238.48	<.001
Leaf Physiology	***A***	7	181	7.88	<.001
	g_s	7	180	6.62	<.001
	E	7	181	15.84	<.001
	WUE	7	181	8.09	<.001
	Ψ_p	7	226	7.95	<.001
	Chl	7	227	4.49	<.001
Shoot Physiology	***K_b***	4	53	7.73	<.001
Root Physiology	***L_r***	4	52	2.5	0.054
	K_r	4	52	14.92	<.001

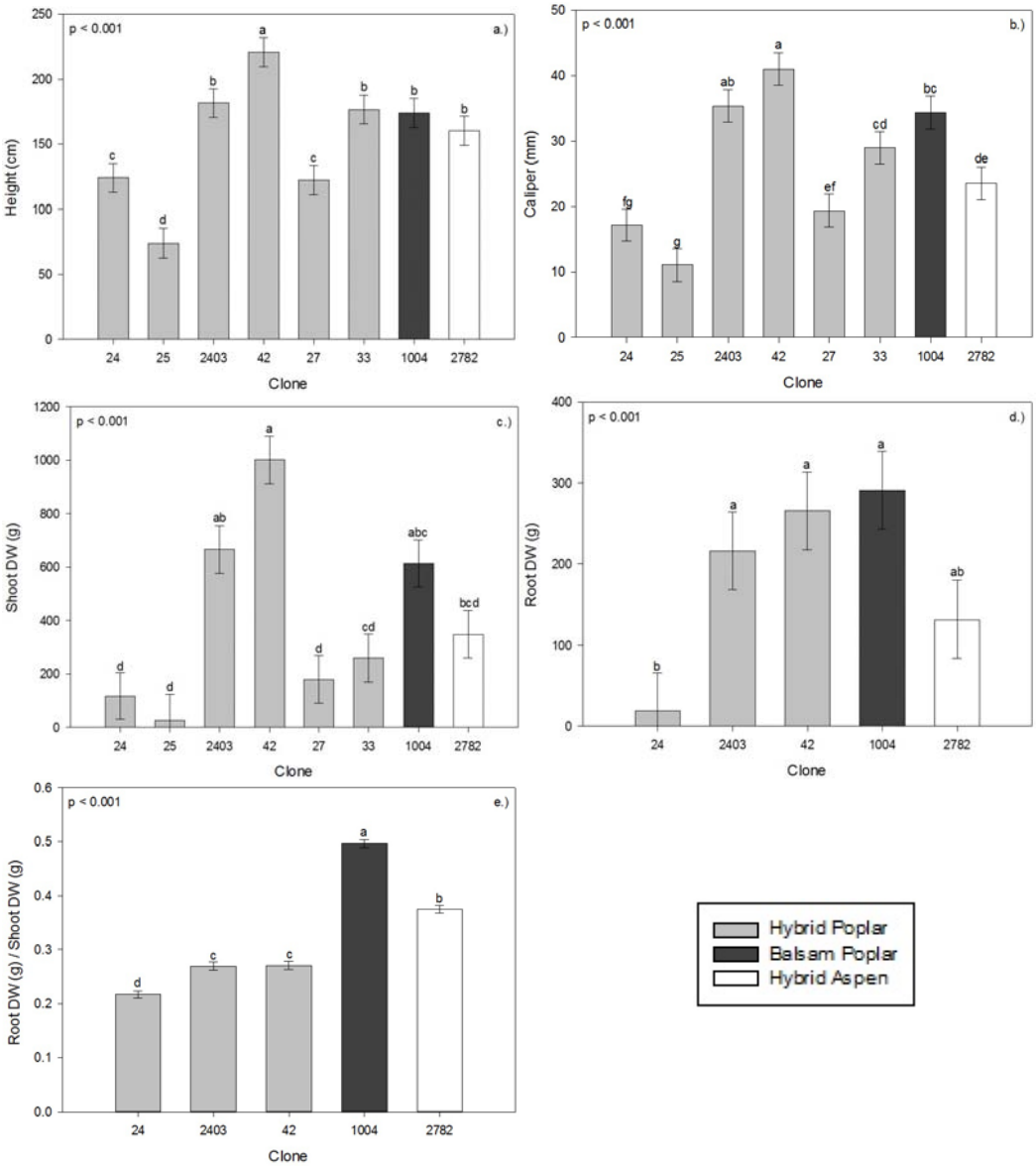

Figure 3.2.1: Means (±SE) of morphological parameters of different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (Hybrid aspen) for the 2011 field season taken in August when plants were in their fourth growing season: height (a), caliper (b), shoot DW (c), root DW (d), and root to shoot ratio (e). Significant differences between values of clones are represented by alphabetical groupings, where $\alpha \leq 0.05$ after a Tukey's adjustment was applied to p-values.

3.2.2 *Leaf Physiological Parameters*

Significant differences between clones were found for all gas exchange parameters including g_s, A, E and water use efficiency (Table 3.2.1, Figure 3.2.2). Significant differences between clones were also found in total chlorophyll concentration and Ψ_p (Table 3.2.1, Figure 3.2.2). Okanese (2403) had the highest A, followed by Assiniboine (25) > balsam (1004) > Northwest (27) > Walker (24) > P38P38 (33) > hybrid aspen (2782) > Berlin (42) (Figure 3.2.2). Notably, the clone with the lowest shoot DW and lowest height had the second highest A rate while the clone with the highest shoot DW and greatest height had the lowest A rate (Figures 3.2.1 and 3.2.2). The three clones with the highest DW and greatest height had the lowest g_s: Berlin (42) > Okanese (2403) > Balsam (1004) (Figures 3.2.1 and 3.2.2). The two clones with the lowest height and lowest shoot DW had the highest g_s rates: Walker (24) and Assiniboine (25) (Figures 3.2.1 and 3.2.2). Clones Walker (24) and Assiniboine (25) also had the highest E rates (Figure 3.2.2). This was followed by clone P38P38 (33) > hybrid aspen (2782) > Berlin (42) > Northwest (27) > Okanese (2403) > balsam (1004) (Figure 3.2.2). The balsam (1004) clone had the highest water use efficiency, followed by Okanese (2403) > Northwest (27) > Berlin (42) > Walker (24) > hybrid aspen (2782) > P38P38 (33) > Assiniboine (25) (Figure 3.2.2). Okanese (2403) had a significantly lower chlorophyll concentration compared with the remaining clones except for the hybrid aspen (2782) clone (Figure 3.2.2). Okanese (2403) had the Ψ_p closest to zero, while balsam (1004) poplar had the lowest (most negative) Ψ_p (Figure 3.2.2). The Ψ_p of clones followed the ranking of Okanese (2403) >

Walker (24) = P38P38 (33) > Berlin (42) >Northwest (27) > hybrid aspen (2782) > Assiniboine (25) > balsam (1004) (least negative to most negative) (Figure 3.2.2).

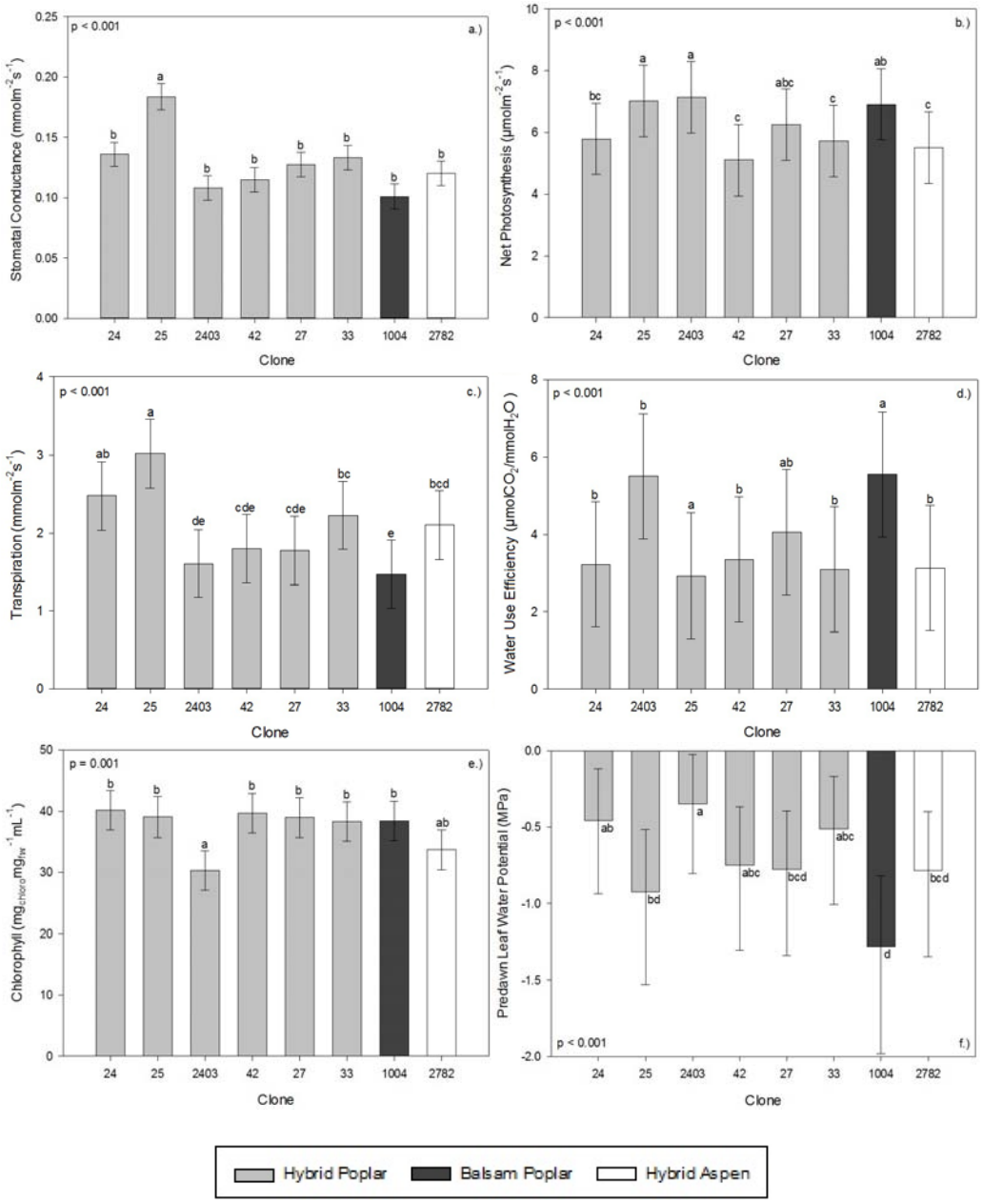

Figure 3.1.2: Means (±SE) of leaf physiological parameters of different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (Hybrid aspen) for the 2010 field season measured in July 2010, when plants were in their third growing season: g_s (a), A (b), E (c), water use efficiency (d), chlorophyll concentration (e),

and Ψ_p (f). Significant differences between values of clones are represented by alphabetical groupings.

3.2.3 *Root Hydraulic Conductance and Conductivity*

Significant differences between clones were found for root hydraulic conductance, but not root hydraulic conductivity (Table 3.2.1 and Figure 3.2.4). Root conductivity values ranged between 1.17^{-2} kg H_2O $s^{-1}MPa^{-1}kg_{root}^{-1}$ (Okanese (2403)) and 8.40^{-3} kg H_2O $s^{-1}MPa^{-1}kg_{root}^{-1}$ (hybrid aspen (2782)) (Figure 3.2.4). Again, the three clones with the greatest shoot DW (Berlin (42), Okanese (2403) and balsam (1004) poplar, Figure 3.2.1), and the two clones with the greatest shoot height (Berlin (42) and Okanese (2403), Figure 3.2.1) had the highest root hydraulic conductance: Berlin (42)>Okanese (2403)>balsam (1004) (Figure 3.2.4). Clone Walker (24), with the lowest height and lowest shoot DW, had the lowest root hydraulic conductance (Figures 3.2.1 and 3.2.4)

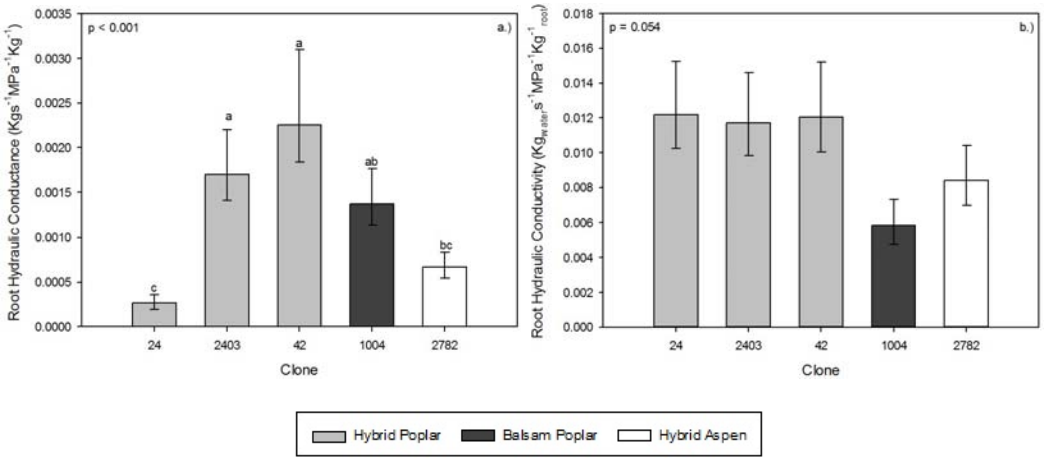

Figure 3.2.3: Means (±SE) of root phyiological parameters of different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (Hybrid aspen) for the 2011 field season taken in August when plants were in their fourth growing season: root conductance (a) and root conductivity (b). Significant differences between values of clones are represented by alphabetical groupings, where $\alpha \leq 0.05$ after a Tukey's adjustment was applied to p-values.

