Effects of drought stress on metabolite profiles of hybrid and pure lines of *Populus* spp.

by

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Abstract

Drought stress is perhaps the most commonly encountered abiotic stress plants experience in the natural environment, and is one of the most important factors limiting plant productivity. In the studies described in this text, drought-stressed poplar trees (*Populus* spp.) were analyzed for their metabolite content using the technique of untargeted metabolite profiling by employing gas chromatography coupled to mass spectrometry (GC/MS). The purpose of these analyses was to characterize the metabolite profile of poplar trees under drought stress in order to assess relative drought resistance and to investigate what mechanisms might be employed in the ability to resist drought. To do this, three independent experiments were carried out, each employing different poplar tree species and drought application protocols. For all three experiments, metabolite profiling identified key metabolites that increased or decreased in relative abundance upon exposure to drought stress. Overall, amino acids, the antioxidant phenolic compounds, catechin and kaempferol, and the osmolytes raffinose and galactinol exhibited increased levels under drought stress, whereas metabolites involved in photorespiration, redox regulation, and carbon fixation showed decreased levels under drought stress. One clone in particular, Okanese, in common with Experiments #1 and #2 described in this thesis, displayed unique responses to the imposed drought conditions. This clone was found to have higher stomatal conductance, transpiration rate, and leaf water potential, and lower growth rate in Experiments #2 and #1, respectively. Okanese also had lower accumulation of osmolytes such as raffinose, galactinol and proline, but higher overall levels of antioxidants such as catechin and dehydroascorbic acid. As such, it was proposed that osmotic adjustment as a mechanism for drought resistance in this clone is not as well developed in comparison to other clones investigated in this thesis, and that a possible alternative mechanism for the enhanced drought resistance displayed by Okanese may be due to differential allocation of resources towards root growth rather than osmotic adjustment.

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Dedication

I dedicate this work to my mom, Christine, and my dad, William, who provided support and inspiration throughout this degree, and who were a constant reminder to me about what is truly important in life. I also dedicate this work to my best friend, Todd, who nourished me with his exemplary cooking and who was always willing to lend me his ear.

CHAPTER 1

Introduction

1.1 Drought is an important limiting factor to plant growth

Drought is one of the most significant abiotic environmental stressors to plants, and water is typically the most limiting resource to plant growth, yield, and productivity (Boyer 1982). Drought can be defined generally as a deficiency in precipitation over an extended period of time, though this general definition lacks the precision to truly understand the effects of drought and its underlying causes. In truth, it is difficult to ascribe drought with a precise definition that is applicable and consistent throughout all regions of the globe. This is because in many instances, drought can probably best be conceptualized as a deficiency in rainfall over a given period of time relative to the statistical multi-period norm for a given region (Anonymous 2010). Different regions, therefore, will have different environmental conditions that define a drought episode. Drought can take different forms, varying in time-span and severity, and due to the effects of global climate change, is likely to become more common and more severe in many parts of the world. It has been postulated by the Intergovernmental Panel on Climate Change (IPCC) that it is very likely (>90%) that all land areas of the globe will warm more than the global average, with amplification at higher latitudes, ultimately resulting in more extreme weather patterns, including drought (IPCC 2007, Hughes 2000). Working Group I of the IPCC also states that although precipitation in southern regions of Canada will likely increase in the winter and spring, precipitation is likely to decrease in the summer months (IPCC 2007). Though the realities of this forecast will have a detrimental effect on many regions and climatic zones, from an anthropocentric viewpoint, areas whose economies depend directly on land-based resources, such as agriculture and forestry, will most certainly suffer.

One way to adapt to proposed climate change models is to select food crops and tree species that have greater capacity to deal with environmental stress, that is, select genotypes for the ability to maintain growth under non-ideal growing conditions. This is an important and worthy aspiration. It has been noted in studies on agricultural crops that there is a large, and generally untapped

genetic potential for yield that has not been fully realized because of the need for better adaptation of plants to the environments in which they are grown (Turner and Begg 1981, Boyer 1982). Because the majority of land upon which plants and trees grow does not provide ideal physicochemical conditions for growth, and because genotypic selection of plants has largely been based on evidence of improved productivity under ideal conditions, a gap exists between actual yield and the genetic potential for yield of many plant species (Boyer 1982, Duvick 2005). Therefore, studies investigating the ability of different plant species or genotypes to adapt to adverse growing conditions will prove useful both in selecting better adapted and regionally specific plants, and in understanding the fundamental mechanisms of adaptation.

Plant responses to osmotic stress due to soil moisture or high vapour pressure deficits occur on several levels. On a large scale (behavioural) level, plants generally will close their stomata to inhibit water loss, thus causing reduced photosynthesis. Over time this results in reduced growth, usually accompanied by a shift in biomass allocation towards the root system, and under severe water deficits, the plant may perish. Plants that can adapt to water deficits will exhibit changes at the morphological, physiological, biochemical and molecular levels in an attempt to balance water lost through transpiration and water uptake by the root system, while still remaining relatively productive. Because the ability to change at various biological levels is essentially a measure of a plant's plasticity, and plasticity is heritable, it should be possible to select for plants that are better adapted to a reduced water regime (Bradshaw 2006, Morgan 1984, Gebre et al. 1997). The following few sections will expand on the subjects of morphological, physiological, biochemical, and molecular level responses to water deficit.

1.2 Morphological responses

Morphological level responses in plants due to reduced water availability are varied and may include one or all of the following changes to plant structure. Growth slows or ceases due to the combined effects of restricted uptake of CO_2 resulting from closed stomata, the lack of sufficient water for cells to expand, the

carbon costs of osmotic adjustment, and increased resource partitioning to roots (Verslues et al. 2006, Tschaplinski et al. 1998, Hopkins and Huner 2004). Consequently, total biomass tends to decrease due to the cessation of growth and abscission of leaves and branches. The ratio of above to below ground biomass also tends to shift; carbon allocation to roots may be favoured in comparison to plants grown under well-watered conditions (Hsiao and Acevedo 1974, Turner and Begg 1981, Tschaplinski et al. 1998). The characteristics of leaves produced under reduced moisture conditions may change to promote an increased barrier to diffusive water vapour loss from tissues. Leaves may become thicker, with epidermal cells that exhibit thicker cuticles, and the abundance, distribution, and size of stomata across the leaf surface may change to favour less densely distributed, smaller stomata that are present predominantly on abaxial leaf surfaces (Ceulemans et al. 1988, Willmer and Fricker 1996, Regier et al. 2009).

Morphological changes due to soil moisture deficit are often the result of physiological responses to decreasing soil, and thus, plant moisture content. Physiological adjustments that would be important for selection strategies and human end use will allow the plant to continue assimilating CO_2, and at the same time minimize water loss and avoid cellular damage. Important physiological acclimation responses include improved stomatal responsiveness and photosynthetic efficiency or improved water-use efficiency (Blake et al. 1984, Silim et al. 2001, Wang et al. 2004). Improved stomatal responsiveness results in more rapid stomatal closure once leaf water potentials fall below a threshold level. A more efficient photosynthetic system is able to function on lower concentrations of intercellular CO_2 (a consequence of stomatal closure), and is able to absorb and metabolize excess energy without essential proteins or enzymes being damaged when photosynthesis is not functioning at its maximum (Hopkins and Huner 2004, Heldt 2005).

1.3 Molecular level responses

Physiological level plant responses to water deficits are driven by the underlying biochemical and molecular changes induced in a particular plant

species. Though comparatively very little is known about the precise mechanisms of gene induction due to osmotic stress, several classes of proteins have been shown to be upregulated in plants experiencing osmotic stress. These include the Late Embryogenesis Abundant (LEA) proteins and the Superoxide Dismutases (SODs).

LEA proteins are a well known family of proteins whose expression is linked to a variety of phenological stages when osmotic stress occurs: post-abscission seed desiccation tolerance, drought tolerance in plants, cold tolerance of seedlings, even dehydration resistance in prokaryotes (Tunnacliffe and Wise 2007, Caruso et al. 2002). They were first discovered to be highly expressed in late stages of embryo development in seeds (Tunnacliffe and Wise 2007, Hong-Bo et al. 2005). Their expression is induced by both the presence of abscisic acid and drought conditions, though the precise mechanism of protein induction is not yet known (Tunnacliffe and Wise 2007). LEA proteins are predominantly found in the cytoplasm and nuclear regions of cells, though specific classes of these proteins may show different localizations, such as the membranes of different cellular compartments (e.g., the group 3 LEA protein, LEAM, is thought to protect the inner mitochondrial membrane under dehydration conditions; and group 2 LEA protein, WCOR410, accumulates at the plasma membrane during cold acclimation) (Tunnacliffe and Wise 2007, Zhang et al. 2007, Hong-Bo et al. 2005, Caruso et al. 2002). One such class of LEAs shown to be important in drought tolerance are the dehydrins, specifically of the LEA D-11 subgroup (Zhang et al. 2007, Caruso et al. 2002). Dehydrins likely confer protective effects to various membrane components of a cell by protecting against reactive oxygen species and stabilization of enzymes such as α-amylase. The defining structural feature of dehydrins is the conserved amino acid sequence (the K-segment) that forms amphipathic alpha helices (between one and eleven helices) throughout the protein (Kosova et al. 2007). These alpha helical regions are thought to play an important role in the interaction of this protein with membranes. Apart from the amphipathic alpha helices, dehydrins are also characterized by random coils that are rich in the aliphatic amino acid glycine and/or other hydrophilic amino acids.

These random coils have the capacity to bind large amounts of water due to the interaction between water and the dipolar peptide bonds, and also contribute to the protein's ability to remain soluble upon exposure to high temperatures (Kosova et al. 2007). Based on the structure of dehydrins, it has been proposed that the protective functions of dehydrins are important in membrane stabilization (the alpha helical regions allow interaction between the protein and membranes), water retention, and enzyme stabilization (the random coil regions bind water and allow interaction with enzymes).

The transcripts of two putative cytoplasmic dehydrins, *peudhn1* and *peudhn2*, were found to be expressed in a *Populus euramericana* clone under drought conditions (Caruso et al. 2002). *Peuhdn1* was sequenced and found to be constitutively expressed in greater quantities in leaves than in roots. Upon exposure to drought conditions (via addition of polyethyleneglycol to the growth media), *peudhn1* progressively increased, then increased further upon rehydration. It was proposed that the progressive increases in abundance of this protein may be linked to drought hardening, and that the constitutive nature of the expression of *peudhn1* overall may be associated with this particular clone's ability to tolerate drought (Caruso et al. 2002).