3.2.4 *Shoot Physiology*

Significant differences between percent conductance loss due to air blockages in branches were found between clones (Table 3.2.1, Figure 3.2.3). The two clones with the greatest height and shoot DW, clones Berlin (42) and Okanese (2403), had the highest percent conductance loss due to air blockages in branches (Figures 3.2.1 and 3.2.3). Hybrid aspen (2782) had the third highest loss in conductance, followed by Walker (24), and balsam (1004) poplar clones, respectively (Figure 3.2.3).

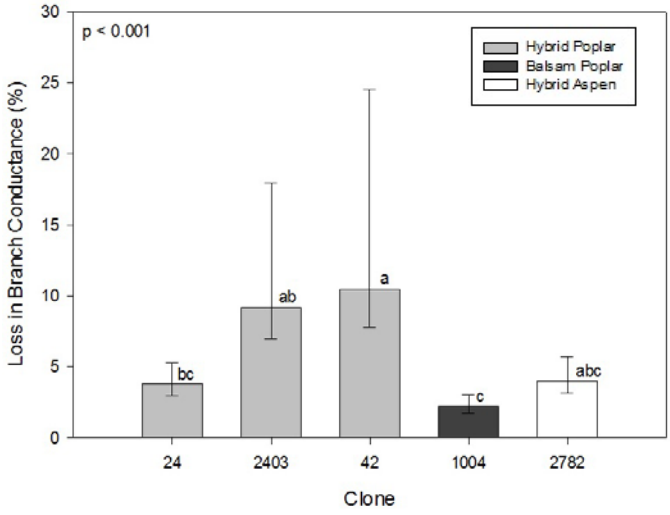

Figure 3.2.4: Means (±SE) of shoot physiological parameters (percent loss in branch conductance) of different poplar clones: 24 (Walker), 25 (Assiniboine), 2403 (Okanese), 42 (Berlin), 27 (Northwest), 33 (P38P38), 1004 (balsam), and 2782 (Hybrid aspen) for the 2011 field season taken in August when plants were in their fourth growing season. Significant differences between values of clones are represented by alphabetical groupings, where $\alpha \leq 0.05$ after a Tukey's adjustment was applied to p-values.

3.2.5 *Correlations between Morphological Parameters and Physiology*

Pearson correlation analyses were performed to identify any correlations between measured morphological and physiological parameters. Because full sets of measurements were only available for five of the eight clones, correlations were performed on Walker (24) (Table 3.2.6 Appendix 1, Figure 3.2.5 Appendix 2), Okanese (2403) (Table 3.2.7 App. 1, Figure 3.2.6 App. 2), Berlin (42) (Table 3.2.8 App. 1, Figure 3.2.7 App. 3), balsam poplar (1004) (Table 3.2.9 App. 1, Figure 3.2.8, App. 2), and hybrid aspen (2782) (Table 3.2.10 App. 1, Figure 3.2.9, App. 2).

Leaf Physiology

Height of Okanese (2403) plants had a significant negative correlation with g_s (Table 3.2.7, App. 1). Walker (24) had a significant negative correlation with g_s for root DW and shoot DW and a positive correlation with root to shoot ratio (Table 3.2.6, App. 1). Walker (24) also had the highest g_s (Figure 3.2.2), lowest root DW, shoot DW, and root to shoot ratio (Figure 3.2.1) of the five analyzed clones. Water use efficiency of Walker (24) had a significant positive correlation to height and caliper (Table 3.2.6, App. 1). The height and caliper of Walker (24) were the lowest of the clones while only clone hybrid aspen (2782) had lower water use efficiency (Figures 3.2.1 and 3.2.2). The Ψ_p of hybrid aspen (2782) had the second lowest value (next to clone balsam (1004)) and the root to

shoot ratio of clone hybrid aspen (2782) was the second highest (next to balsam (1004) poplar) (Figures 3.2.1 and 3.2.2).

Stem Hydraulic Conductance

Berlin (42) was the only clone to show a significant correlation between morphological parameters and percent loss in stem conductance due to air blockage (Table 3.2.8, App. 1). Shoot DW, root DW, and caliper of Berlin (42) were positively correlated with percent loss in stem conductance due to air blockage (Table 3.2.8, App. 1). Berlin (42) had the highest percent loss in stem conductance due to air blockage (Figure 3.2.3), shoot DW and caliper, and the second highest root DW (Figure 3.2.1).

Root Hydraulic Conductance and Conductivity

Both Okanese (2403) and balsam (1004) clones had a significant positive correlation between caliper and root hydraulic conductance (Tables 3.2.7 and 3.2.9 App. 1). Shoot DW and root to shoot ratio of clones Berlin (42), Okanese (2403), and hybrid aspen (2782) had significant positive correlations with root conductance (Table 3.2.7, 3.2.8 and 3.2.9, App. 1). Clones Berlin (42) and Okanese (2403) had both the highest shoot DW and root hydraulic conductance (Figures 3.2.1 and 3.2.4). Root DW's of clones Berlin (42) and Okanese (2403) was also had a significant positive correlation with root hydraulic conductance (Table 3.2.7 and 3.2.8 App. 1). Shoot and root DW of clone hybrid aspen (2782) also had a significant positive correlation with root hydraulic conductivity (Table

3.2.10, App. 1). Hybrid aspen (2782) had the second lowest root hydraulic conductivity of the clones (Figure 3.2.4).

4. Discussion

4.1 Biomass:

Similarly to the present study, growth performance differences have been commonly found among different poplar clones (Rhodenbaugh and Pallardy 1993, Barigah et al. 1994, Scarascia-Mugnozza et al. 1997, Bunn et al. 2004, Bonhomme et al. 2008).

In another field study conducted several years earlier in a field adjacent to my experiment, P38P38 and Walker clones were classified as high performers based on their high above ground growth (Walker was considered a reference clone, but had an average growth that higher than that of the high performance clones) whereas Berlin was an average performer (Schreiber et al. 2011). In my study, Berlin was considered a high performer based on its high above ground growth (height, caliper and shoot dry weight), clone P38P38 an average performer and Walker a poor performer. The study by Schreiber et al. (2011) was conducted on trees that were substantially older than the trees used in this experiment (hybrid poplar were 16 and aspen 11-years old compared to 3- and 4-years old in my study). These discrepancies suggest that age and growth conditions may influence the growth of the clones and the clones may not, therefore, always grow consistently according to the trends found in my study. As the clones age, their height, caliper and shoot mass rankings may change and be more similar to the

findings of Schreiber et al. (2011). For instance, the shoot and height rankings between the first and second field seasons of my experiment did not remain exactly the same. Also, Berlin clone has been noted as having poor growth in unfavourable soils which could account for the different classification of growth between my experiment and that by Shreiber et al. (2011) (Arango-Velez et al. 2011).

A study by Arango-Velez et al. (2011) reported, based on Al-Pac's genetic field trials, that clones Okanese and Balsam have relatively high growth rates, the growth of clone P38P38 is variable depending on conditions, clone Walker had better growth with well drained soils, and clones Berlin, Northwest and Assiniboine have low growth (in unfavourable soils). In my study, Okanese and Berlin showed high growth rates (suggesting favourable soil conditions due to the high growth of clone Berlin which tends to have low growth in unfavourable soils), balsam, P38P38 and Northwest had average growth rates, and Assiniboine and Walker low growth rates. The low growth of Walker could have been due to its suggested intolerance of poorly drained soils and the high occurrence of soil water exceeding field capacity in my experiment. The growth differences between clones found in my experiment did not agree with previous field studies and may suggest that the field site used had favourable soil conditions (high growth of clone Berlin), poorly drained soils (the poor performance of clone Walker), or other unknown factors causing the lower growth of clone balsam and the differences in above ground growth of the clones between my study and other Al-Pac genetic field trials.

Differences in physiological responses and growth are likely attributable to genetic differences between these highly complex hybrid poplar clones. The first potential way in which the similarities and differences in physiological responses and biomass production of the different clones can be explained is through genetics. A study by Talbot et al. (2011) used a genotyping assay to distinguish between four compatible *Populus* species (*Populus balsamifera* L., *Populus deltoides* Bartr. ex Marsh, *Populus laurifolia* Ledeb. and *Populus nigra* L.) from across the Canadian prairies including four of the clones used in this experiment (Northwest, Walker, Assiniboine, and Okanese). Northwest, Walker and Assiniboine were the clones with the lowest above ground growth (height, caliper, shoot dry weight) in the experiment; Northwest was determined by Talbot et al. (2011) to be about 50% *P. balsamifera*, 49% *P. deltoides* and 1% *P. nigra*, Walker 53% *P. nigra*, 3% *P. laurifolia*, and 44% *P. deltoides*, and Assiniboine 23% *P. deltoides*, 1% *P. laurifolia*, and 76% *P. nigra*. Also, one of the highest performing clones, Okanese, was found by Talbot et al. (2011) to be 51% *P. nigra*, 26% *P. laurifolia*, 8% *P. balsamifera*, and 15% *P. deltoides*. The only general trend that could be found was that Okanese, the clone with the greatest shoot dry weight, caliper and height of the four clones, had the lowest proportion of *P. deltoides* and the highest proportion of *P. laurifolia*. Notably, although this particular clone was not analyzed, the balsam poplar (*P. balsamifera*), hybrid aspen, and P38P38 clones which all had average growth (height, caliper and shoot dry weight) and are assumed to not contain genes from *P. deltoides*, *P. laurifolia*, nor *P. nigra*; while the clone (Berlin) with the greatest height and overall above

ground biomass production, was a cross between *P. nigra* and *P. laurifolia* and theoretically contains predominantly the genes of those two parent species. It is possible, based on the results from this experiment and those of Talbot et al. (2011), that the presence of genes from *P. deltoides* had a negative effect on hybrid poplar growth while the presence of genes from *P. laurifolia* has a positive effect on hybrid poplar growth and contributing more to the observed hybrid vigour. This could be due to *P. deltoides* adaptation to, and tendency to grow best in, well drained soils. Whereas *P. laurifolia* is adapted to, and has a tendency to grow best in, moist to wet soils (Hortipedia 2011). In my experiment, there were a high proportion of the growth seasons where the field was visibly above field capacity (Cooper 1990, Van Haverbeke 1990).

As noted in previous sections, during much of both the first and second field season of this study, soil moisture was very high and often times exceeding soil capacity. It is not unreasonable to expect that the trees in this study expressed responses to flooding conditions. Common flooding responses by clones include production of hypertrophied lenticels, decreased leaf initiation and development, reduced height, diameter, and shoot weight, chlorosis and abscission of leaves, die-back of roots, and reduced protein and chlorophyll concentrations (Gong et al. 2007, Neilsen et al 2008, Guo et al. 2011, Du et al. 2012, Luquez et al. 2012). Kozlowski (1997) summarizes that flooding affects soils by: altering soil structure, depleting oxygen as water occupies the pores in soil previously occupied by oxygen, accumulating carbon dioxide, inducing anaerobic decomposition of organic matter and reducing iron and manganese. Flooding, in

general, inhibits the gas exchange between the soil and atmosphere (Smit and Stachowiak 1988). As with drought, under flooding conditions if stomatal conductance is not reduced, and roots are inhibited in their ability to take up water (due to anoxic conditions), water stress is likely to occur (Gong et al. 2007).

Key adaptations in flooding tolerance include production of hypertrophied lenticels, aerenchyma tissue, and adventitious roots (Kozlowski 1997, Du et al. 2012). Because of the scope of this project, flooding adaptations were not investigated although some clones in this experiment may have had these adaptations. Adventitious roots increase water absorbed by roots (Kozlowski 1997). Just as has been summarized previously about drought, flood tolerance varies greatly among species, genotypes, age, gender, duration, and other study conditions (Kozlowski 1997, Caoa and Connerb 1999, Neilsen et al. 2008, Luquez et al. 2012). For example, of 16 investigated *P. deltoides* clones, a range of flooding tolerance responses were found; eight were resistant, four were moderately tolerant and four were intolerant (Caoa and Connerb 1999). This suggests that even within a species, flood tolerance can vary greatly. A study by Luquez et al. (2012) revealed that a five-year field trial experiment and greenhouse experiment had opposite results of groups of poplar cultivars in their responses to flooding. Those which were flooding tolerant in the field were more susceptible to flooding in the greenhouse study. In some poplar (*Populus angustifolia*) male and female genotypes grew differently under flooding conditions although they grew similarly under control conditions (Neilsen et al. 2008).

4.2 Role of Leaves in Biomass Production via Photosynthesis, Stomatal Conductance, Water Use Efficiency, Chlorophyll Concentration, and Leaf Predawn Water Potential

4.2.1 *Photosynthesis*

Although there were no significant differences between clones in season one, the clones with the greatest mean shoot masses (balsam, Okanese and Berlin) had the highest net photosynthetic rates. These trends were not apparent in season two although there were significant differences between clones in the mean photosynthetic rate. Notably, the clone with the lowest mean shoot biomass and height had the second highest net photosynthetic rate and the clone with the highest mean shoot biomass and height had the lowest net photosynthetic rate. Many previous studies have demonstrated that poplars tend to vary in photosynthesis rates based on genetic differences (Dickmann et al. 1992, Rhodenbaugh and Pallardy 1993, Barigah et al. 1994, Bunn et al. 2004, Voltas et al. 2006, Fichot et al. 2010).