SODs are another class of protein that has been found to be activated in studies of drought stress response in poplar (Lei et al. 2007). It is known that an array of environmental stresses (including drought stress) can cause the formation of oxygen radicals originating from the photosynthetic electron transport systems, which are very destructive to enzymes and membranes (Wang et al. 2004). SODs are able to catalyze the dismutation of two superoxide free radicals with two protons to form oxygen and hydrogen peroxide, one of the first steps in scavenging for reactive oxygen species. Hydrogen peroxide is still not a particularly friendly substance to the cellular atmosphere, and further antioxidant proteins, such as catalase, are involved in inhibiting reactive oxygen species from initiating further damage within the cell (Wang et al. 2004, Lei et al. 2007).

1.4 Biochemical level responses

Other molecular responses to moisture deficit stress occur at the biochemical level, and many biochemical mechanisms share analogies with the protective mechanisms of proteins. These responses involve osmotic adjustment, stabilization of cellular structures, and redox regulation. Osmotic adjustment allows the cell to lower its solute potential which helps in water retention; small organic molecules contributing to osmotic adjustment do so by increasing in concentration, thus increasing cellular osmolarity. Compounds that stabilize cellular structures such as membranes and proteins do so not necessarily by accumulating to high concentrations, but by protecting the electrical charge of functional groups of those structures. Redox regulation is important in protecting the cell against oxidative damage by absorbing free radicals that are produced in response to the water stress. It has been noted that metabolic responses are the closest of all the –omics technologies to phenotype, and that the biochemical phenotype of an organism is ultimately the result of interactions between genotype and environment (Bino et al. 2004, Seger and Sturm 2007, Dettmer et al. 2007, Hall 2005). Therefore responses to stress may represent the interface between the genetic predisposition of plants to tolerate the stress, and the environmental influence of the stress on the plant. If this is so, then upon application of stress conditions or a change in environment, one should be able to see changes in metabolic profiles of stressed plants. The changes to the metabolic profile may highlight pools of metabolites involved in biochemical pathways important to stress acclimation or cellular maintenance, and may indicate which pathways have been perturbed by the stress.

Among metabolites involved in osmotic adjustment, sugars and polyols are considered particularly important. They are osmotically active compounds, that is, they are involved in the osmotic adjustment of tissue in order to maintain relative water content (Jouve et al. 2004, Guerrier et al. 2000). Osmotically active solutes act to increase cellular osmolarity, thus attempting to maintain a negative water potential gradient in plant tissue below that of the soil environment. Osmotic adjustment allows cells to maintain turgor and continue growing during

exposure to drought or osmotic stress and helps cells to recover more quickly after a period of such stress (Cayley et al. 1992). It has been shown that *Populus tremula* L. cultures subjected to salt (NaCl) stress accumulated sucrose, mannitol, and raffinose (Jouve et al. 2004). Sucrose and mannitol may accumulate simply due to retardation of sugar transfer from the mesophyll to the phloem and reduction of growth due to stress, and sucrose is a known feed-back inhibitor of Rubisco. Raffinose is proposed to be an osmoprotectant, with the capacity for enzyme and membrane stabilization (Jouve et al. 2004, Taji et al. 2002). In poplar hybrids, a variety of sugars, polyols, and their derivatives were observed to accumulate in response to drought stress, including glucose, fructose, sucrose, malic acid, myoinositol, and salicin (Kozlowski and Pallardy 2002). It is known that the solutes involved in osmotic adjustment will vary significantly depending on the species and their inherent resilience to drought stress, and the length and intensity of drought exposure.

Other small organic molecules have been implicated in the plant biochemical response to drought stress. Organic acids represent a class of small metabolically active organic compounds that may well have protective properties against drought stress. Malic acid has been observed in several studies (Alvarez et al. 2008, Kozlowski and Pallardy 2002, Patonnier et al. 1999) to increase in concentration under drought conditions. However, it has been noted that the reasons for malic acid accumulation in the apoplast may not be due to drought stress *per se*, but rather as a stomatal regulatory system that works synergistically with abscisic acid (Wilkinson and Davies 2002). Malate may instead accumulate during times of high photosynthetic rates, and indicate that the photosynthetic machinery is saturated, thus inducing stomatal closure in an effort to conserve water. Apoplastic sucrose is also suspected to perform a similar role as described for malate in addition to being osmotically active (Wilkinson and Davies 2002). Another organic acid with an important and central role in the plant drought stress response is abscisic acid (Davies et al. 2005, Wilkinson and Davies 2002). Abscisic acid is a plant hormone; it is a small organic molecule that can be synthesized in the roots and shoots of plants, and it

can also be obtained “pre-synthesized” by some plants via influx from the soil environment (Davies et al. 2005). Abscisic acid can be stored in the symplast of cells or transported in the xylem in its glucose ester form, which causes it to be inactive. Abscisic acid promotes stomatal closure by binding to a receptor on the external surface of guard cell plasma membranes and inducing a series of signal transduction cascades beginning with a cytosolic increase in the concentration of calcium ion (Ca^{++}) and the formation of H_2O_2, nitric oxide (NO) , and the effector molecule inositol triphosphate (IP3). The increase in calcium ion concentration causes a reduction in guard cell turgor, and thus stomatal closure, due to the loss of potassium (K^+) and chloride (Cl^-) ions (Wilkinson and Davies 2002, Wilkinson and Davies 2010).

The abscisic acid stress signal is complicated by the differing physiological environments of the cellular compartments through which it passes on its way towards guard cells, as well as by “cross-talk” with other hormones and metabolites. Leaves, it seems, are capable of fine tuning their response to abscisic acid both by establishing pH gradients and by accumulating solutes such as malate (Davies et al. 2005). The precise mode by which pH gradients within a plant are created is unknown, however, leaf apoplastic pH was shown to be affected by soil nutrient status and the leaf-to-air vapour pressure difference (Davies et al. 2005).

Abscisic acid, as its name suggests, is an acid, and therefore its activity can be modulated by the pH in a given cellular compartment. For example, the concentration of abscisic acid in the xylem of a leaf is known to have a greater effect on stomatal closure if the apoplastic environment around the guard cells is slightly more basic (pH 7.0) than usual (pH 6.0) (Wilkinson and Davies 2002). This fact suggests that a change in xylem pH may change the plant’s perception of abscisic acid concentration. This subtlety seems to have confounded some previous experiments that have attempted to relate the total amount of abscisic acid in leaf tissue to the extent of stomatal closure observed (Wilkinson and Davies 2002).

Another class of osmotically active metabolites that has been the subject of much research in the past and present are the amino acids, particularly proline. Proline has been shown to accumulate in drought-stressed poplar leaves along with total amino acid content (Lei et al. 2007, Jouve et al. 2004). However, not all studies have shown that increases in free proline content parallel a response to drought (Tchaplinski and Tuskan 1994, Delauney et al. 1993). It seems that increases in amino acid content may not be a global plant response to drought stress, or it may be that the method in which the drought stress treatment is applied affects the types of osmotica that accumulate. Gebre et al. (1997) found that greenhouse grown poplar clones (*P. deltoides* Bartr.) subjected to water deficits did not accumulate significant amounts of proline in leaf tissue, which was consistent with a previous study that subjected poplar hybrids (*P. trichocarpa* x *deltoides*) to drought in a field trial (Tschaplinski and Tuskan 1994). Tschaplinski and Tuskan (1994) noted that the accumulation of amino acids as a form of osmotic adjustment to drought stress is more likely observed in agronomic crops rather than long-lived woody species such as poplar, where the predominant form of osmotic adjustment is in the form of carbohydrates. Apart from the apparently limited accumulation of proline due to drought in woody species such as poplar, proline has been shown to be a potent osmotic adjuster in herbaceous and crop species, though there is considerable variation in the accumulation of nitrogenous compounds within this group of species as well (Alvarez et al. 2008).

1.5 Poplars as model organisms

Poplar trees (*Populus* spp.) are ecologically important tree species, often serving a role as a vegetative pioneer species, a role that is qualitatively linked to their inherently fast growth rates which are among the fastest of temperate trees (Eckenwalder 1996). Some species of poplar inhabit large areas of land, pointing to their ability to adapt to diverse environmental conditions within a species (Farmer 1996). In the northern hemisphere, poplar species are all single-trunked, deciduous, and dioecious, and can reproduce both sexually, via wind pollination, and vegetatively by means of root-borne sucker shoots (Eckenwalder 1996).

Because most poplar species have fast growth rates, are soboliferous (able to produce sucker shoots), and reach maturity relatively quickly (within 4-6 years) compared to other tree species, they have found popular use in both the agriculture and forestry industries. Agriculturally, poplars are mainly used as a shelterbelt species, and in forestry, as a source of fibre production (Bekkaoui et al. 2003, Roden et al. 1989). They therefore command significant economical and commercial interest. Poplars possess a number of unique attributes that make them a “model organism” including: rapid growth rates and the ability to hybridize and transform them using modern molecular biology techniques; they are relatively easy to grow from stem cuttings and tissue culture; and they represent the first tree species to have a fully sequenced genome (Han et al. 1996, Sjodin et al. 2009, Tuskan et al. 2006).

This thesis will describe three independent experiments carried out with the broad intention to better understand and characterize the metabolic effects of drought stress on poplar genotypes. Several areas of interest will be investigated, and these include: 1) what metabolites show perturbation under drought conditions and what roles do they likely play in the drought stress response in poplar, 2) how do the patterns of drought response as indicated by the metabolite profile differ for the different poplar clones studied (e.g. are there poplar clone(s) that stand out as having a unique response to drought application), and 3) does the response to drought indicate a possible mode of drought resistance for the poplar clones studied. Each of the three experiments represent a slightly different way of designing and applying a drought stress treatment, the results of which may provide insight into establishing an overarching mechanism(s) of drought adaptation, rather than metabolic effects that may be specific to a single particular drought application protocol. The thesis is concluded in Chapter 4, with a discussion of overall results, and how the results from this thesis may assist in the selection of superior hybrid poplar clones that will be best adapted to current and future climate conditions.