Growth and maintenance of a tree ultimately depend on the rate by which the canopy of the tree can capture light and assimilate carbon dioxide through photosynthesis (Wolfe et al. 1998, Dillen et al. 2010). Therefore, the correlation found between root mass and the photosynthetic rate of the balsam poplar clone, and the clones with the highest biomass having the highest photosynthetic rates in

season one, is not surprising. Similar results were reported by Voltas et al. (2006) where correlations were found between stem growth and photosynthesis of *Populus xeuramericana* clones during their second year of growth under field conditions. Barigah et al. (1994) also found in their field experiment of poplar clones (hybrid *P. trichocarpa x P. deltoides*, which were the higher biomass producing clones, and *P. xeuramericana*, and pure *P. trichocarpa*) during the first field season, the poplar clones with the greatest shoot dry weight, height and caliper had high photosynthetic rates and four out of five investigated clones had a correlation between leaf photosynthesis and above ground biomass (Barigah et al. 1994).

However, what is surprising in my experiment is that there were no significant correlations between growth parameters (shoot and root mass, root to shoot ratio, height and diameter) and net photosynthesis except for root mass of the balsam poplar clone in season one. Some studies have shown relationships between photosynthesis and biomass while others have not, leading to a lack of a clear relationship between the two factors (Dillen et al. 2010).

One explanation for the poorly correlated relationship between biomass production and photosynthesis is the sensitivity of instantaneous photosynthesis rate measurements to environmental variation (Dillen et al. 2010). Voltas et al. (2006) caution that because of the large diurnal changes in net photosynthesis and stomatal conductance that have been observed in several field studies, interpreting instantaneous photosynthetic measurements should be done carefully. Dillen et al. (2010) reason that the lack of a clear relationship could possibly be due to

several factors, specifically instantaneous measurements may not represent the trend of photosynthesis that occurs throughout a day or growth season (climate factors which influence photosynthesis can fluctuate immensely even over the course of an hour), and the lack of consideration for the leaf area over which photosynthesis is occurring (higher leaf area of a whole tree will have more photosynthesis taking place than a tree with a lower leaf area at the same photosynthetic rate) (Barigah et al. 1994). Barigah et al. (1994) found significant positive relationships between photosynthetic capacity and above ground biomass for four of the five poplar clones they investigated with a clone of *P. xeuramericana* being the exception. Despite its high photosynthetic capacity, that particular clone was found to have low growth and a relatively low leaf area. This would imply that not only do clones need a high photosynthesis rate to produce biomass, but they need to couple that photosynthesis rate with a large canopy over which photosynthesis is occurring. The two clones with the highest above ground biomass production in the experiment by Barigah et al. (1994) were credited as having higher production due to a larger leaf area and a high photosynthetic performance; the two clones with the lowest photosynthetic rates and low leaf area production had overall low above ground biomass production. In my experiment, similar relationships can be assumed as the clones with the highest observable leaf area had the greatest above ground biomass production while clones that had observably low overall leaf area produced little biomass despite having relatively high photosynthesis rates (ie. Assiniboine in season two).

Flooding has been found to cause decreased photosynthetic rates; however, this does not occur in all poplar (Kozlowski 1997, Neilsen et al. 2008, Rood et al. 2010, Guo et al. 2011, Luquez et al. 2012). For example, a study by Guo et al. (2011) revealed that 10 of 13 clones had reduced growth rates under drought conditions which was correlated to a reduction in photosynthesis rates. Net photosynthesis and stomatal conductance decreased significantly with flooding, but both recovered when flooding ended with photosynthesis recovery lagging behind stomatal conductance (Gong et al. 2007). A study by Gong et al. (2007) revealed that all 14 of the investigated poplar clones reduced their stomatal conductance and net photosynthesis rates under flooding conditions although more sensitive clones reached their lowest values more quickly and were more greatly affected. The differences in net photosynthesis rates may have been due to the flood like conditions that persisted during a good proportion of both field seasons.

It is worth noting that in the two previously mentioned field studies by Voltas et al. (2006) and Barigah et al. (1994) which showed correlations between biomass and photosynthesis, photosynthesis measurements were repeated several times (once weekly during the field season and nine times during the field season, respectively). In my experiment, photosynthesis measurements were made only once (per individual tree) which may have reduced the likelihood of measurements that were representative of the full field season. Other studies have also reported a lack of correlation between photosynthesis rate and biomass production. For example, a greenhouse study of hybrid poplar clones when they

were 6-10 weeks old by Rodenbaugh and Pallardy (1993) showed no correlation between biomass production and photosynthesis rate. In that study, photosynthesis measurements were only taken once. Also, a field study of three year old hybrid poplar clones by Bunn et al. (2004) found that leaf development is more important than photosynthetic rate in determining biomass production when photosynthesis measurements were only taken three times (Bunn et al. 2004). The low repetition in photosynthesis measurements in experiments by Bunn et al. (2004) and Rodenbaugh and Pallardy (1993) as well as my own may have contributed to the lack of correlation between photosynthetic rates and biomass production. Overall, as conducted in my experiment, photosynthetic rates are not a good predictor of biomass production under field conditions of the examined hybrid poplars.

4.2.2 *Stomatal Conductance*

Photosynthesis requires gas exchange between the leaf and the surrounding atmosphere through stomatal pores; the rate at which the gas exchange occurs is known as stomatal conductance (Silim et al. 2009). During both seasons, significant differences were found between clones in stomatal conductance. Many studies have found genetic differences in stomatal conductance of poplar (Blake and Tschaplinski 1984, Dickmann et al. 1992, Rhodenbaugh and Pallardy 1993, Voltas et al. 2006).

As previously stated, my study includes five hybrids of the *Tacamahaca* and *Aigeiros* sections: Walker, Assiniboine, Northwest, Berlin and Okanese, a hybrid

from the section *Tacamahaca*: P38P38, a pure poplar from the section *Tacamahaca*: balsam, and a hybrid aspen clone from the section *Populus*. During the first field season, the hybrid aspen clone had a significantly lower stomatal conductance than all other clones. The aspens of the *Populus* section are upland "dier" habitat species compared with the riparian cottonwoods of the *Tacamahaca* and *Aigeiros* sections more commonly found near river systems (Braatne et al. 1996, Eckenwalder 1996, Cooke and Rood 2007). Stomatal closure or reduced stomatal conductance is a way in which plants can regulate cavitation risk, and reduce water loss (Shulte and Hinckley 1987, Huckin et al. 2005). In drier habitats, where water is more limiting than riparian habitats, reduced stomatal conductance rates would give plants an adaptive advantage. Stomatal traits are believed to be heritable (Pearce et al. 2005). As such, it is not surprising that the hybrid aspen clone had the lowest stomatal conductance during the first field season.

During both seasons, the three clones with the highest mean shoot dry mass had three of the lowest stomatal conductance rates and the clones with the lowest mass had the three highest stomatal conductance rates. This trend suggests that relatively low stomatal conductance is associated with higher biomass production, although there were few significant correlations between biomass and stomatal conductance. This could possibly be related to high water use efficiency as water use efficiency is dependent upon the ratio of productivity and water loss through stomata, yet I did not find many correlations between water use efficiency

(discussed later) and biomass production, and the direct relationship between stomatal conductance and water use efficiency was not investigated.

Flooding has been found to cause decreased stomatal conductance; however, this does not occur in all poplar (Kozlowski 1997, Rood et al. 2010, Guo et al. 2011, Luquez et al. 2012). Flooding has been found to cause stomatal closure during flooding periods, however, stomatal conductance has been found to recover quickly once flooding has ended (Gong et al. 2007, Du et al. 2008). A study by Gong et al. (2007) revealed that all 14 of the investigated poplar clones reduced their stomatal conductance and net photosynthesis rates under flooding conditions although more sensitive clones reached their lowest values more quickly and were more greatly affected. It is possible that the clones in this study that had the lowest stomatal conductance were adapted to reduce their stomatal conductance under drought conditions.

During the first season, there were no significant correlations between stomatal conductance and biomass production, but in season two, height of Okanese plants, and shoot and root mass of Walker plants were negatively correlated with stomatal conductance, again suggesting that high biomass production is associated with low stomatal conductance, and positively correlated with high root to shoot ratio. Clone Walker had the highest mean stomatal conductance rate as well as the lowest mean root mass, shoot mass and root to shoot ratio (of the five analyzed clones). It can therefore be concluded from these results that high stomatal conductance tends to have a negative effect on biomass production, although it is not always a significant relationship, and some clones

have a closer relationship between stomatal conductance and biomass production than others. Other studies have also shown that stomatal conductance of hybrid poplars is not correlated with biomass production (Fichot et al. 2010, Voltas et al. 2006). The inconsistent correlation between biomass production and stomatal conductance across seasons for clones Walker and Okanese suggests that there may have been another unknown factor influencing the relationship.

A field study by Al Afas et al. (2006) found that stomatal density of hybrid poplar and pure poplar (with *P. nigra*, *P. trichocarpa* and *P. deltoides* parentage) was positively correlated with biomass production and was determined by parentage while (although not significantly correlated) clones with smaller stomata tended to have higher biomass production and clones with longer stomata tended to have lower biomass production. A field study by Pearce et al. (2005) found a significant correlation between stomatal conductance and stomatal density. The field study by Pearce et al. (2005) found that stomatal density influenced differences in stomatal conductance in riparian poplar species and that there was variation in stomatal characteristics within and among poplar species and hybrids (Pearce et al. 2005). It is therefore possible, based on this evidence of heredity of stomatal traits, that the stomatal traits related to high biomass are commonly shared heritable traits of highly productive clones and does not directly influence biomass production which could leading to a lack of correlation between biomass production and stomatal conductance seen in my experiment (Pearce et al. 2005, Al Afas et al. 2006). Since stomatal density has been shown to be correlated with both stomatal conductance (Pearce et al. 2005) and biomass

production (Al Afas et al. 2006), stomatal density may be a better stomatal trait to use as an indicator of biomass production.

Another study conducted by Arango-Velez et al. (2011) conducted in a greenhouse under optimal conditions investigated stomatal control of gas exchange under drought stress and found that stomatal responses under mild drought stress could not explain growth rate differences in the same studied clones as the current experiment and other factors may be causing the differences. In the same study by Arango-Velez et al. (2011), Assiniboine trees had the most sensitive stomata to drought, followed by P38P38 and Berlin, then Okanese and Northwest. Balsam poplar, and Walker clones had the least sensitive stomata to drought (Arango-Velez et al. 2011). Although they were the same clones in both experiments the sensitivity of stomatal closure determined by Arango-Velez et al. (2011) did not parallel the stomatal conductance found under field conditions in this experiment suggesting that the physiology of stomata are different between optimal greenhouse conditions and highly variable field conditions.

Based on Ohm's law, as it applies to water flow in plants, maximum stomatal conductance is limited by specific leaf area and whole plant hydraulic conductance (Fichot et al. 2010, Tyree & Ewers 1991; Meinzer 2002). As such, stomatal conductance is influenced by shoot and root conductance (discussed later) which may prove to have stronger influences on biomass production than instantaneous stomatal conductance.

Although it was not a very good indicator of above ground growth potential of hybrid poplar, stomatal conductance was the strongest indicator of productivity

of the measured leaf physiology traits (as compared to photosynthesis, water use efficiency, chlorophyll concentration and leaf predawn water potential) for the studied hybrid poplar clones.

4.2.3 *Water Use Efficiency*

Water use efficiency is an index that can be compared between trees where a given unit of productivity is obtained for a given unit of water used in the process. In this experiment, water use efficiency was taken as the ratio between photosynthesis rate and transpiration rates. During the first season, there were no significant differences between clones in water use efficiency, but there were significant differences in the second season. During the first season, water use efficiency and shoot mass of the balsam clone were positively correlated, and during the second season, water use efficiency of Walker was positively correlated to height and caliper. Other studies have found that water use efficiency does not correlate well with measurements of biomass production (Voltas et al. 2006, Bonhomme et al. 2008).

Water use efficiency is reported to be highly variable between poplars (Dickmann et al. 1992, Marron et al. 2005, Bonhomme et al. 2008). In a study of 31 *Populus deltoides* × *Populus nigra* clones, Marron et al. (2005) found that water use efficiency did not correlate with total biomass suggesting that productivity and water use efficiency are independent traits which lends to the possibility for selection of both traits, high water use efficiency and high productivity (Dillen et al. 2010). This could be useful in selecting clones which

will produce the greatest biomass under limited water conditions or drought. Similar findings were reported by Monclus et al. (2005). A study by Blake et al. (1983) found that of 17 poplar clones analyzed, water efficient clones shared one or more adaptation to reduce water loss: prodominent cutical ledges or hairs above stomata openings, earlier partial stomata opening as water use efficiency was correlated with both the degree and duration of stomatal opening, or smaller or less dense stomata on the adaxial surface of upper leaves. Although it is possible that the more water efficient clones shared these characteristics, stomatal size and leaf characteristics were not investigated. Monclus et al. (2005) found that stomatal density was negatively correlated with biomass and positively correlated with carbon isotope discrimination (which were not correlated with each other) in *P.* x*euramericana;* as such Monclus et al. (2005) suggest that carbon isotope discrimination can be used as an index for selection of high productivity and high water use efficiency. Carbon isotope discrimiation may be a more useful measurement in future experiments as it measures the integrated seasonal water use efficiency instead of an instantaneous measure as was measured in this experiment, and as described earlier may explain the lack of correlation between water use efficiency and biomass production.