CHAPTER 2

Materials and Methods

2.1 Experiment #1

2.1.1 Plant material

Four hybrid poplar (*Populus* spp.) clones were used in this study. The poplar clone Walker is a hybrid of *Populus deltoides* var. *occidentalis* and *P.* x *petrowskyana*, and the other three hybrids represent progeny from controlled and uncontrolled crosses with Walker. The detailed genetic backgrounds of all four hybrid poplar clones are:

1. *Populus* x cv. 'Walker' (*P. deltoides* W. Bartr. ex Marsh. var. *occidentalis* Rydb. x *P*. x *petrowskyana* Schneid. (*P. laurifolia* Ledeb. x *P. nigra* L. var. *italica* DuRoi)),
2. *Populus* x 'Okanese' (formerly 'WP-69', *P.* 'Walker' x *P. petrowskyana*),
3. *Populus* x 'WP-86V-86' (*P.* 'Walker' x *P. petrowskyana*),
4. *Populus* x 'Katepwa' (a progeny line resulting from open-pollination of *P.* x 'Walker')

Dormant, un-rooted, hardwood cuttings approximately 10 cm long from each of the hybrid poplar clones were rooted in one gallon pots containing perennial mix (West Creek Farms Ltd, Fort Langley, BC, Canada) using STIM-ROOT No. 2 rooting powder (Nu-Gro IP Inc., Brantford, ON, Canada). Plants were grown under natural light in the University of British Columbia horticulture greenhouse between May to September 2008, at an average temperature of 22.9°C and average relative humidity of 59.6%. Plants were allowed to grow until they reached a height of approximately 90 cm (118 days) when they were transferred to a flood table where fertigated water (containing 15-5-15 N:P:K plus micronutrients for continuous feeding of approximately 100 ppm N) was applied automatically each day at 10:00 am.

2.1.2 Experimental design and sampling

The experiment was set up as a completely randomized design with the four hybrid poplar clones being randomly assigned and subjected to either a well-watered or water withdrawal drought treatment. In addition, one third of trees assigned to each of well-watered and drought treatments were also assigned to a

predetermined, destructive harvest schedule at 7 days after drought initiation (DAD), 9 DAD, and 14 DAD. Throughout the experiment, plants assigned to well-watered and drought treatments were interspersed amongst each other on a single flood table. Every second day, plants were randomly rotated on the flood table.

At 0 DAD, the trees assigned to the drought treatment were placed atop plastic pedestals approximately 40 cm tall in order to raise them completely above the water level of the flood table, thus withdrawing them from any source of water (Figure 2.1). At 7 DAD and 9 DAD, one third of trees receiving drought and well-watered treatments were destructively harvested at midday for each harvest event. After the 9 DAD harvest, all trees subject to the water withdrawal treatment were taken off the plastic pedestals and placed back on the flood table to receive a 5 day re-watering period. The last third of all the trees were then harvested at midday at 14 DAD.

Prior to each harvest, relative leaf number was ascertained using an index leaf of 30 mm (Larson and Isebrands 1971). One leaf of leaf plastochron index (LPI) equal to six and indicative of a mature, fully expanded leaf was harvested from each tree and immediately flash frozen in liquid nitrogen.

2.1.3 Physiological measurements

Average growth rate per day for each tree was calculated from stem height measurement taken every other day starting from 0 DAD. Leaf water potential was measured at midday for each tree on the day of harvest using a pressure bomb (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Average percent soil water content (% SWC) was calculated gravimetrically from pot weight measurements taken for a subset of four trees from each of the four clones for each of control and drought treatments every other day starting at 2 DAD and on the days of harvest.

2.1.4 Sample preparation

Frozen leaves were ground in liquid nitrogen using clean mortar and pestle. The frozen ground leaf tissue was then placed in 2 mL screw-cap tubes and freeze-dried for five days. Freeze-dried tissue was then weighed into tared 2

mL lock-cap microcentrifuge tubes to an approximate volume of 0.5 mL, and stored at -80°C until metabolite extraction.

2.1.5 Metabolite extraction and derivatization

Each sample was extracted in 1300 µL solvent mix (3% distilled, deionized water in methanol, with the internal standard ortho-anisic acid (0.62 mg/mL), for 15 minutes at 70°C on an orbital shaker set to 1400 rpm. Following centrifugation for 10 minutes at 17,000 g, 200 µL of supernatant was transferred to a 1.5 mL lock-cap microcentrifuge tube, to which was added 130 µL chloroform and 270 µL distilled, deionized water. This mixture was shaken gently for approximately 10 seconds and centrifuged for 5 minutes at 17,000 g to allow phase separation. 400 µL of the upper polar phase was transferred to a new 1.5 mL lock-cap microcentrifuge tube and dried overnight at 30°C in a Vacufuge (Eppendorf).

For sample derivatization, each sample was resuspended in 50 µL pyridine containing 20 mg/mL methoxyamine HCl and incubated at 37°C for 2 hours on an orbital shaker set at 1100 rpm. Following a brief centrifugation step (1 minute at 17,000 g) to settle condensation, 10 µL of an n-alkane standard mixture consisting of 20 µL/mL each of C12 and C15 and 5 mg/mL each of C19, C22, C28, C32, and C36, and 70 µL of N-methyl-N-trimethylsilyltriflouroacetamide (MSTFA) was added. This was followed by incubation at 37°C for 30 minutes on an orbital shaker set at 1100 rpm. Samples were filtered through filter paper into GC/MS vials, and allowed to sit at room temperature prior to GC/MS analysis. A version of this protocol for metabolite extraction and derivatization has previously been described in Robinson et al. (2005), and was adapted for use in this experiment.

2.1.6 GC/MS analysis

A ThermoFinnigan Trace GC-PolarisQ ion trap MS was used for GC/MS analysis. This instrument was fit with an AS2000 auto-sampler and a split injector (Thermo Electron Co., Waltham, MA, USA). The GC was equipped with a Restek Rtx-5MS column made from fused silica, with a length of 30 m, an interior diameter of 0.25 mm, and a stationary phase of 5% diphenyl 95% dimethyl polysiloxane. The GC conditions were set as follows: inlet temperature 250°C,

He as a carrier gas with a constant flow rate of 1 mL/min, injector split ratio 10:1, resting oven temperature at 70°C, and the GC/MS transfer line at a temperature of 300°C. Following the injection of 1 µL of sample, the oven temperature was held at 70°C for 2 minutes, and then ramped to 325°C at a rate of 8°C/min. The temperature was held at 325°C for six minutes before cooling to the initial resting oven temperature of 70°C. Mass spectrometric analysis was conducted in positive electron ionization mode with an ionization potential of 70 eV. The foreline was evacuated to 40 mTorr, He gas flow into the vacuum chamber was set to a rate of 0.3 mL/min, and the source temperature was held at 230°C. The detector signal was recorded from 3.35 to 35.5 minutes after the injection of sample, and ions were scanned across the range of 50-650 mass units with a total scan time of 0.58 s.

2.1.7 Data processing and metabolite identification

Raw data files were processed using the R package XCMS as has been previously described by Smith et al. 2006. Briefly, this involved identification of peaks in the metabolite profile within the chromatographic domain. Following identification of peaks, grouping of peaks across samples was carried out within the mass domain and an iteration of retention time correction was done based on peak grouping; peaks were then regrouped across samples. This automated process was done entirely within the XCMS framework, the algorithm of which is based on the assumption that each metabolite detected by the mass spectrometer was represented by at least two highly correlated m/z signals. Only the m/z signals that showed high intensity correlation (>95%) and highly similar retention time (difference in median retention time after retention time correction <0.03 seconds) with at least one other m/z peak, were retained. Therefore, groups of m/z peaks believed to originate from the same metabolite were formed and the m/z signal with the highest intensity of such a group was selected as the representative signal for the corresponding metabolite. Thus, the ouput of XCMS is a data matrix consisting of number of samples x number of peaks found using XCMS with the signal intensity for a given peak in a given corresponding sample in the body of the data matrix. The accuracy of XCMS was verified and

metabolite identification was aided by using NIST AMDIS (Automated Mass spectral Deconvolution and Identification System) (National Institutes of Standards and Technology (NIST), Gaithersburg, MD, USA). Metabolite identification was carried out using the NIST MS-Search software equipped with the NIST mass spectra library. Additional libraries used in metabolite identification were the Max Planck Institute Trimethylsilane (TMS) library (http://www.mpimp-Golm.mpg.de/mms-library/index-e.html), the Golm Metabolome Database (http://www.mpimp-Golm.mpg.de/csbdb/gmd/gmd.html) (Kopka *et al*. 2005), and the Mansfield UBC Laboratory TMS derivatized mass spectral library (containing 513 known compounds). In general, an identification probability of 800 and the specific metabolite retention indices were used as a guide for determining metabolite identity. An identification probability value of 800 represents a good match, and using this value in combination with observed metabolite retention indices is a conservative approach to metabolite identification. Employing a high identification probability value favours identification of compounds with unique mass spectra over those with common mass spectra, but ensures that identifications of both types of compounds are correct. Metabolites that were identified with identification probability values lower than 800 were ascertained in consultation with Dr. Rebecca Dauwe (pers. comm.).

2.1.8 Statistical analysis

All peaks representing identified and unknown metabolites were first expressed as a proportion of the internal standard, and then normalized to the unextracted, freeze-dried weight of each tissue sample. The internal standard compound was added to compensate for slight variations that may have occurred in sample extraction and machine operating parameters over time. Therefore, the intensity of metabolite peaks was compared to the intensity of the internal standard compound and will be discussed in terms of signal intensity in the following sections. All peaks were then subjected to Welch's t-tests (α level set at 0.05). Examination of the number of significant t-tests for important treatment and genotype comparisons provided a basis for variable reduction and interpretation.

The exact procedure for variable reduction was as follows: 1) the number of significant t-tests for water treatment and clone comparisons was counted for each metabolite; 2) metabolites were considered to show high significance if they displayed a number of significant comparisons that was greater than the product of (number of comparisons) · 0.05 · 4 (four was chosen as an arbitrary factor to increase the level of significance a metabolite had to show over the random chance that a comparison might be found to be significant at an α level set at 0.05); 3) compare the metabolites that exhibited a high number of significant comparisons across all three independent but associated experiments described in this thesis. Therefore metabolites that showed significance in comparisons of water treatment and clone across all three experiments were chosen for further analysis using general linear modeling.

The general linear model for the three factorial experimental design (with 24 possible factor level combinations) is fixed and is represented by:

$$y_{ijk}=\mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijk},$$

where factor A corresponds to the clones with i levels, factor B corresponds to the water treatment with j levels, factor C corresponds to the harvest time with k levels, ij is the eight possible interactions between clone and treatment, ik is the 12 possible interactions between clone and harvest time, jk is the six possible interactions between treatment and harvest time, ijk is the 24 possible interactions between clone, treatment, and harvest time, and εijk is the random error. The clones, the water treatments, and the harvest times are all fixed factors.

The null hypothesis was that leaf water potential, average growth rate, percent soil water content, and metabolite intensity of each of the selected metabolites for all clones would be the same regardless of treatment. The type I error rate (α) was set at 0.05 for all tests. Logarithmic transformations of leaf water potential, malic acid, galactinol, and phenylalanine and power transformations of proline and tryptophan were necessary in order to satisfy assumption of normality. Analyses of variance were computed for: leaf water potential, average growth rate per day, percent soil water content, and metabolite

intensity. Differences between means were tested using Bonferroni adjusted t-tests. All analyses were performed using SAS software package (SAS Institute, Inc., Cary, NC, USA), R (R Development Core Team 2009), and Microsoft Office Excel 2007 (Microsoft, Seattle, WA, USA).

2.1.9 Metabolite profile visualization

Metabolite profile visualization was carried out using the conditional formatting function in Microsoft Office Excel 2007 (Microsoft, Seattle, WA, USA). Log_2 (droughted/well-watered) fold changes were plotted for each clone species at each day of harvest for each metabolite.

2.2 Experiment #2

2.2.1 Preface

Plant material and sampling, as well as the physiological measurements for this experiment were carried out at the University of Toronto by Sherosha Raj (Campbell Laboratory). All further sample processing and analyses were carried out at the University of British Columbia by Genoa Barchet.

2.2.2 Plant material and sampling

Two hybrid poplar (*Populus* spp.) clones were used in this study. Both clones are hybrids of *Populus deltoides* var. *occidentalis* and *P.* x *petrowskyana*. The hybrid poplar genetic backgrounds employed in this study were Walker (*Populus deltoides* W. Bartr. ex Marsh. var. *occidentalis* Rydb. x *P*. x *petrowskyana* Schneid. (*P. laurifolia* Ledeb. x *P. nigra* L. var. *italica* DuRoi)) and Okanese (formerly 'WP-69', *P.* 'Walker' x *P. petrowskyana*). Dormant, un-rooted hardwood cuttings (approximately 25 cm in length) were sourced from two sites: Prairie Farm Rehabilitation Administration (PFRA) Shelterbelt Centre, Indian Head, SK and Alberta-Pacific Forest Industries Inc. (Al-Pac), Boyle, AB. They were rooted in 1 m long x 10.5 cm diameter opaque PVC pipe containers in Sunshine Mix #1 (Sun Gro Horticulture Inc., Bellevue, WA, USA) and grown in growth chambers under a 16 hour:8 hour light:dark photoperiod (minimum PPFD of 200 µmol m^{-2} s^{-1}). Temperatures ranged from 22°C maximum daylight temperature to 17°C minimum night temperature with relative humidity of 55-

65%. Trees were watered to field capacity every two days and fertilized with 600 mL of 20:20:20 N:P:K once every two weeks for nine weeks in addition to the week before the drought experiment. After nine weeks of unstressed growth, the drought experiment was initiated. Drought conditions were applied to half of the trees for each treatment combination by withdrawing application of water; control trees were watered normally. After a period of 13 days (11 days for Walker trees originating from Saskatchewan), mature, fully expanded leaves (with leaf plastochron index (LPI) equal to six) were harvested from each tree at predawn (1 hour prior to light period) and midday (middle of light period).

2.2.3 Physiological measurements

Stomatal conductance (mol CO_2 m^{-2} s^{-1}) and transpiration rate (g H_2O m^{-2} min^{-1}) were measured at midday for a subset of five trees for each treatment combination using a LI-6400XT portable photosynthesis system (LI-COR Biosciences, NE, USA).

2.2.4 Sample shipping and preparation

After harvesting of samples, the leaves were weighed to determine fresh mass, then were freeze-dried and weighed again to determine dry mass. Samples were then wrapped whole in aluminum foil, placed into zip-lock plastic bags containing desiccant, and then couriered from the University of Toronto to the University of British Columbia. At the University of British Columbia, the samples were placed at -80°C until further processing.

Freeze-dried whole leaves were ground to a fine powder using a clean mortar and pestle. The ground, freeze-dried tissue was then weighed into preweighed 2 mL lock-cap microcentrifuge tubes (to an approximate volume of 0.5 mL) and stored at -80°C until metabolite extraction.

2.2.5 Metabolite extraction and derivatization

Metabolite extraction and derivatization was carried out for each sample as described for Experiment #1 above (section 2.1.5). The only exception was that 0.25 mg/mL ribitol was used as an internal standard rather than 0.62 mg/mL ortho-anisic acid.

2.2.6 GC/MS analysis

GC/MS analysis was performed using the same method as that described for Experiment #1 above (section 2.1.6).

2.2.7 Data processing and metabolite identification

Data processing and metabolite identification were performed as described for Experiment #1 above (section 2.1.7).

2.2.8 Statistical analysis

All peaks representing identified and unknown metabolites were first expressed as a proportion of the internal standard compound, and then normalized to the unextracted, freeze-dried weight of each tissue sample. The internal standard compound was added to compensate for slight variations that may have occurred in sample extraction and machine operating parameters over time. Therefore, the intensity of metabolite peaks was compared to the intensity of the internal standard compound and will be discussed in terms of signal intensity in the following sections. Each peak was then subjected to Welch's t-test to identify metabolites showing statistically significant mean differences between selected tree groups (α level set at 0.05).

Examination of the number of significant t-tests for important treatment and genotype comparisons provided a basis for variable reduction and interpretation. The exact procedure for variable reduction was as follows: 1) the number of significant t-tests for water treatment and clone comparisons was counted for each metabolite; 2) metabolites were considered to show high significance if they displayed a number of significant comparisons that was greater than the product of (number of comparisons) · 0.05 · 4 (four was chosen as an arbitrary factor to increase the level of significance a metabolite had to show over the random chance that a comparison might be found to be significant at an α level set at 0.05); 3) compare the metabolites that exhibited a high number of significant comparisons across all three independent, but associated experiments described in this thesis. Therefore, metabolites that showed significance in comparisons of water treatment and clone across all three experiments were chosen for further analysis using general linear modeling. The

general linear model for the four factorial experimental design is fixed with a total of 16 possible factor level combinations. The clones, the water treatments, the harvest times, and the sources of origin are all fixed factors.

The null hypothesis was that stomatal conductance, transpiration rate, and metabolite intensity of each of the selected metabolites for all clones will be the same regardless of treatment. The α level was set at 0.05 for all tests. Power transformation of galactinol was necessary in order to satisfy assumption of normality. Analyses of variance were computed for: stomatal conductance, transpiration rate, and metabolite intensity. Differences between means were tested using Bonferroni adjusted t-tests. All analyses were performed using SAS software package (SAS Institute, Inc., Cary, NC, USA), R (R Development Core Team 2009), and Microsoft Office Excel 2007 (Microsoft, Seattle, WA, USA).

2.3 Experiment #3

2.3.1 Preface

Plant material and sampling, as well as the physiological measurements for this experiment were carried out at the University of Toronto by Erin Hamanishi (Campbell Laboratory). All further sample processing and analyses were carried out at the University of British Columbia by Genoa Barchet.

2.3.2 Plant material

Six lines of pure *Populus balsamifera* clones were employed in this study. The clones employed were:

1. *Populus balsamifera* 'AP947'
2. *Populus balsamifera* 'AP1006'
3. *Populus balsamifera* 'AP1005'
4. *Populus balsamifera* 'AP2278'
5. *Populus balsamifera* 'AP2298'
6. *Populus balsamifera* 'AP2300'

Dormant, un-rooted, hardwood cuttings (approximately 25 cm in length) were sourced from Alberta-Pacific Forest Industries Inc. (Al-Pac), Boyle, AB. The

cuttings were rooted in 1 m long x 10.5 cm diameter opaque PVC pipe containers in Sunshine Mix #1 (Sun Gro Horticulture Inc., Bellevue, WA, USA) and grown in growth chambers under a 16 hour:8 hour light:dark photoperiod (minimum PPFD of 200 µmol m^{-2} s^{-1}). Temperatures ranged from 22°C maximum day time temperature to 17°C minimum night temperature with relative humidity of 55-65%. Trees were watered to field capacity every two days and fertilized with 600 mL of 20:20:20 N:P:K once every three weeks for nine weeks, after which time the drought experiment was initiated. Drought conditions were applied to half of the trees for each treatment combination by withdrawing application of water; control trees were watered as during the nine weeks prior to the drought experiment initiation. Trees were destructively harvested after a period of 15 days; mature, fully expanded leaves (with leaf plastochron index (LPI) equal to six) were harvested from each tree at predawn (1 hour prior to light period) and midday (middle of light period).

2.3.3 Physiological measurements

Stomatal conductance (mol CO_2 m^{-2} s^{-1}) and net photosynthetic rate (µmol m^{-2} s^{-1}) were measured at midday for a subset of three trees for each treatment combination using a LI-6400XT portable photosynthesis system (LI-COR Biosciences, NE, USA).

2.3.4 Sample shipping and preparation

Samples were treated as described for Experiment #2 above (section 2.2.4).

2.3.5 Metabolite extraction and derivatization

Samples were extracted and derivatized as described for Experiment #1 above (section 2.1.5).

2.3.6 GC/MS analysis

Samples were analyzed using GC/MS as described for Experiment #1 above (section 2.1.6).

2.3.7 Data processing and metabolite identification

Raw data was processed and peaks were identified as described for Experiment #1 above (section 2.1.7).