4.2.4 *Chlorophyll*

Significant differences between clones in chlorophyll concentration were found for both field seasons. In both seasons, differences were found between the clones with high above ground growth (height, caliper and shoot biomass) and

the clones with low above ground growth suggesting that chlorophyll concentration is related to the level of above ground productivity of hybrid poplars; however, larger growing clones had lower chlorophyll concentration than the smaller clones. In the first season chlorophyll concentration was negatively correlated with height and caliper for Okanese and root mass of with the balsam clone. The negative trend between growth and chlorophyll is difficult to explain as chlorophyll is necessary for photosynthesis. It is possible that larger clones have more leaf area and require less chlorophyll per unit area to undergo the required amount of photosynthesis as the total chlorophyll in the canopy in larger clones may be higher than or equal to the chlorophyll in smaller clones. For example Marron et al. (2002) found that leaves of a *Populus* x*euramericana* clone Luisa Avanzo grew more rapidly than that of a similar clone Dorskamp (also a *P.* x*euramericana*), which exhibited a higher specific leaf area and had a lower chlorophyll concentration per unit area.

Reductions in leaf chlorophyll concentration in response to drought has been found to be related to an adaptation to reduce sunlight absorption resulting in lesser destruction of the photosystem by photooxidation (Neilsen et al. 2008, Guo et al. 2011, Du et al. 2012, Luquez et al. 2012). Reduced chlorophyll concentrations in flooded trees have also been associated with lower levels of photosynthesis (Du et al. 2012). Flooding has specifically been found to inhibits, chlorophyll concentration of *P. deltoides* (Caoa and Connerb 1999). In both field seasons, especially during the second season, the Okanese clone had low leaf

chlorophyll concentrations compared to the other clones. This may have been due to the flood susceptibility of the chlorophyll of its *P. deltoides* parent clone.

There was very few significant correlations between chlorophyll and growth of clones suggesting that chlorophyll concentration would not serve as a good indicator of productivity potential of hybrid clones.

4.2.5 *Leaf Water Potential*

During the night, photosynthesis does not occur and transpiration occurs at very minimal rates; therefore, during this time, the water potential gradient across a plant equilibrates with soil water potential. Under good water conditions, leaf water potential equilibrates to zero, yet will be negative during water deficit stress when leaf water potential is limited by soil moisture availability (Améglio et al. 1999, Intrigliolo and Castel 2006). However, predawn disequilibrium was found in seven of 15 investigated species (not including two of the investigated temperate forest species *Pinus palustris and Quercus marilandica*) even under well watered conditions which was partially attributed to high concentrations of leaf apoplastic solutes, yet a full explanation is unknown (Donovan et al. 2001). The disequilibrium found by Donovan et al. (2001) resulting from high apoplastic solute concentrations may help to explain why the predawn water potential measurements taken in this experiment did not equal zero under well watered conditions.

Significant differences between clones in leaf predawn water potential were found for both field seasons. During the second field season, the clones with high

above ground biomass production (Okanese and Berlin) had significantly different predawn water potentials compared with the other clones. Predawn water potential was positivity correlated with root to shoot ratio of Okanese during the first field season and negatively correlated with shoot and root mass of the hybrid aspen clone.

Flooding has been found to reduce leaf water potential over time with faster decreases in leaf water potential occurring in less tolerant plants (Du et al. 2012). The previously referenced drought study by Arango-Velez et al. (2011) which, in part, investigated the leaf water potential of most of the same clones investigated in this study revealed leaf water potentials of stressed trees that were similar to the values obtained in my study. It is possible that given the effects that flooding can have on the water status of a tree by inhibiting the water uptake function of roots, the effects of flooding on the trees in my study were similar to the drought effects seen in the study by Arango-Velez et al. (2011) thus causing predawn leaf water potential values as low as the ones in my study.

The positive correlations between root to shoot ratio and predawn water potential for Okanese clones is likely due to the lack of the trees' ability to take up enough water during the night to equilibrate the water potential of the leaves with the soil. By increasing the root to shoot ratio, suggested by the positive correlation between root to shoot ratio and predawn water potential, the trees would be able to take up more water resulting in a predawn water potential of zero and a complete equilibration with soil water potential. The lower leaf water

potential would limit the duration that the plant could maintain open stomata for photosynthesis.

Based on the lack of a significant correlation between any of the other clones and biomass parameters with leaf pre-dawn water potential, it can be concluded that overall the level of water availability and the ability of the clones to recover the water lost during the day was not limiting their growth.

4.3 Stem Hydraulic Conductance

Although stem hydraulic conductance measurements were not investigated for the first field season, the second field season showed an interesting trend in percent loss of branch conductance due to air blockage. First, there were significant differences between the clones in percent loss of branch conductance. Second, the two clones with the highest mean height and shoot biomass (Okanese and Berlin) had the highest percent loss of shoot conductance, which was significantly higher than the rest of the clones. Third, shoot mass, root mass and caliper of Berlin (with the highest mean percent loss in branch conductance) were all positively correlated with percent loss in branch conductivity.

This result is in agreement with the study by Arango-Velez et al. (2011) who found that balsam poplar had a low sensitivity to xylem cavitation, Walker was moderately resistant, and both Okanese and Berlin were highly sensitive to xylem cavitation. Hybrid aspen was not studied by Arango-Velez et al. (2011). Notably, the sensitivity of these clones to xylem cavitation directly corresponded to their drought resistance. The balsam poplar clone had the highest drought

resistance, while the Walker clone was moderately drought resistant and Okanese and Berlin clones had low drought resistance (Arango-Velez et al. 2011). My results are in accordance with the greenhouse study by Arango-Velez et al. (2011) and suggest that a trade-off exists between high above ground productivity and xylem cavitation resistance. The similarity of results between my study and that of Arango-Velez et al. (2011) suggests that the field conditions of my study induced stress similar to those in the study by Arango-Velez et al. (2011). The water stress applied to the trees in the study by Arango-Velez et al. (2011) under drought conditions was similar to the water stress conditions of the trees in my study because of flooding conditions. Also, a study by Schreiber et al. (2011) conducted on trees grown in the same area as the current trial, found that hybrid poplars (riparian species) were more vulnerable to cavitation than trembling aspen (non-riparian species), yet in my study, two of the clones (balsam and Walker) were less sensitive to cavitation than the hybrid aspen clone. Other studies have found clonal differences in xylem resistance to cavitation, associated with thicker double walls, but the resistance to cavitation is not always the same as the trend in my experiment; Fichot et al. (2010) found that more cavitation-resistant *P. deltoides* x *P. nigra* hybrid genotypes grew faster than less resistant genotypes of *P. deltoides* x *P. nigra* under field conditions. Five of eight clones in my experiment shared at least one parent species with the hybrids in the study by Fichot et al. (2010).

The loss of hydraulic conductance in branches is due to embolisms (air pockets) or complete cavitation of xylem vessels (Tyree and Ewers, 1991).

Embolism occurs when there is a breakage in the water column; as water evaporates from the surface of a leaf during transpiration and more water molecules are pulled up the xylem if the water uptake of the tree is not high enough to replace the water lost during transpiration a breakage will occur (Sperry et al. 1988; Tyree and Sperry, 1989; Tyree and Ewers, 1991; Windt et al. 2006). Embolism and cavitation can lead to a lack of water supply to the canopy of the tree, which will eventually kill the whole tree (Sperry et al. 1988).

Based on the studies to date, it is reasonable to hypothesize that percent loss in branch conductance would be associated with a decrease in plant biomass. This, however, was not the trend seen in my experiment. My results suggest that the larger an individual tree is, the more likely it is to develop embolisms and have a loss of conductance, a trade-off exists between xylem resistance to cavitation and growth potential, and that the degree of embolism seen in this experiment did not appear to be limiting the growth of trees. It is also important to consider the age of the trees used in this experiment (four year old trees by the second field season) because as the clones age and grow, the relationship between cavitation resistance and biomass production may change. Stomatal closure, one way in which plants can regulate the risk of cavitation (creating a safety margin between the water potential which causing stomatal closure and that which causes cavitation) is an important factor influencing percent loss in conductance of branches, but was not investigated in this experiment (Huckin et al. 2005).

4.4 Root Hydraulic Conductance and Conductivity

Water uptake by trees is a passive process where transpiration by the canopy essentially pulls water up through a tree from the roots and soil (Steudle and Peterson 1998, Marjanović et al. 2005). A root system with high conductance is therefore required to support a large canopy. Variation in the physiology of roots across or within tree species is common and widely accepted (Friend et al. 1991, Brunner and Godbold 2007). In both seasons, the three clones which had the greatest shoot biomass also had the highest mean root conductance and the clone with the lowest shoot biomass had the lowest mean root conductance. Overall, my results showed a trend of increasing above ground biomass production with an increase in root conductance. The relatively high conductance of roots of the high producing clones was necessary for the clones to reach a relatively high level of above ground biomass production. Root hydraulic conductance was positively correlated with shoot mass for four of the five clones analyzed: balsam, Walker, Berlin, and hybrid aspen during the first field season and three of the clones for the second field season: Walker, Berlin, and hybrid aspen further supporting the conclusion that increased root conductance is required to support greater shoot biomass. Root hydraulic conductance was positively correlated with root mass for two clones in the second season: Okanese and Berlin, the two clones with the highest root hydraulic conductance, and four of the five clones in the first season: balsam, Berlin, hybrid aspen, and Walker. This suggests that the high root hydraulic conductance and differences in growth that therefore resulted from the higher conductance may have been due to the

sheer size of the root systems themselves. This is also suggested by the lack of significant differences in root conductivity between the clones in the second field season. As there were significant differences in the size of the root systems in the second season, but not in the conductivity of the root systems, the differences in root hydraulic conductance were likely due to the differences in root size.

Flooding affects roots by inhibiting root formation, branching, growth of existing roots, and causes the root system to decay (Kozlowski 1997, Gong et al. 2007). The depletion of soil oxygen levels caused by flooding interferes with proper root metabolism and plant physiological processes (Kozlowski 1997, Guo et al 2011). Kozlowski (1997) summarized that several studies have shown that stomatal closure by flooded plants is associated with a decrease in root hydraulic conductivity. As previously stated, flooding causes a decrease in soil oxygen and an increase in soil carbon dioxide levels, this has been found to increase resistance to water flow through roots, which in turn would likely reduce the hydraulic conductance of the roots (Luquez et al. 2012, Smit and Stachowiak 1988). Root tolerance to flooding is a large factor in determining a plants overall tolerance to flooding and survival under flood conditions (Gong et al. 2007).

P. deltoides was mentioned previously to likely have a negative influence as a parent contributing genes to the clones in this study (Walker, Okanese, Assiniboine and Northwest) (Talbot et al. 2011). Another study found that flooding inhibits root growth of *P. deltoides* (Caoa and Connerb 1999). This may explain the low root mass and root hydraulic conductance of the Walker clone. Also, as hybrid aspen is a poplar clone adapted to an upland habitat, the level of

flooding in this experiment may explain the low root weight and hydraulic conductance of the hybrid aspen clone as well.

My results indicate that although roots can be considered the most understudied part of a tree, they might be the most important factor in determining plant biomass production in hybrid poplar (Brunner and Godbold 2007, Gou et al. 2010). As root systems have typically been understudied in the past, the lack of knowledge about the different root systems of each clone may be a considerable factor in my inability to draw conclusions about performance. Although it was not a perfect indicator that correlated strongly with all clones, root conductance was the strongest physiological indicator of biomass production for the hybrid poplar clones investigated in this study.

5. Conclusions

Based on the shoot mass, caliper, and height of the clones over the two growth seasons, clones showed general trends in above ground productivity. Clones 24, 25, and 27 had low above ground biomass productivity, clones 33, 2782, and 1004 had average above ground biomass productivity and clones 2403 and 42 had the highest above ground biomass productivity. Although no single physiological parameter could explain the differences in growth of the clones, many trends were revealed that suggest the physiology associated with high, average, and low above ground productivity is different and the different growth strategies employed may be used as selection parameters for future clone breeding and selection (Table 5.1).

Table 5.1: Morphological and physiological traits that contributed to the growth differences between high (Berlin and Okanese), average (hybrid aspen, balsam, and P38P38), and low (Walker, Assiniboine, Northwest) above ground biomass producing clones.