2.3.8 Statistical analysis

All peaks representing identified and unidentified metabolites were first expressed as a proportion of the internal standard compound, and then normalized to the unextracted, freeze-dried weight of each tissue sample. The internal standard compound was added to compensate for slight variations that may have occurred in sample extraction and machine operating parameters over time. Therefore, the intensity of metabolite peaks was compared to the intensity of the internal standard compound and will be discussed in terms of signal intensity in the following sections. All peaks representing identified and unidentified metabolites were subjected to Welch's t-tests (α level set at 0.05). Examination of the number of significant t-tests for important treatment and genotype comparisons provided a basis for variable reduction and interpretation. The exact procedure for variable reduction was as follows: 1) the number of significant t-tests for water treatment and clone comparisons was counted for each metabolite; 2) metabolites were considered to show high significance if they displayed a number of significant comparisons that was greater than the product of (number of comparisons) · 0.05 · 4 (four was chosen as an arbitrary factor to increase the level of significance a metabolite had to show over the random chance that a comparison might be found to be significant at an α level set at 0.05); 3) compare the metabolites that exhibited a high number of significant comparisons across all three independent, but associated experiments described in this thesis. Therefore, metabolites that showed significance in comparisons of water treatment and clone across all three experiments were chosen for further analysis using general linear modeling. The general linear model for the three factorial experimental design (with 24 possible factor level combinations) is fixed and is represented by:

$$y_{ijk} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijk},$$

where factor A corresponds to the clones with i levels, factor B corresponds to the water treatment with j levels, factor C corresponds to the harvest time with k levels, ij is the 12 possible interactions between clone and treatment, ik is the 12 possible interactions between clone and harvest time, jk is the four possible

interactions between treatment and harvest time, ijk is the 24 interactions between clone, treatment, and harvest time, and εijk is the random error. The clones, the water treatments, and the harvest times are all fixed factors.

The null hypothesis was that stomatal conductance, net photosynthetic rate, and metabolite intensity of each of the selected metabolites for all clones would be the same regardless of treatment. The α level was set at 0.05 for all tests. Logarithmic transformation of succinic acid and power transformations of galactinol and raffinose were necessary in order to satisfy assumption of normality. Analyses of variance were computed for: stomatal conductance, net photosynthetic rate, and metabolite intensity. Differences between means were tested using Bonferroni adjusted t-tests. All analyses were performed using SAS software package (SAS Institute, Inc., Cary, NC, USA), R (R Development Core Team 2009), and Microsoft Office Excel 2007 (Microsoft, Seattle, WA, USA).

2.4 Figure for Experiment #1

Figure 2.1 Experimental set up of Experiment #1 showing arrangement of plants on flood table and the elevation of drought treatment trees (foreground) on plastic pedestals above the water level.

CHAPTER 3

Results

3.1 Methodology

The technique of metabolic profiling has been utilized in previous studies for a broad array of functional genomic endeavours, including genotype distinction and chemotyping (Robinson et al. 2005, Merchant et al. 2006). The purpose of the experiments described in this text was to better understand and characterize the metabolic effects of drought stress on poplar genotypes. To this end, the technique of metabolite profiling using gas chromatography/mass spectrometry (GC/MS) was employed to separate and identify compounds in well-watered and droughted *Populus* spp. trees. Compounds of interest were further analyzed using analysis of variance. These analyses produced metabolic patterns that indicated this method's validity in the context of previous results reported in the literature, and ability to represent true differences in metabolite content and thus physiological status for the clones studied in this thesis. Two very clear examples of this were the metabolite levels of galactinol and raffinose in Experiments #2 and #3. Each of these metabolites in these independent experiments exhibited clear diurnal patterns with increases observed at midday and under drought stress regardless of genotype (Figure 3.36, Figure 3.37). In addition, patterns of proline accumulation in Experiment #1 were also very clearly indicated (Figure 3.13). These patterns of accumulation due to diurnal cycles and/or drought stress have been well documented in the literature (e.g. Buchi et al. 1998, Nakamichi et al. 2009, Taji et al. 2002) and the clarity of their expression in the three independent experiments outlined in this thesis provided an indication of the reliability and stability of the methods of drought application, sampling/handling, and metabolite analysis protocols.

3.2 Experiment #1 results

Experiment #1 is an analysis of four hybrid poplar clones, Walker, Okanese, WP-86V-86, and Katepwa, which were each subjected to seven and nine days of water withdrawal, followed by five days of recovery (day 14). The experimental design for this experiment is a three-factorial design. The results described below will provide information on the physiological behaviour and the

behaviour of select metabolite species involved in drought stress response, behaviours which were investigated using analysis of variance. In addition, whole metabolite profile visualization was carried out by constructing a heat map based on $\log_2$ (fold changes) for droughted vs well-watered metabolite levels.

3.2.1 Physiological measurements

The analysis of variance for percent soil water content (% SWC) exhibited a treatment x harvest time interaction. As the time during which trees were subjected to drought treatment lengthened, % SWC decreased towards 50% dry mass. Well-watered trees displayed a relatively constant % SWC. Comparisons were made between each time of measurement from 2DAD to 14DAD and between each treatment for 7DAD, 9DAD, and 10DAD to 14DAD. At field capacity, % SWC was measured to be approximately 250%. This value remained relatively constant throughout the experiment for well-watered trees, with one exception: a significant increase at 10DAD to approximately 270% soil water content. Droughted trees showed a continual decrease in % SWC at each day of measurement (with significant differences observed between 2DAD and 4DAD, and between 4DAD and 6DAD), reaching approximately 50% soil water content on 9DAD. Upon re-watering on 9DAD, % SWC increased back to well-watered levels by 14DAD (Figure 3.1).

Average midday leaf water potentials (Ψw(l)) for well-watered trees of all clones were similar (about -0.75 MPa) on all days of measurement, with no significant differences observed between clones or time of harvest (Figure 3.2). However, for trees subject to drought stress, average Ψw(l) decreased by about 100% on 7DAD (to approximately -1.5 MPa) and 300% on 9DAD (to approximately -3 MPa) for all clones except for Okanese, which remained roughly at -0.75 MPa throughout the experiment. After five days of recovery under well-watered conditions (14DAD), average Ψw(l) for clones displaying significant decreases had returned to pre-drought levels. Under droughted conditions, all clones (except Okanese) showed significantly different average Ψw(l) when comparing 7DAD and 9DAD, and 9DAD and 14DAD; WP-86V-86 and Katepwa also showed significant differences between 7DAD and 14DAD (Table 3.1).

Okanese did not exhibit significant differences in average Ψw(l) between any of the three harvest times, nor between the two water treatments (Table 3.2).

As expected, the average growth rates for the time frames immediately prior to each harvest were reduced for all clones at all harvest times under drought conditions (Figure 3.3). Under well-watered conditions, the growth rates were variable between clones, with Okanese and Katepwa displaying the slower rates of growth and Walker displaying the fastest rate of growth (Walker exhibited an average growth rate that is approximately 1.5 times faster than both Okanese and Katepwa). Under droughted conditions, however, no significant differences were observed for any of the clones or between 7DAD and 9DAD. Growth rates showed a trend towards recovery at 14DAD when they increased over those at 9DAD more than three fold. Between well-watered and water stressed conditions, all comparisons of average growth rates were significantly decreased.

3.2.2 Metabolite profile

The metabolites chosen for further study in this experiment were: succinic acid, glycolic acid, malic acid, threonic acid, quinic acid, galactinol, raffinose, and fructose. These metabolites were selected from the metabolite profile for further analysis using analysis of variance because they were found to be highly significantly different across treatment and clone comparisons for each of the three experiments described in this thesis. Additionally, proline, phenylalanine, tryptophan, catechin, kaempferol, and dehydroascorbic acid were chosen for further analysis based on their observed fold changes from a profile-wide heat map (Figure 3.4). Many of these metabolites represent compounds thought to play protective or adjustment roles in plants under stress.

Succinic acid, glycolic acid, and galactinol each displayed three-way interactions between clone, treatment, and harvest time. Succinic acid and glycolic acid each showed decreasing trends in relative abundance, while galactinol showed an increasing trend under drought conditions (Figure 3.5, Figure 3.6, Figure 3.10).

Under well-watered and drought conditions, no significant differences were observed for succinic acid within the same clone at different harvest times.

However, significant differences were observed between different clones at the same harvest times under well-watered conditions, with Walker showing at least 50% higher levels than levels of the other three clones at 7DAD and 9DAD (Figure 3.5). Under drought conditions, only one significant difference was observed between Walker and Katepwa at 7DAD (Table 3.3). Significant differences between water treatments were observed for Walker at 9DAD and 14DAD (a reduction of approximately 50% that of well-watered levels in each case), and for WP-86V-86 at 14DAD (about 45% that of well-watered levels) (Table 3.4).

Glycolic acid also did not exhibit any significant differences between harvest times within the same clone under either of the water treatments (Table 3.5). Significant differences were observed between Walker and Katepwa at 7DAD (Walker having about 160% higher levels than Katepwa), and Walker and Okanese at 14DAD (Walker having about 135% higher levels than Okanese) under well-watered conditions (Figure 3.6). Between water treatments, Walker showed a significant difference at 7DAD (reduction to 40% that of well-watered conditions), and Okanese showed significant differences at 7DAD and 9DAD (reductions to about 30% that of well-watered conditions in each case) (Table 3.6).

Galactinol showed an increasing trend under drought conditions (Figure 3.10). No significant differences were observed for this metabolite under well-watered conditions for any relevant comparisons (Table 3.8). Under drought conditions, however, significant differences were apparent within clones at different harvest times and between clones at the same harvest time. For Walker, significant differences occured between 7DAD and 9DAD, with galactinol levels six times greater at 7DAD than 9DAD. Significant differences between clones on 7DAD were observed for Walker and Okanese, and Walker and Katepwa (Walker showing approximately 275% greater levels than Okanese and Katepwa in each case); on 9DAD between Walker and Katepwa, and WP-86V-86 and Katepwa (Katepwa showing approximately 230% and 50% greater levels than Walker and WP-86V-86, respectively). Significant differences between water treatments were

observed on 7DAD for Walker (a 280% increase under drought conditions) and WP-86V-86 (a 180% increase under drought conditions), and on 9DAD for Okanese (a 190% increase under drought conditions) and Katepwa (a 380% increase under drought conditions) (Table 3.9, Figure 3.10).