Above Ground Biomass Production	Stomatal Conductance	Root Conductance	Loss in Branch Conductance
High	Low	High	High
Medium	Medium	Medium	Medium
Low	High	Low	Medium

In this investigation, it is likely that the general lack of a significant relationship between the gas exchange measurements: net photosynthesis, stomatal conductance, and water use efficiency with biomass is due to the instantaneous nature of the measurements which tend to not account for the full capacity of the canopy of the trees, the duration of the day in which gas exchange is taking place, and the fluctuations in gas exchange across a growing season. Instantaneous gas exchange rates are therefore not a good indicator of biomass production in the studied hybrid poplars. However, out of all the gas exchange measurements, one trend is worth noting: stomatal conductance tends to have a negative effect on biomass production, although it is not always a significant relationship, and some clones have a closer relationship between stomatal conductance and biomass production than others. Although it was not a very good indicator of above ground growth potential of hybrid poplar, stomatal conductance was the strongest indicator of productivity of the measured leaf

physiology traits for the studied hybrid poplar clones. Water use efficiency was not a good predictor of biomass production; however, studies have shown that carbon isotope discrimination can be used as an index for water use efficiency (Monclus et al. 2005) and may be a more useful measurement in future experiments as it measures the seasonal water use efficiency instead of an instantaneous measure as used in this investigation. Chlorophyll concentration of leaves proved to also be a poor indicator of productivity as there was very little significant correlation between chlorophyll and growth of clones. Pre-dawn water potential of leaves showed that in general, the level of water availability and the ability of the clones to recover the water lost during the day was not limiting their growth.

Clones which have been shown through past studies to have low xylem safety to cavitation had a positive trend of increasing cavitation with increased above ground biomass. My results suggested there is a trade-off between xylem safety and growth potential in the investigated hybrid poplars. Although the percent of xylem conductance in branches lost to cavitation may not be a good indicator of productivity, under optimum growing conditions (such as those during this field experiment) clones with low xylem safety may have higher growth then those with higher xylem safety.

Overall, the most consistent physiological indicator of biomass production for the poplar clones investigated in this study was root hydraulic conductance. I conclude that the strongest physiological factor influencing the growth of the hybrid poplar clones was root hydraulic conductance. As such, root hydraulic

conductance may be a physiological feature that could be used as a screening tool for breeding programs targeting high above ground productivity such as height and shoot biomass.

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Appendix 1

Table 3.1.5: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), root conductance (transformed data, K_r), and root conductivity (transformed data, L_r), for clone 24.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	0.120	0.450	0.495	0.321	0.522
	0.710	0.142	0.102	0.336	0.100
g_s	-0.051	-0.120	0.090	0.086	0.252
	0.875	0.711	0.781	0.803	0.454
E	-0.253	0.059	0.126	0.122	0.064
	0.428	0.856	0.696	0.721	0.852
WUE	0.194	0.438	0.499	0.322	0.529
	0.547	0.154	0.099	0.334	0.094
Chl	-0.410	-0.368	-0.404	-0.491	0.373
	0.165	0.217	0.171	0.105	0.232
Ψ_p	0.155	0.047	0.391	-0.218	0.582
	0.669	0.897	0.264	0.545	0.078
K_r	-0.210	-0.382	-0.611	-0.699	0.479
	0.490	0.198	0.027	0.012	0.115
L_r	-0.148	-0.244	-0.538	-0.492	0.183
	0.647	0.444	0.071	0.104	0.568

* Significant correlations are highlighted.

Table 3.1.6: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), root conductance (transformed data, K_r), and root conductivity (transformed data, L_r), for clone 2403.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	0.497	0.509	0.451	0.435	0.359
	0.120	0.110	0.164	0.181	0.279
g_s	0.035	-0.076	0.204	0.124	0.481
	0.918	0.823	0.547	0.717	0.135

E	-0.143	-0.118	0.394	0.479	-0.286
	0.675	0.730	0.230	0.137	0.394
WUE	0.429	0.408	0.089	0.025	0.435
	0.188	0.213	0.796	0.942	0.181
Chl	-0.730	-0.609	-0.348	-0.315	-0.513
	0.007	0.035	0.267	0.319	0.088
Ψ_p	0.504	0.566	0.069	-0.401	0.996
	0.137	0.088	0.849	0.251	<.0001
K_r	-0.575	-0.590	-0.542	-0.548	-0.652
	0.051	0.043	0.069	0.065	0.022
L_r	-0.376	-0.370	-0.399	-0.221	-0.594
	0.229	0.236	0.199	0.491	0.042

* Significant correlations are highlighted.

Table 3.1.7: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), root conductance (transformed data, K_r), and root conductivity (transformed data, L_r), for clone 42.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	S_m	R_m	R_m:S_m
A	0.400	0.392	0.231	0.260	0.027
	0.197	0.208	0.470	0.414	0.934
g_s	0.351	0.256	-0.245	-0.307	0.075
	0.264	0.423	0.443	0.331	0.817
E	0.555	0.409	0.157	0.156	0.090
	0.061	0.187	0.627	0.629	0.781
WUE	0.116	0.254	0.285	0.337	-0.011
	0.720	0.425	0.369	0.284	0.972
Chl	0.441	0.380	0.462	0.531	0.140
	0.151	0.224	0.131	0.076	0.665
Ψ_p	0.206	0.151	0.305	0.160	0.531
	0.569	0.677	0.391	0.659	0.115
K_r	-0.281	-0.407	-0.589	-0.670	-0.053
	0.376	0.189	0.044	0.017	0.870
L_r	0.079	-0.027	-0.215	-0.338	0.232
	0.807	0.934	0.502	0.283	0.469

* Significant correlations are highlighted.

Table 3.1.8: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*),

caliper (C), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (A), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE), predawn water potential (transformed data, Ψ_p), chlorophyll content (Chl), root conductance (K_r), and root conductivity (L_r), for clone 1004.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	0.561	0.406	0.510	0.638	-0.253
	0.058	0.190	0.091	0.035	0.452
g_s	0.165	0.089	0.160	0.124	-0.230
	0.608	0.782	0.620	0.717	0.497
E	0.197	0.101	-0.038	0.224	-0.480
	0.539	0.756	0.907	0.508	0.135
WUE	0.451	0.323	0.591	0.565	0.074
	0.142	0.306	0.043	0.070	0.830
Chl	-0.268	-0.285	-0.405	-0.626	0.565
	0.400	0.370	0.192	0.039	0.070
Ψ_p	-0.143	-0.166	-0.221	-0.417	0.055
	0.713	0.670	0.568	0.264	0.889
K_r	0.524	0.454	0.688	0.813	-0.351
	0.081	0.139	0.013	0.002	0.289
L_r	-0.170	-0.143	0.077	-0.218	0.444
	0.617	0.676	0.823	0.520	0.171

* Significant correlations are highlighted.

Table 3.1.9: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (H), caliper (C), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (A), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE), predawn water potential (transformed data, Ψ_p), chlorophyll content (Chl), root conductance (transformed data, K_r), and root conductivity (transformed data, L_r), for clone 2782.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	0.021	0.006	0.322	0.186	0.169
	0.947	0.986	0.307	0.562	0.600
g_s	0.285	0.112	-0.002	0.142	-0.083
	0.370	0.729	0.994	0.659	0.799
E	-0.321	-0.359	0.027	-0.249	0.366
	0.309	0.253	0.935	0.435	0.242
WUE	0.035	0.021	0.269	0.340	-0.118
	0.915	0.949	0.397	0.279	0.714

Chl	-0.506	-0.405	-0.413	-0.513	-0.140
	0.093	0.191	0.182	0.088	0.664
Ψ_p	0.316	0.338	0.670	0.801	-0.012
	0.374	0.339	0.034	0.005	0.975
K_r	-0.455	-0.524	-0.781	-0.915	-0.242
	0.137	0.080	0.003	<.001	0.449
L_r	-0.088	-0.215	-0.406	-0.553	0.031
	0.787	0.502	0.190	0.062	0.924

* Significant correlations are highlighted.

Table 3.2.6: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), root conductance (transformed data, K_r), and root conductivity (L_r), for clone 24.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	S_m	R_m	R_m:S_m
A	0.420	0.404	0.294	0.294	-0.498
	0.154	0.171	0.330	0.330	0.083
g_s	-0.141	-0.320	-0.711	-0.711	0.697
	0.663	0.311	0.010	0.010	0.012
E	-0.366	-0.462	-0.257	-0.257	0.273
	0.218	0.112	0.397	0.397	0.367
WUE	0.561	0.679	0.456	0.456	-0.474
	0.046	0.011	0.117	0.117	0.102
Chl	-0.307	-0.228	-0.257	-0.257	0.487
	0.307	0.454	0.396	0.396	0.091
Ψ_p	0.376	0.139	0.172	0.172	-0.354
	0.206	0.652	0.574	0.574	0.235
L_b	0.074	0.125	0.035	0.035	0.238
	0.820	0.700	0.914	0.914	0.456
K_r	0.064	-0.098	-0.213	-0.213	0.168
	0.843	0.763	0.506	0.506	0.602
L_r	-0.353	-0.193	-0.193	-0.193	0.278
	0.261	0.548	0.549	0.549	0.383

* Significant correlations are highlighted.

Table 3.2.7: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s),

transpiration (E), water use efficiency (WUE), predawn water potential (transformed data, Ψ_p), chlorophyll content (Chl), percent loss in branch conductance (L_b), root conductance (transformed data, K_r), and root conductivity (L_r), for clone 2403.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	C	S_m	R_m	R_m:S_m
A	0.040	0.135	-0.189	-0.189	-0.167
	0.901	0.676	0.557	0.557	0.604
g_s	-0.631	-0.538	-0.463	-0.463	0.399
	0.028	0.071	0.129	0.129	0.199
E	-0.378	-0.372	-0.269	-0.269	0.559
	0.225	0.233	0.398	0.398	0.059
WUE	0.033	0.101	-0.071	-0.071	-0.215
	0.920	0.755	0.827	0.827	0.502
Chl	-0.180	-0.084	-0.242	-0.242	0.097
	0.575	0.795	0.449	0.449	0.763
Ψ_p	-0.124	-0.181	-0.151	-0.151	0.035
	0.701	0.573	0.639	0.639	0.915
L_b	0.393	0.357	0.118	0.118	0.200
	0.207	0.254	0.716	0.716	0.533
K_r	-0.497	-0.586	-0.669	-0.669	0.833
	0.100	0.045	0.017	0.017	0.001
L_r	-0.181	-0.074	0.126	0.126	-0.360
	0.575	0.818	0.697	0.697	0.250

* Significant correlations are highlighted.

Table 3.2.8: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (H), caliper (C), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (A), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE), predawn water potential (transformed data, Ψ_p), chlorophyll content (Chl), percent loss in branch conductance (L_b),root conductance (transformed data, K_r), and root conductivity (L_r), for clone 42.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	C	S_m	R_m	R_m:S_m
A	0.046	0.304	-0.211	-0.195	0.186
	0.887	0.337	0.511	0.543	0.563
g_s	-0.176	-0.142	-0.316	-0.311	-0.151
	0.584	0.661	0.316	0.326	0.639
E	-0.332	-0.064	0.065	0.026	0.003
	0.292	0.845	0.840	0.936	0.992

WUE	0.211	0.174	-0.222	-0.197	0.110
	0.511	0.589	0.488	0.540	0.733
Chl	-0.136	-0.548	-0.038	-0.054	-0.043
	0.674	0.065	0.906	0.868	0.894
Ψ_p	-0.330	0.283	-0.240	-0.240	0.429
	0.295	0.373	0.452	0.452	0.164
L_b	0.372	0.612	0.708	0.701	-0.435
	0.260	0.046	0.015	0.016	0.182
K_r	-0.597	0.076	-0.719	-0.759	0.579
	0.052	0.825	0.013	0.007	0.062
L_r	0.070	-0.400	0.109	0.136	0.047
	0.837	0.223	0.750	0.689	0.891

* Significant correlations are highlighted.

Table 3.2.9: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), percent loss in branch conductance (L_b), root conductance (transformed data, K_r), and root conductivity (L_r), for clone 1004.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	0.164	0.089	0.054	0.054	0.272
	0.612	0.784	0.869	0.869	0.393
g_s	0.123	0.002	-0.053	-0.053	0.006
	0.704	0.995	0.869	0.869	0.985
E	-0.117	0.056	0.503	0.503	0.361
	0.718	0.862	0.096	0.096	0.249
WUE	0.225	0.065	-0.392	-0.392	-0.249
	0.482	0.840	0.207	0.207	0.435
Chl	-0.112	-0.334	-0.323	-0.323	-0.102
	0.728	0.289	0.306	0.306	0.752
Ψ_p	-0.007	-0.140	-0.303	-0.303	-0.051
	0.984	0.664	0.339	0.339	0.875
L_b	0.192	0.350	0.503	0.503	0.457
	0.551	0.265	0.095	0.095	0.135
K_r	-0.471	-0.660	-0.541	-0.541	-0.507
	0.143	0.027	0.086	0.086	0.111
L_r	0.095	0.197	-0.056	-0.056	0.007
	0.782	0.562	0.871	0.871	0.985

* Significant correlations are highlighted.

Table 3.2.10: Pearson correlation coefficients and their associated significant values for correlations between the measured biomass variables: height (*H*), caliper (*C*), shoot mass (transformed data, S_m), root mass (R_m), and root to shoot ratio (R_m:S_m) with physiological measurements: photosynthesis (*A*), stomatal conductance (g_s), transpiration (*E*), water use efficiency (*WUE*), predawn water potential (transformed data, Ψ_p), chlorophyll content (*Chl*), percent loss in branch conductance (L_b), root conductance (transformed data, K_r), and root conductivity (L_r), for clone 2782.