Malic acid exhibited a clone x harvest time interaction. Walker was the only clone that displayed significant within clone differences, with 60% higher levels of malic acid at 7DAD as compared to 9DAD. Between clone differences were significant on 7DAD for Walker and Katepwa (Walker having about 120% greater abundance than Katepwa), Okanese and WP-86V-86 and Katepwa (Okanese having about 60% and 150% greater concentrations than WP-86V-86 and Katepwa respectively), and WP-86V-86 and Katepwa (about 50% greater levels for WP-86V-86) (Figure 3.7). Okanese displayed at least twice the level of malic acid as all other clones at 9DAD, and at 14DAD, Katepwa showed at least 35% lower response as compared to all other clones.

In addition to the clone x harvest time interaction, malic acid also exhibited a water treatment main effect. Malic acid was significantly decreased under drought stress conditions regardless of clone, reduced to approximately 75% when compared to well-watered conditions (Figure 3.7).

Fructose exhibited two two-way interactions: clone x treatment and harvest time x treatment (Figure 3.12). No relevant comparisons were found to be significant in the harvest time x treatment interaction and therefore will not be further explored in this thesis. The clone x treatment interaction revealed that only Walker exhibited a significant decrease under drought stress (to 65% that of well-watered conditions) for fructose for all between treatment comparisons. All other clones exhibited non-significant increases in fructose signal intensity. Between clone comparisons showed that under well-watered conditions, Walker had significantly higher levels of fructose than Okanese and Katepwa (90% and 65% higher levels, respectively); under droughted conditions, WP-86V-86 had significantly higher levels of fructose than Walker and Okanese (55% and 45% higher levels, respectively).

Both raffinose and threonic acid displayed treatment and harvest time main effects. Raffinose exhibited a highly significant increase of about 130% under droughted conditions (Figure 3.11). Threonic acid displayed a significant decrease under drought conditions to about 65% that of well-watered conditions (Figure 3.8). Both metabolites displayed similar patterns for the harvest time main effect, remaining relatively constant at 7DAD and 9DAD, and showing a significant decrease of 30% at 14DAD.

Quinic acid exhibited two main effects, that of clone and harvest time. The harvest time main effect was similar to that of raffinose and threonic acid, remaining relatively constant at 7DAD and 9DAD, and displaying a significant decrease (approximately 23%) at 14DAD (Figure 3.9). The clone main effect showed that Walker and WP-86V-86 each contained significantly higher levels (at least 40% higher) than Okanese and Katepwa.

Another organic acid and derivative of ascorbic acid, dehydroascorbic acid, displayed slight decreasing trends under drought conditions, though these decreases were not found to be statistically significant for any of the clones (Figure 3.18). The individual clones, however, displayed interesting differences in the overall levels of dehydroascorbic acid, with Okanese displaying the highest level of dehydroascorbic acid, more than twice that of any other clone, regardless of water treatment.

Three of the amino acids that displayed interesting patterns in the heat map and were further analyzed using analysis of variance displayed significant increases in abundance under drought conditions (Figure 3.4). Proline and phenylalanine exhibited remarkably similar changes under drought stress as shown in the three-way interaction of clone x treatment x harvest time (Figure 3.13, Figure 3.14). All clones except for Okanese displayed increasing trends for both of these metabolites over the duration of the drought stress treatment. In contrast, Okanese displayed decreasing trends for both proline and phenylalanine over time, though the concentrations for these metabolites were still higher (about 1000 times higher for proline and ten times higher for phenylalanine at 7DAD) under drought conditions. Tryptophan also increased

over the duration of the drought treatment, in addition to exhibiting differences between clones (Figure 3.15). WP-86V-86 displayed the highest levels of tryptophan under drought conditions, more than twice as high as any of the other clones regardless of treatment.

Another trend illustrated by the heat map comparison was the apparent increase of antioxidant phenolic compounds, catechin and kaempferol. Each of these metabolites showed clone main effects as well as treatment x harvest time interactions (Figure 3.16, Figure 3.17). Overall, Okanese showed the highest catechin and lowest kaempferol levels in comparison to all other clones. Katpewa showed the highest kaempferol levels, more than twice the level of all other clones regardless of treatment. Over the duration of the drought treatment, an increasing trend was observed for both catechin and kaempferol. Although kaempferol did not exhibit any statistically significant increases, catechin showed a statistically significant increase at 9DAD, to about four times the level of well-watered conditions. At 14DAD, catechin exhibited a slight decrease for droughted trees, but was still significantly higher than trees under well-watered conditions.

3.3 Experiment #2 results

Experiment #2 is an analysis of two hybrid poplar clones, Walker and Okanese, which were each sourced from Alberta (AB) and Saskatchewan (SK), subjected to 13 days of water withdrawal (11 days for Walker sourced from SK), and harvested at two timepoints, predawn and midday. The experimental design for this experiment, therefore, is a complex four-factorial design. The results described below will provide information on the physiological behaviour and the response of key metabolites involved in drought stress.

3.3.1 Physiological measurements

Overall, Walker exhibited approximately 35% lower stomatal conductance than did Okanese. Since stomatal conductance is a derivative of transpiration taking into consideration the leaf-to-air vapour pressure gradient, this pattern was also reflected in transpiration rates. Application of drought conditions resulted in approximately 40% reduction in stomatal conductance for trees sourced from

Alberta, and an approximately 60% reduction for trees sourced from Saskatchewan. Again, transpiration rate mirrored this pattern (Figure 3.19, Figure 3.20).

3.3.2 Metabolite profile

The metabolites chosen for further study in this experiment were: succinic acid, glycolic acid, malic acid, threonic acid, quinic acid, galactinol, raffinose, and fructose. These metabolites were selected from the metabolite profile for further investigation using analysis of variance because a high number of comparisons between water treatment and clones were found to be significantly different (using simple t-tests) and the number of significant tests was found to be high for each of the three experiments described in this thesis. Furthermore, many of these metabolites also represent compounds thought to play protective or adjustment roles in plants under stress.

Two and three-way interactions involving these metabolites are shown and discussed if they involve water treatment as a significant factor.

Overall, the abundance of succinic acid declined under drought treatment. This pattern of decline was statistically significant for Okanese sourced from Alberta (a decline of approximately 25%), and Walker sourced from Saskatchewan (a decline of approximately 20%) (Figure 3.21). Malic acid showed a reduction under drought conditions, though these declines were only significant for Okanese at both predawn (decline of about 25%) and midday (decline of about 30%) harvest times (Figure 3.23).

Glycolic and threonic acids both showed decreasing trends in abundance under drought stress. Analysis of variance for each of these metabolites showed a four-way interaction, meaning that for each clone harvested at different times of the day, and sourced from different locations, the metabolites showed significant and unique variations in relative abundance. Glycolic acid showed significant decreases for Okanese sourced from Alberta at both predawn (approximately 68% of well-watered conditions) and midday (approximately 77% of well-watered conditions) harvest times (Figure 3.22). Walker sourced from Alberta did not show decreases for glycolic acid at either of the harvest times. No significant

differences were observed for glycolic acid levels at the predawn harvest for trees sourced from Saskatchewan, though Okanese again showed a significant reduction in glycolic acid levels (to approximately 62% of well-watered conditions) when harvested at midday (Figure 3.22).

Threonic acid displayed similar patterns to the patterns exhibited by glycolic acid (Figure 3.24). Okanese sourced from Alberta showed significant declines in threonic acid levels at both harvest times (declines to about 67% and 78% that of well-watered conditions for predawn and midday harvest times, respectively), while Walker sourced from both Alberta and Saskatchewan was not significantly altered in levels of threonic acid. Okanese sourced from Saskatchewan showed significant declines (to approximately 67% that of well-watered conditions) at the midday harvest time, but not at predawn.

Quinic acid also displayed decreasing trends under drought stress (Figure 3.25). The only significant decrease, however, was for droughted trees sourced from Alberta and harvested at predawn, with decreases to approximately 75% that of well-watered trees from the same harvest time and source, regardless of clone.

Galactinol showed increasing trends under drought conditions, regardless of clone. This increasing trend was found to be statistically significant for Walker sourced from Saskatchewan, which showed an increase in galactinol levels of more than 200% (Figure 3.26). Raffinose also displayed an increasing trend for both clones, with Walker showing a significant increase to about 175% the level of well-watered trees. Overall, raffinose levels were significantly increased at midday over predawn levels (by about 130%), and were significantly increased under drought conditions at midday over midday well-watered samples (by about 80%) (Figure 3.27).

Fructose showed increasing trends, regardless of clone, at both harvest times from trees sourced from Alberta, though these trends were not found to be statistically significant. The predawn harvest time for trees sourced from Saskatchewan showed a significant increase of about 60%; there were no

significant differences in fructose levels between well-watered and droughted trees from midday harvests sourced from Saskatchewan (Figure 3.28).

3.4 Experiment #3 results

Experiment #3 is an analysis of six pure *Populus balsamifera* clones, AP947, AP1006, AP1005, AP2278, AP2298, and AP2300, which were each subjected to 15 days of water withdrawal, and harvested at predawn and midday. The experimental design for this experiment is a three-factorial design. The results described below will provide information on the physiological behaviour and the response of key metabolite species involved in drought stress, behaviours which were investigated using analysis of variance.

3.4.1 Physiological measurements

Stomatal conductance significantly decreased for all clones under drought conditions. The clone that experienced the greatest decrease in stomatal conductance was AP1006 (down by 94%) and the clone that experienced the least decline in stomatal conductance was AP2298 (down by 50%) (Figure 3.29).

Net photosynthetic rate also showed general decreases, though the observed changes were not as significant as stomatal conductance (Figure 3.30). All clones tended to decrease photosynthetic rate under drought conditions; only AP1006 exhibited a significantly decreased rate in comparison to well-watered conditions (Table 3.16).

3.4.2 Metabolite profile

The metabolites chosen for further study in this experiment were: succinic acid, glycolic acid, malic acid, threonic acid, quinic acid, galactinol, raffinose, and fructose. These metabolites were selected from the metabolite profile for further analysis using analysis of variance because they were found to be highly significantly different across treatment and clone comparisons for each of the three experiments described in this thesis. Many of these metabolites also represent compounds thought to play protective or adjustment roles in plants under stress.

Succinic acid exhibited a clone x treatment x harvest time interaction and showed overall declines in abundance for clones under drought stress at both predawn and midday harvest times (Figure 3.31). Absolute decreases were greatest for trees harvested at the predawn time point, with the exception of AP2300 which did not show a significant decline in succinic acid at predawn (Table 3.17). The greatest absolute declines at both predawn and midday harvest times were observed for AP947 (with declines of 50% and 65% for predawn and midday harvest times, respectively) and AP2298 (with declines of 59% and 65% for predawn and midday harvest times, respectively).

Glycolic acid also exhibited a clone x treatment x harvest time interaction with a decreasing trend observed for trees under drought conditions at both harvest times (Figure 3.32). Three exceptions to this trend were found with AP947 and AP2300 at predawn, and AP1006 at midday, all of which showed an increasing trend under drought stress, though these increases were not statistically significant (Table 3.19). The greatest decrease in glycolic acid under drought stress was observed for AP1005 at predawn, displaying a dramatic decrease to 7% that of well-watered conditions. AP1005 also exhibited significant decreases at midday, down to 18% that of well-watered conditions. Overall, a greater number of the clones experienced significant declines of glycolic acid at the midday harvest time than for the predawn harvest time, for which only AP1005 showed a significant reduction. At the midday harvest time, all clones showed significant reductions except for AP1006 and AP2298.

Interestingly, threonic acid mirrored remarkably well the patterns observed for glycolic acid (Figure 3.34). However, statistical analysis of this metabolite's abundance revealed less significance for between treatment comparisons (Table 3.23). AP2278 is the only clone that exhibited significant reductions in threonic acid at both predawn and midday (to 41% and 27% that of well-watered trees at predawn and midday, respectively). As for glycolic and succinic acids, AP2300 displayed an increasing trend for threonic acid at the predawn harvest time.

Malic acid was another organic acid that displayed decreasing trends under drought stress. The greatest decrease was observed for AP2278, with a

decline of 50% under drought conditions (Figure 3.33). All other differences between treatments were not statistically significant (Table 3.21). Overall, clones AP947 and AP2298 displayed the highest levels of malic acid under well-watered conditions. Upon drought exposure, however, between clone distinctions arose for AP1005 and AP2278 and the other four clones, with the former displaying the greatest decreasing trend for malic acid abundance.

Quinic acid displayed a clone main effect and a treatment x harvest time effect. The treatment x harvest time effect indicated that levels of quinic acid decreased under drought stress, and that this effect was greater at midday than at predawn (Figure 3.35). The clone main effect indicated that AP2300 had the greatest overall abundance in comparison to all other clones, and this was statistically significant in comparison to AP947 and AP1005.

Raffinose and galactinol each showed similar increasing trends under drought stress and at midday harvest times (Figure 3.36, Figure 3.37). Overall, under drought stress conditions, galactinol levels increased to about 164% that of well-watered conditions, whereas raffinose increased to about 275% that of well-watered conditions. All clones exhibited significant between harvest time comparisons except AP947 for raffinose. Overall, clones did not tend to differ in either galactinol or raffinose levels within the same harvest time.

Fructose displayed two interactions, an interaction for clone x treatment, and another interaction for treatment x harvest time (Figure 3.38). Overall, this metabolite showed decreasing trends under drought conditions, down by about 24% from well-watered conditions at midday. However, only AP1005 and AP2278 showed significant decreases in fructose levels under drought conditions, each showing decreases of about 40% that of well-watered conditions (Table 3.24).

3.5 Figures

3.5.1 Experiment #1 figures

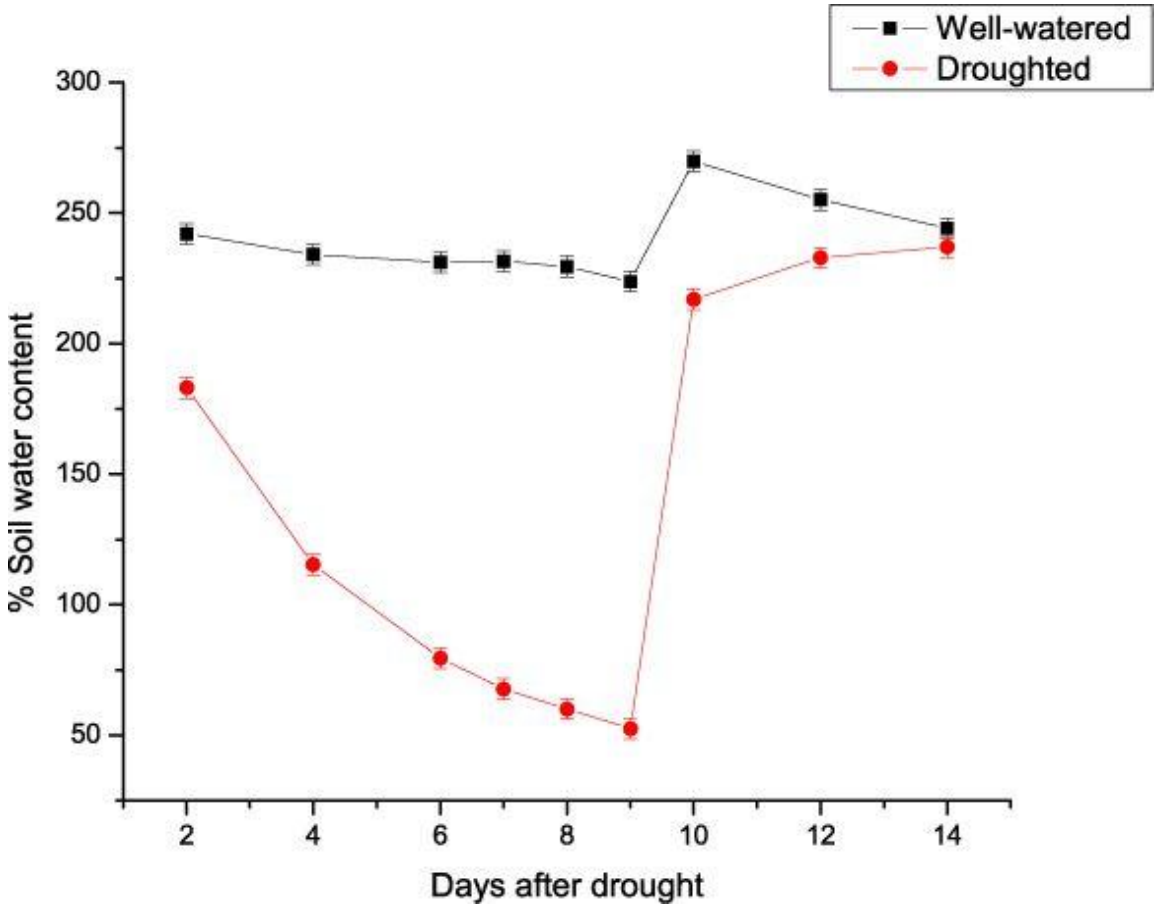

Figure 3.1 Percent soil water content (% SWC) showing a two-way interaction between harvest time and treatment for well-watered and droughted trees. Error bars represent standard errors of the mean, n = 4.

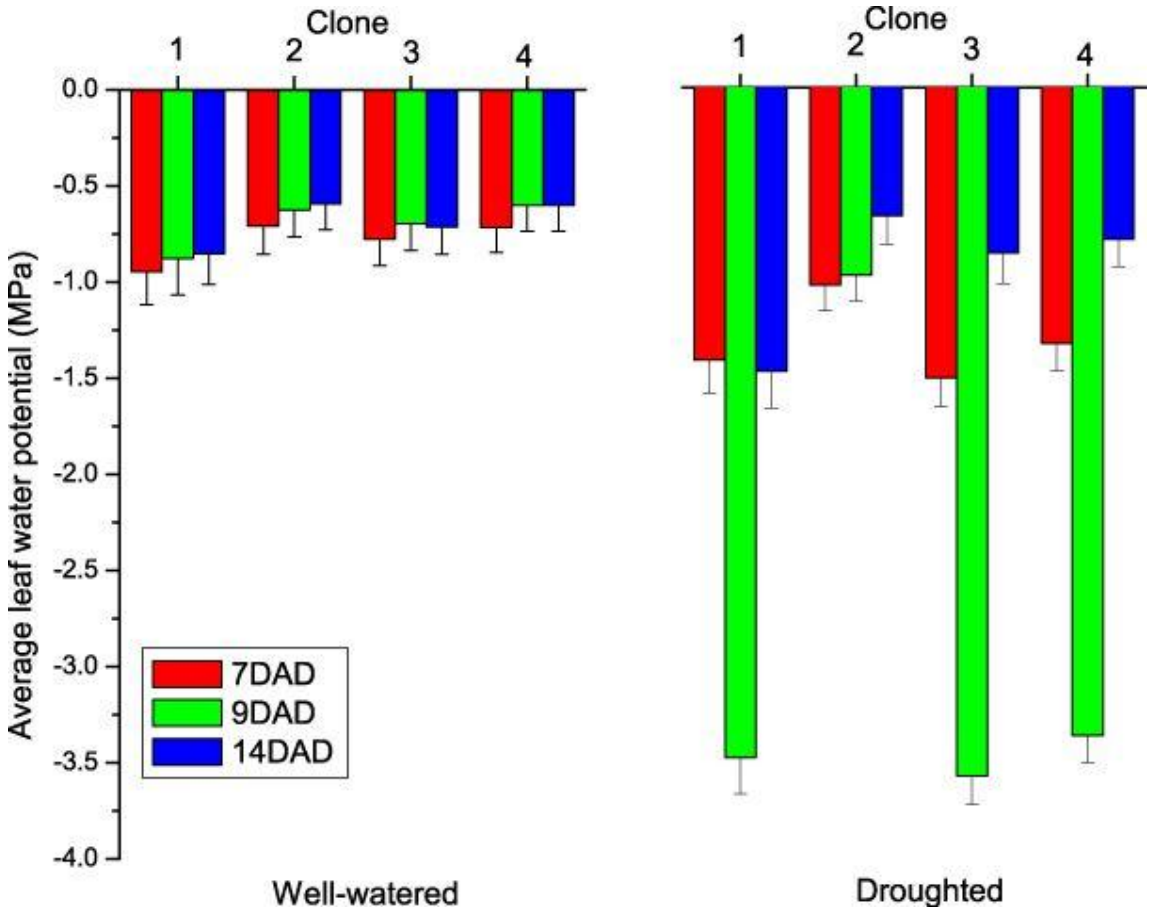

Figure 3.2 Leaf water potential (MPa) showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars represent standard errors of the mean, n ≥ 5. Three trees perished as a result of drought treatment, these samples were completely removed from any further analysis. Significant comparisons are shown in Table 3.1 and Table 3.2.