Pearson Correlation Coefficients
Prob > |r| under HO: Rho=0

	H	***C***	***S_m***	***R_m***	***R_m:S_m***
A	-0.075	0.257	-0.109	-0.206	-0.016
	0.828	0.446	0.736	0.521	0.960
g_s	-0.500	-0.448	-0.445	-0.325	-0.567
	0.118	0.167	0.147	0.303	0.054
E	-0.213	-0.526	-0.095	0.029	-0.224
	0.529	0.097	0.768	0.930	0.484
WUE	0.017	0.457	-0.057	-0.144	0.027
	0.959	0.158	0.860	0.655	0.934
Chl	0.171	-0.109	0.507	0.007	0.301
	0.616	0.750	0.093	0.985	0.343
L_b	0.149	0.140	0.375	0.440	0.324
	0.662	0.682	0.230	0.152	0.304
K_r	-0.458	-0.300	-0.605	-0.514	-0.608
	0.157	0.371	0.037	0.087	0.036
L_r	-0.562	-0.188	-0.597	-0.598	-0.555
	0.072	0.580	0.041	0.040	0.061

* Significant correlations are highlighted.

Appendix 2

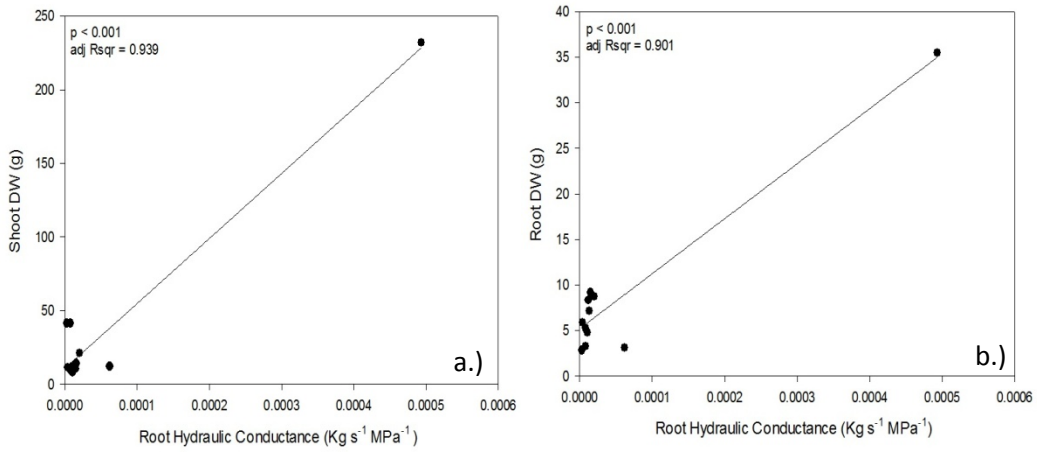

Figure 3.1.4: The positive linear relationships between root hydraulic conductance and shoot dry weight (a) and root dry weight (b) of clone Walker (24) during the third growth season.

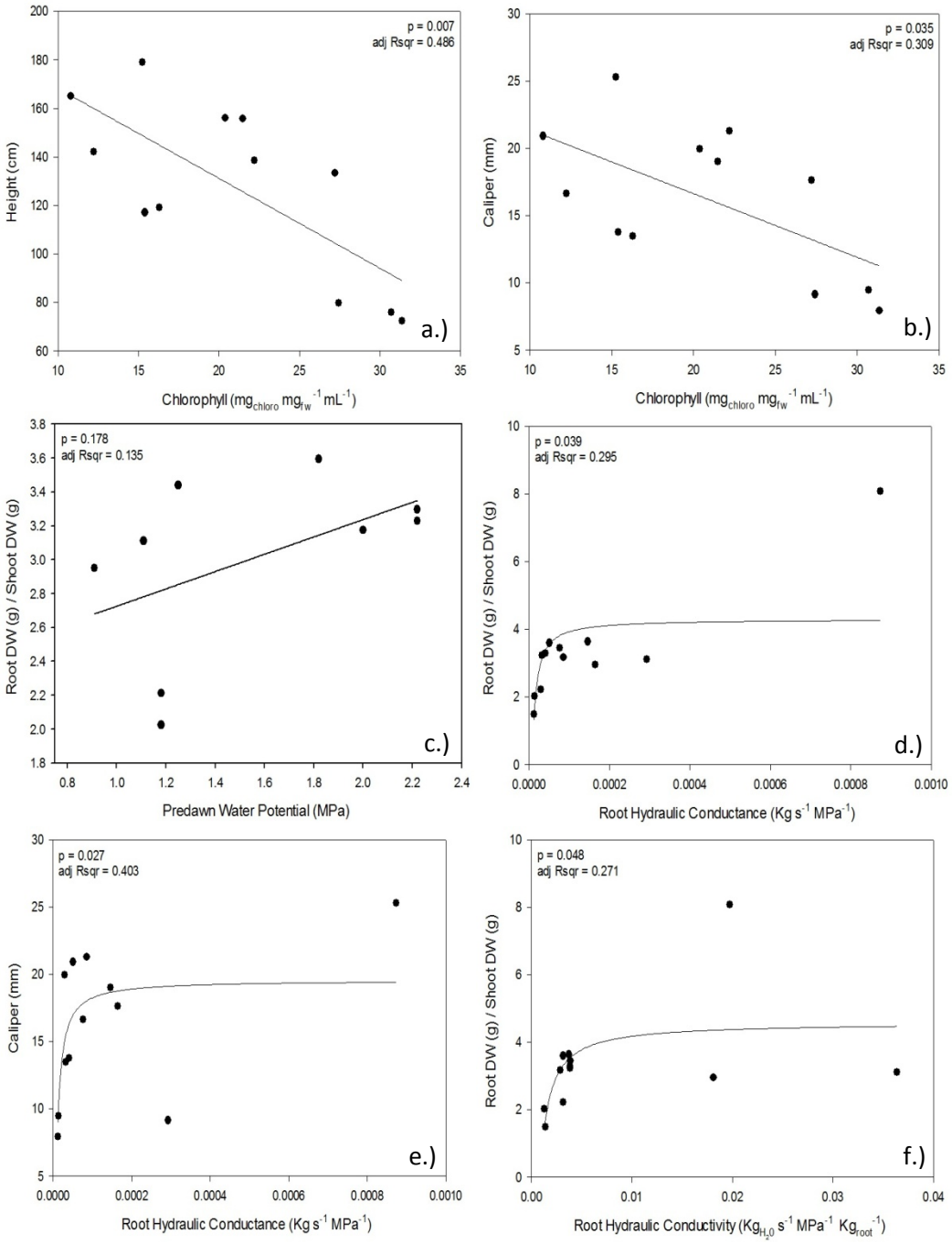

Figure 3.1.5: The negative linear relationships between chlorophyll concentration and height (a) and caliper (b), positive linear relationship between predawn water potential and root dry weight (c), positive non-linear relation between root hydraulic conductance and root dry weight (d) root hydraulic conductance and caliper (e) and root hydraulic conductivity and root dry weight (f) of clone Okanese (2403) during the third growth season.

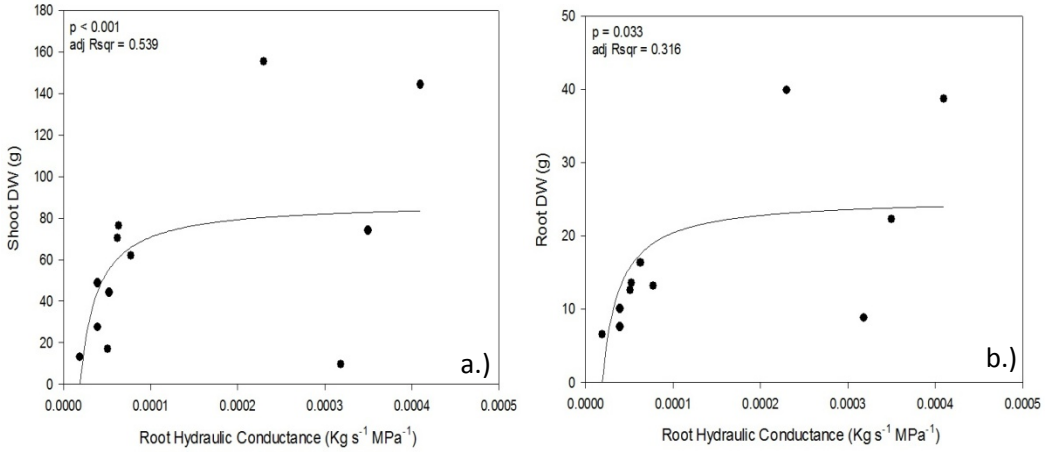

Figure 3.1.6: The positive non-linear relationships between root hydraulic conductance and shoot dry weight (a) and root dry weight (b) of clone Berlin (42) during the third growth season.

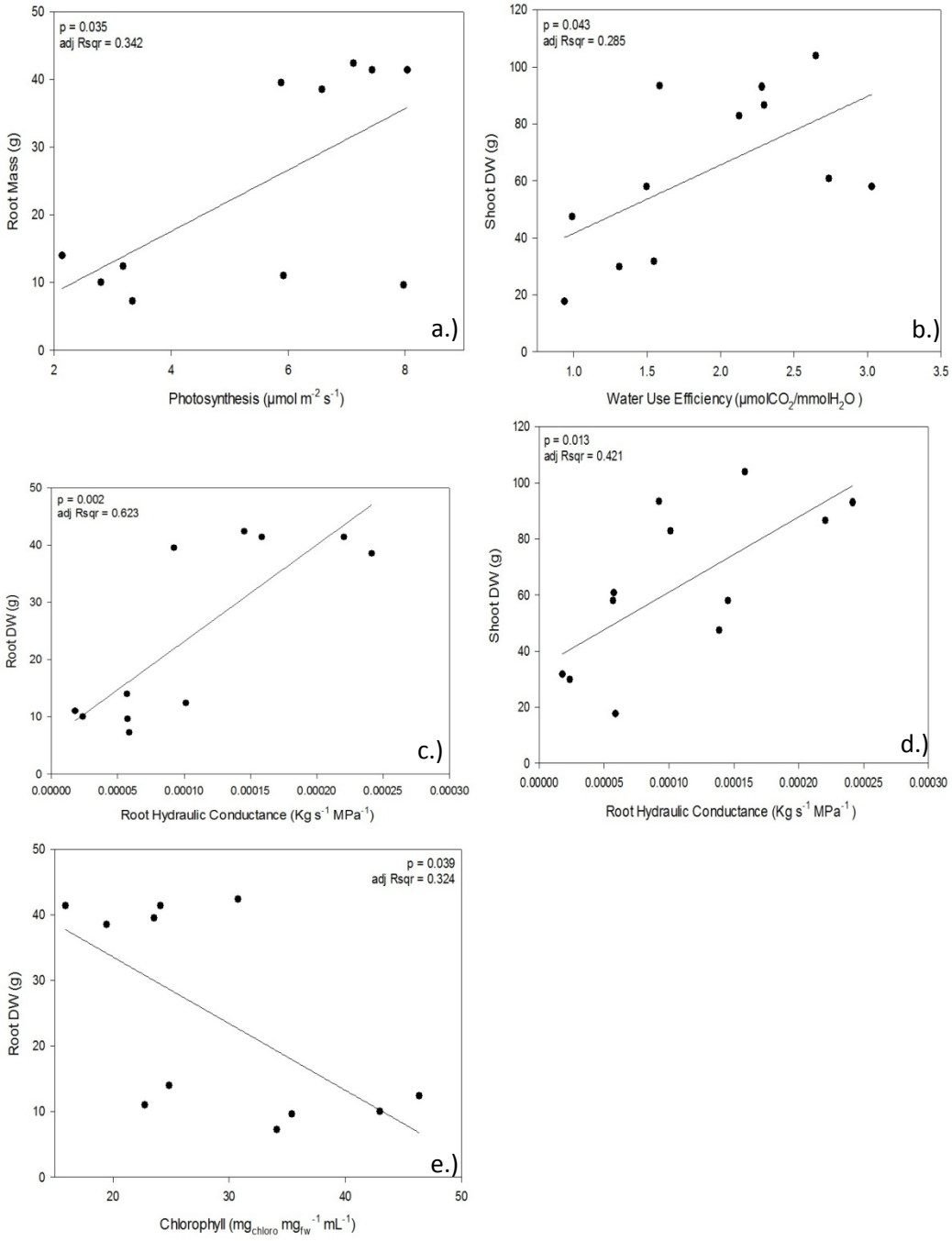

Figure 3.1.7: The positive linear relationships between photosynthesis and root dry weight (a), water use efficiency and shoot dry weight (b), root hydraulic conductance and root dry weight (c), root hydraulic conductance and shoot dry weight (d), and negative linear relationship between chlorophyll concentration and root dry weight of balsam clone (1004) during the third growth season.

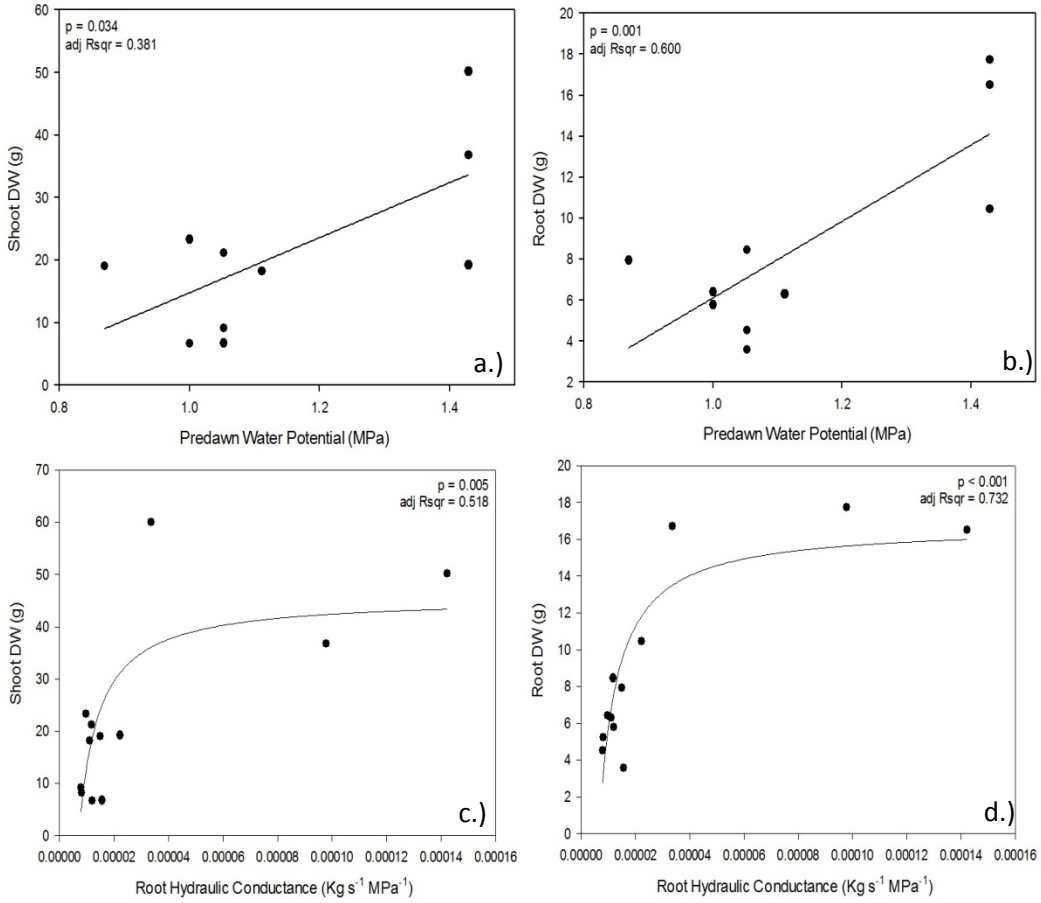

Figure 3.1.8: The positive linear relationships between predawn water potential and shoot dry weight (a) and root dry weight (b), positive non-linear relationship between root hydraulic conductance and shoot dry weight (c), and root dry weight (d) of the hybrid aspen clone during the third growth season.

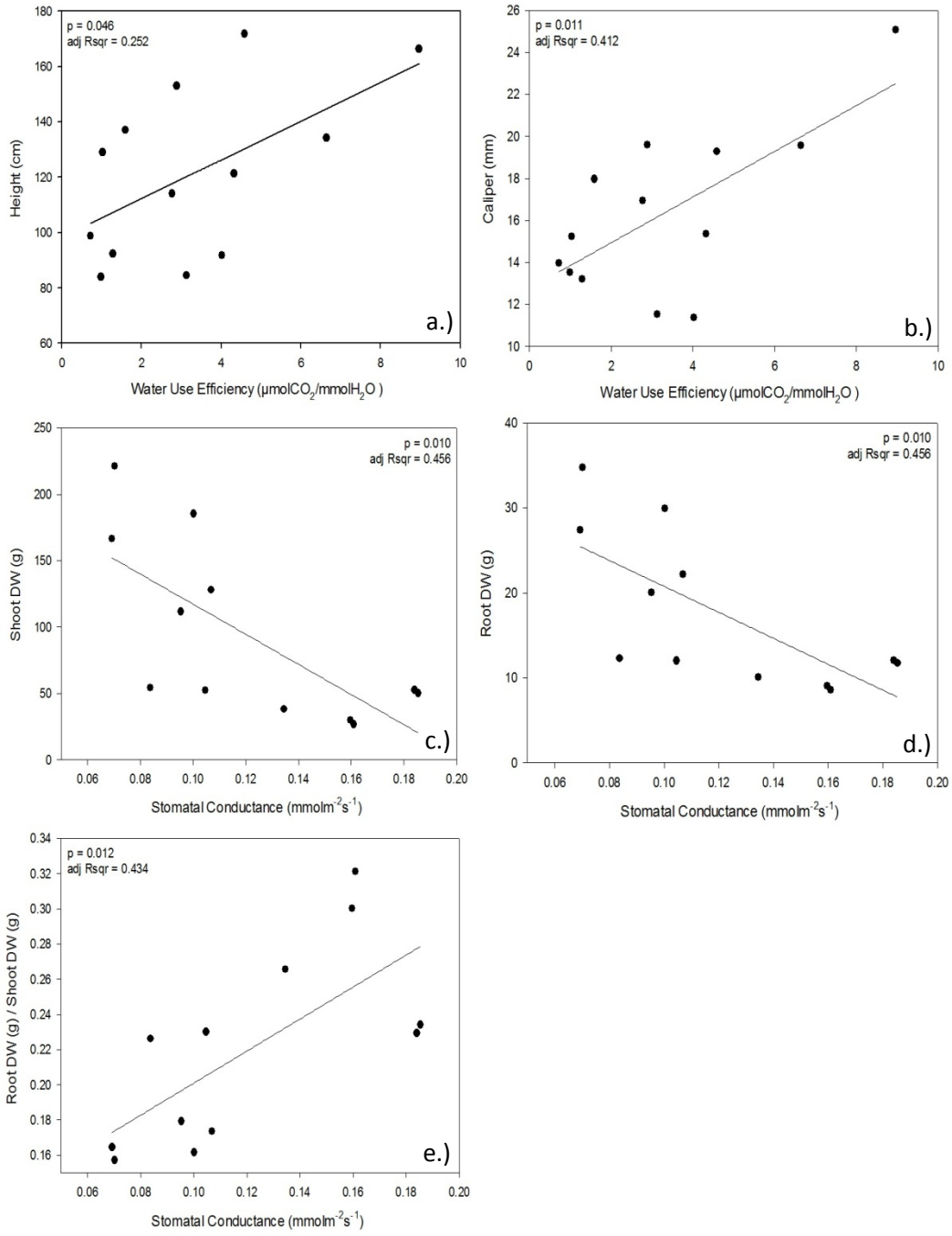

Figure 3.2.5: The positive linear relationships between water use efficiency and height (a) and caliper (b), negative linear relationship between stomatal conductance and shoot dry weight (c) and root dry weight (d), and positive linear relationship between stomatal conductance and root to shoot ratio of clone Walker (24) during the fourth growth season.

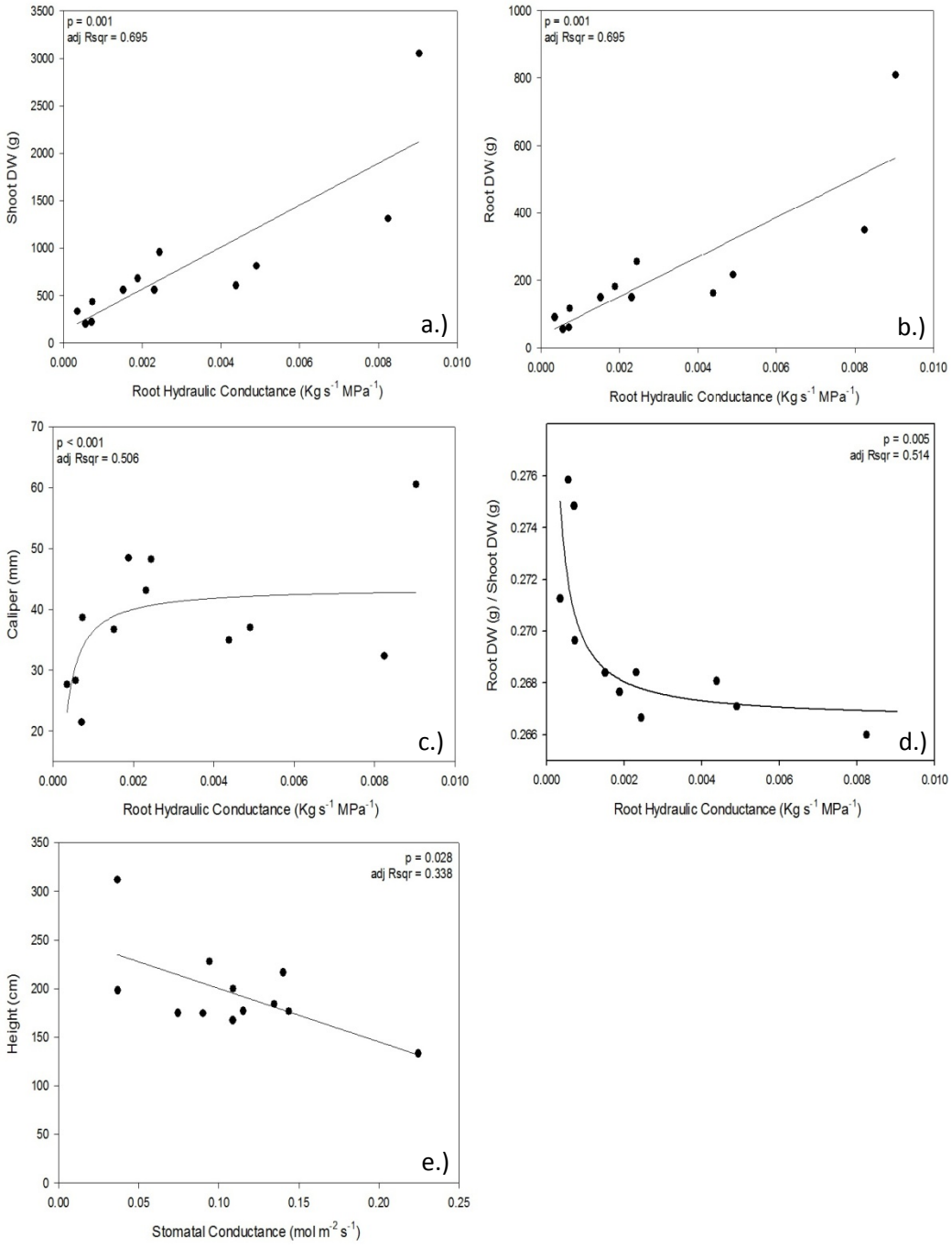

Figure 3.1.6: The positive non-linear relationships between root hydraulic conductance and shoot dry weight (a) and root dry weight (b), positive non-linear relationship between root hydraulic conductance and caliper (c), negative non-linear relationship between root hydraulic conductance and root to shoot ratio (d), and stomatal conductance and height (e) of clone Okanese (2403) during the fourth growth season.

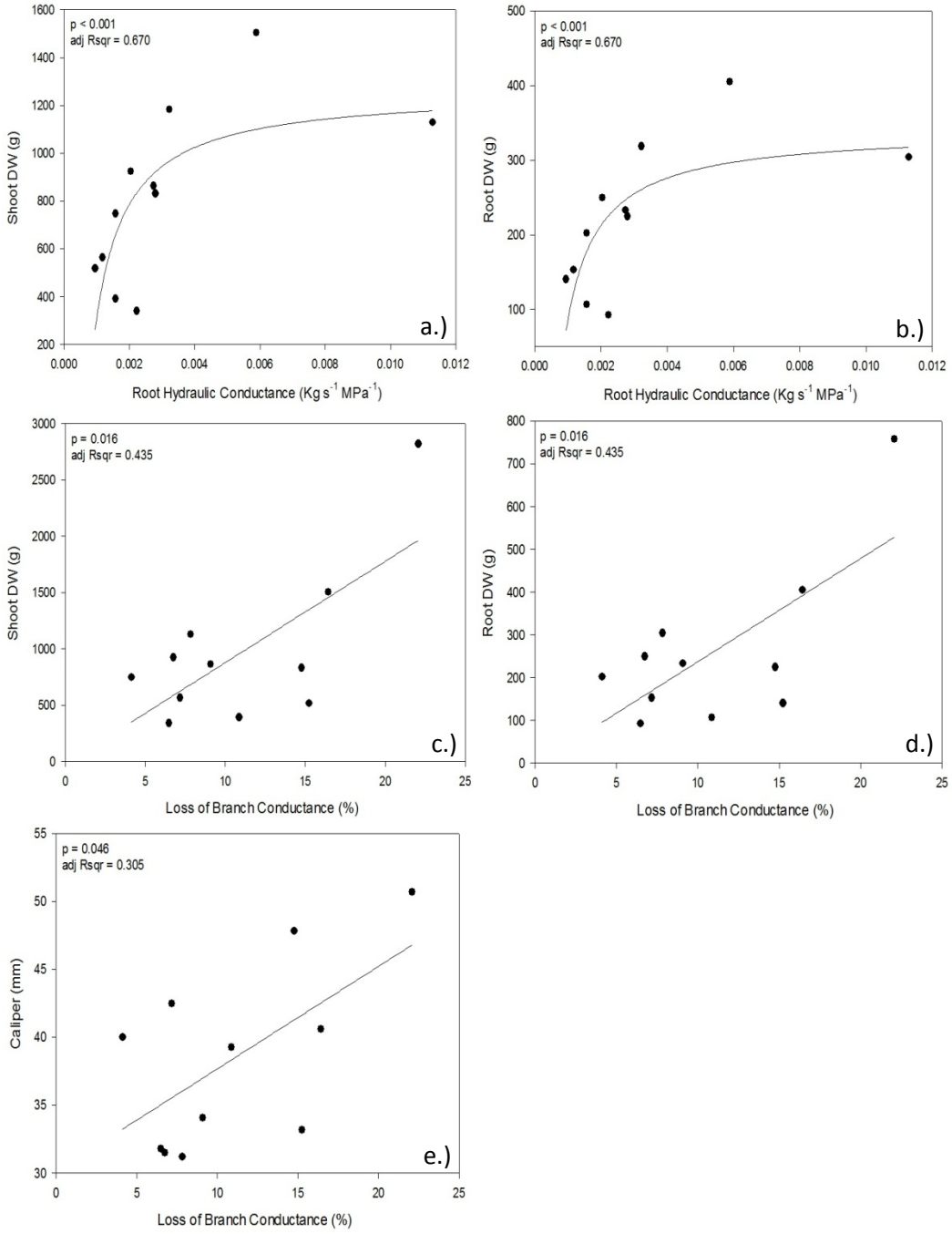

Figure 3.1.7: The positive non-linear relationships between root hydraulic conductance and shoot dry weight (a) and root dry weight (b), positive linear relationship between loss in branch hydraulic conductance and shoot dry weight (c), root dry weight (d), and caliper of clone Berlin (42) during the fourth growth season.

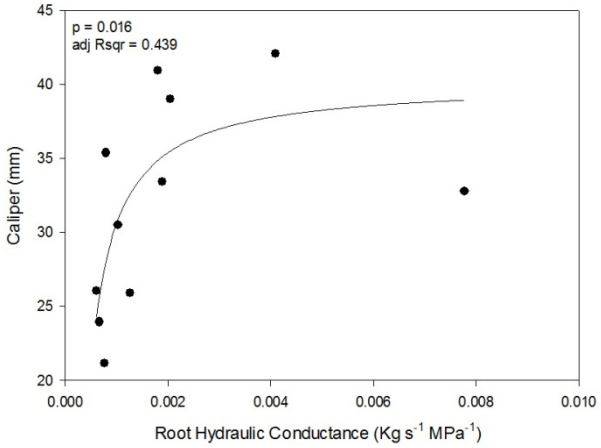

Figure 3.1.8: The positive non-linear relationship between root hydraulic conductance and caliper of balsam clone (1004) during the fourth growth season.

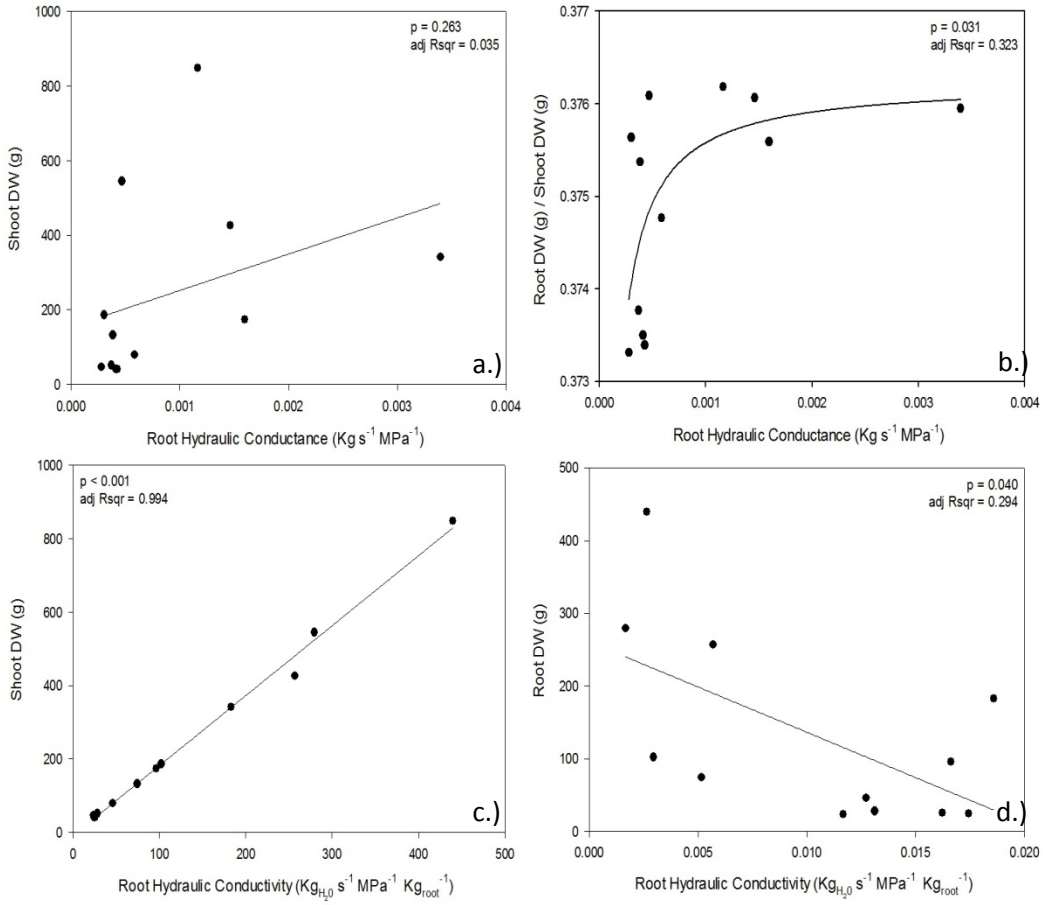

Figure 3.1.9: The positive linear relationships between root hydraulic conductance and shoot dry weight (a), non-linear relationship between root hydraulic conductance and root to shoot ratio (b), positive linear relationship between root hydraulic conductivity and shoot dry weight (c), negative linear relationship between root hydraulic conductivity and root dry weight (d), of hybrid aspen clone (2782) during the fourth growth season.

Appendix 3

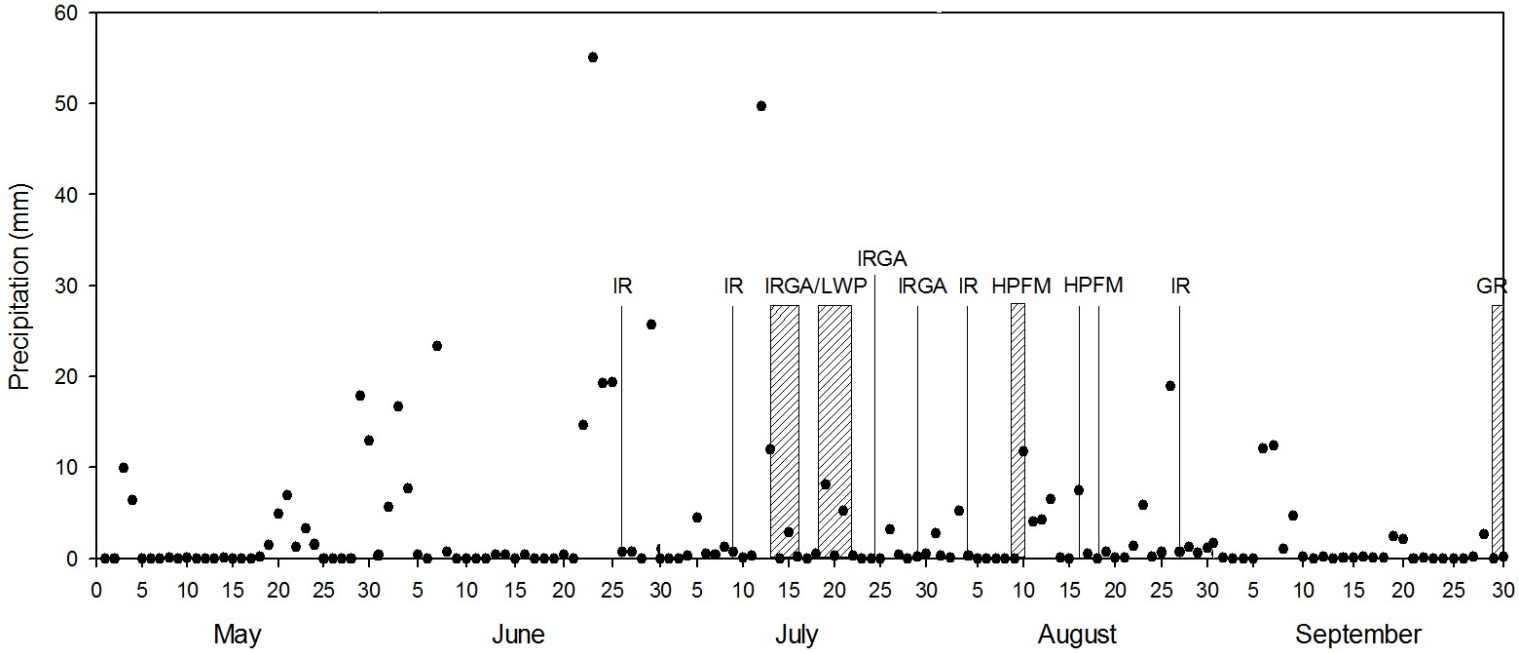

Figure 2.5.1: Daily precipitation during the first field season (third growing season, 2010), and schedule of measurements taken and irrigation treatments applied: IR – irrigation, IRGA – photosynthesis, stomatal conductance, water use efficiency, and transpiration, LWP – Leaf predawn water potential, HPFM – root hydraulic conductance and conductivity, and shoot and root dry weight measurements were collected, and GR – height and caliper.

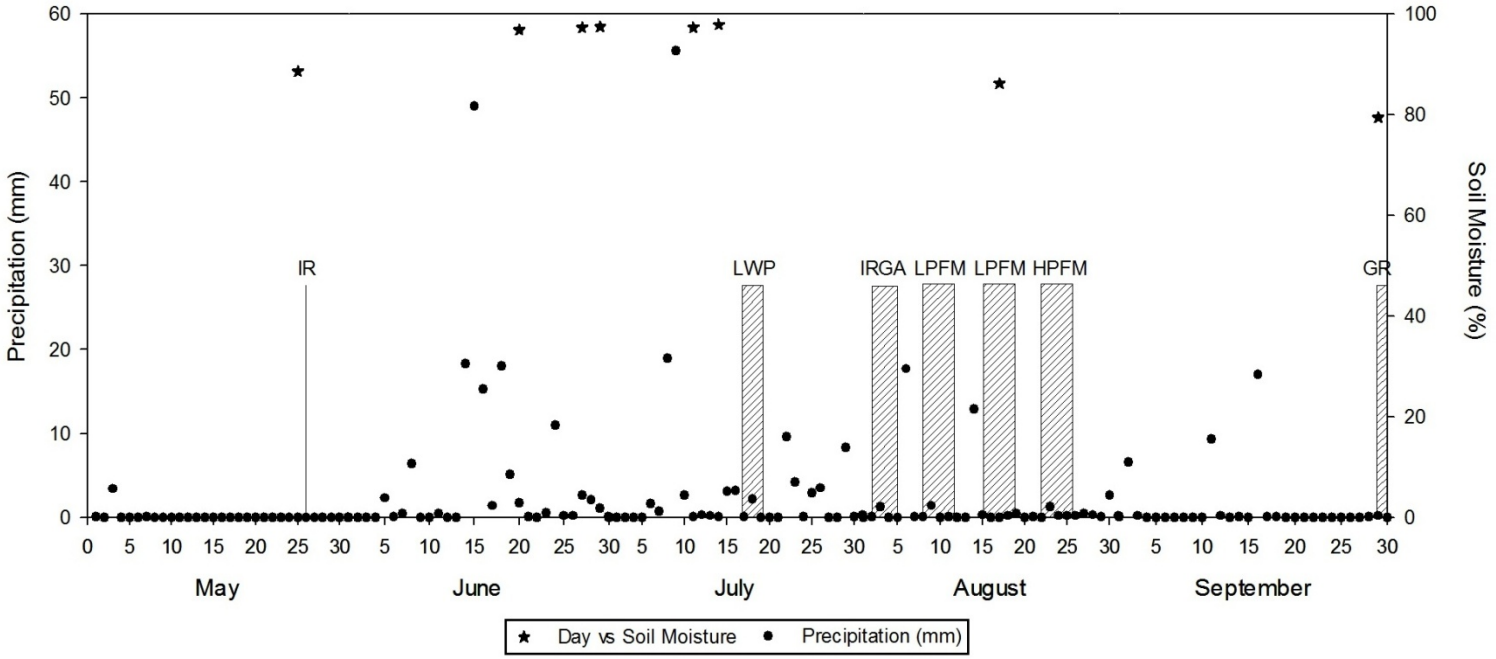

Figure 2.5.2: Daily precipitation and soil moisture measurements during the second field season (fourth growing season, 2011), and schedule of measurements taken and irrigation treatments applied: IR – irrigation, IRGA – photosynthesis, stomatal conductance, water use efficiency, and transpiration, LWP – Leaf predawn water potential, LPFM – percent loss in branch hydraulic conductance, HPFM – root hydraulic conductance and conductivity, and shoot and root dry weight measurements were collected, and GR – height and caliper. Soil moisture remained visibly above field capacity for the majority of the time period between June 14th and August 10th. Only one irrigation treatment was applied in May due to the high precipitation levels in June and soil saturation levels that remained visibly high throughout the entire growing season.