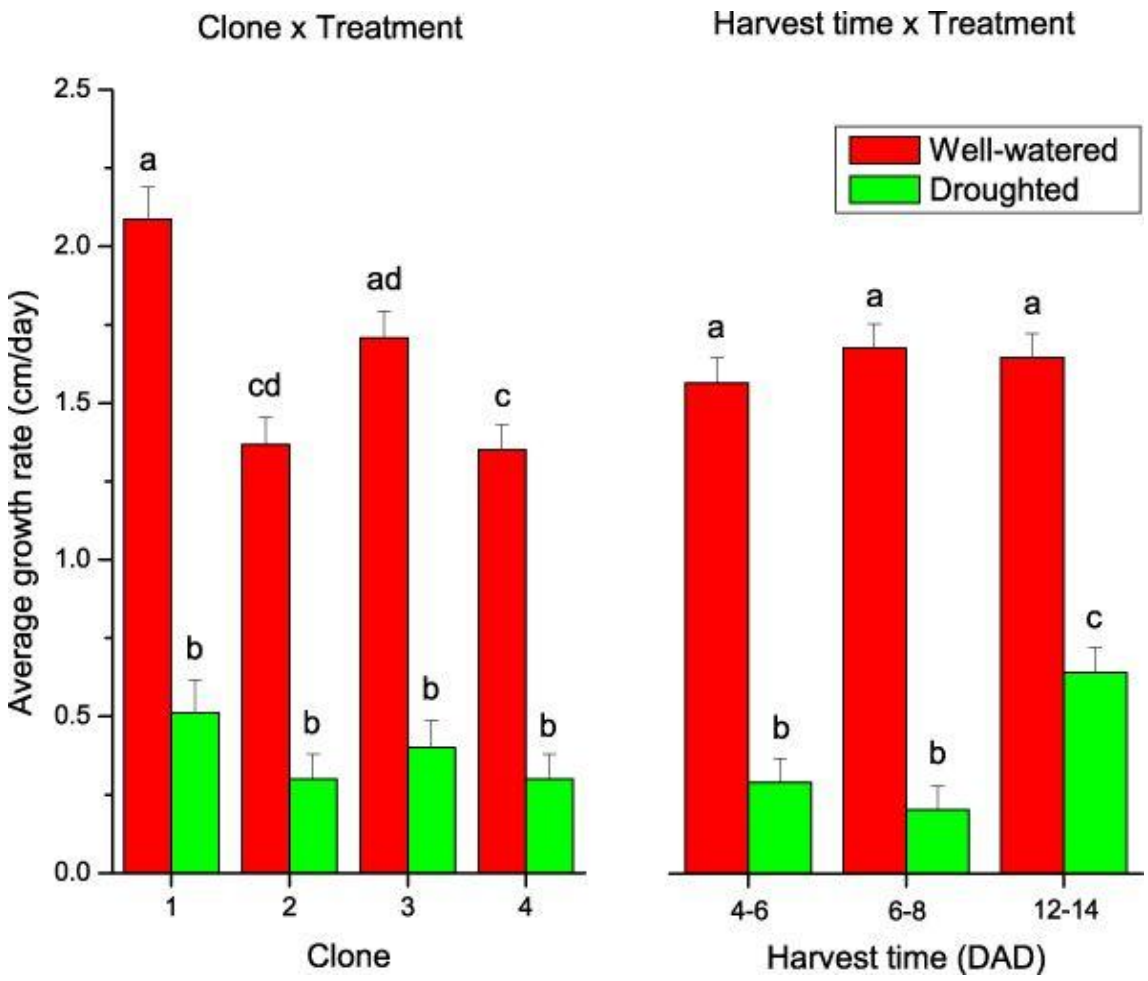

Figure 3.3 Growth rate (cm/day) showing two two-way interactions between clone and treatment and between harvest time and treatment. The clone x treatment interaction shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees under well-watered and drought conditions. The harvest time x treatment interaction shows growth rates measured over the time range immediately prior to the harvest times of seven days after drought (7DAD), 9DAD, and 14DAD. Error bars represent standard errors of the mean, $n \geq 5$. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0031$ (clone x treatment) or $p \leq 0.0056$ (harvest time x treatment).

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
L-Valine	4.5	5.2	2.2	6.7	3.6	2.4	8.4	7.0	1.2	8.5	8.4	2.8	AA
L-Leucine	4.9	5.2	0.0	5.0	2.9	0.2	7.6	7.2	1.5	9.5	9.5	1.7	AA
L-Alanine	1.0	-0.2	-2.1	-0.3	-0.4	0.6	1.5	1.8	-2.3	2.9	1.1	-0.8	AA
L-Glutamate	0.1	-0.2	-0.7	0.5	-0.4	0.7	0.8	1.2	-0.2	0.8	2.1	0.0	AA
L-Glutamine	0.6	2.9	-4.7	4.0	2.5	2.2	4.0	4.3	4.5	8.9	5.7	0.6	AA
L-Proline (1)	7.3	9.1	-0.8	9.9	4.5	-5.0	11.8	13.1	10.2	11.1	12.0	2.8	AA
L-Proline (2)	3.1	4.5	0.4	3.6	2.7	1.4	5.1	5.7	2.6	4.3	5.5	1.6	AA
L-Isoleucine	5.2	6.5	4.1	5.6	4.2	2.6	7.7	8.1	1.8	8.0	8.5	3.0	AA
L-Threonine	3.1	4.7	4.1	4.0	2.5	1.5	5.1	5.9	0.9	5.7	6.0	1.4	AA
L-Phenylalanine	1.6	3.2	-2.5	3.4	1.9	0.3	4.9	5.5	1.4	5.1	5.4	0.8	AA
L-Tryptophan	8.7	8.5	6.5	10.2	8.1	7.1	9.0	9.0	6.2	7.7	10.3	6.8	AA
Glycine	1.4	0.6	-1.1	2.3	1.5	2.3	1.5	2.0	0.1	0.5	3.0	0.9	AA
L-Serine	2.4	7.0	4.0	2.4	1.0	0.6	2.4	3.4	3.0	3.7	4.6	3.4	AA
Pyroglutamic acid	0.3	0.7	0.4	1.1	0.7	2.0	2.2	2.7	-0.3	1.9	2.9	1.7	AA
M116T624_NI_Amino_acid	0.1	-0.3	0.7	-0.7	0.1	1.7	0.2	0.0	-0.7	-1.3	-0.3	1.1	AA
M186T636_NI_Amino_acid	0.1	-0.2	0.1	-0.6	-0.3	0.2	-0.1	-0.6	-1.1	0.1	-0.8	0.0	AA
M142T657_NI_Amino_acid	-2.9	-3.9	-4.1	0.9	-1.9	-0.2	0.5	-0.6	-0.5	0.6	-0.7	-1.9	AA
M172T842_NI_Amino_acid	-0.1	-1.0	1.0	-0.4	-0.2	-0.2	0.4	-0.5	0.2	-0.6	0.5	-0.2	AA
Phosphoric acid	-0.1	1.5	-0.1	-1.0	-0.1	0.5	-0.7	0.7	0.6	-0.8	0.0	0.2	IA
Citric acid	-0.8	0.0	0.8	0.9	1.1	-0.7	-1.0	-1.2	0.9	-0.6	-1.8	0.5	OA
2-Ketoglutaric acid	-0.1	-0.8	-1.1	-0.4	0.1	0.5	-1.1	-1.6	0.0	-1.0	-1.4	-0.5	OA
Succinic acid	-0.6	-1.0	-1.1	-0.6	-0.3	0.4	-0.4	-0.4	-0.7	-0.4	-0.3	0.1	OA
Fumaric acid	-3.7	-2.5	-0.3	-2.0	-2.3	1.2	-2.4	-0.9	-2.2	-1.2	-0.6	0.3	OA
Malic acid	-0.5	0.1	-0.8	-0.6	-0.1	0.0	-0.1	-0.1	-0.2	-0.3	-0.3	-0.2	OA
Glycolic acid	-1.3	-1.1	-1.3	-1.8	-1.6	0.1	-0.6	-1.0	-1.0	-0.7	-0.3	-0.7	OA
Dehydroascorbic acid	1.9	1.2	2.5	-0.3	0.1	-0.2	-2.3	-3.0	1.4	-0.4	-0.7	1.5	OA

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
Threonic acid	-0.7	-0.9	-1.6	-1.0	-0.8	-0.1	-0.2	-0.1	-0.8	-0.4	-0.2	-0.8	OA
Tartaric acid	0.0	0.0	-0.5	-0.7	-0.3	0.6	-0.4	0.3	-0.4	-0.1	0.4	-0.1	OA
2,3-Dihydroxybenzoic acid	0.4	-1.7	-0.7	0.8	-0.5	0.2	0.2	-0.2	-0.3	-0.1	0.1	-0.6	OA
Ribonic acid	-0.3	0.0	-0.4	-0.5	-0.4	0.7	0.0	0.3	-0.2	0.0	0.2	0.3	OA
Shikimic acid	0.2	-0.9	-0.4	-0.8	-1.0	0.4	-0.7	0.3	-0.2	-0.8	-0.3	-0.3	OA
Quinic acid	0.2	0.1	-0.3	-0.7	-0.3	0.3	-0.2	0.3	0.0	-0.2	-0.1	-0.3	OA
Caffeic acid	-0.1	-0.7	-0.6	0.6	-0.8	0.5	0.2	0.3	-0.1	-0.4	0.0	-0.5	OA
3-Caffeoylquinic acid	0.1	2.7	1.3	2.4	3.4	-1.2	-0.1	0.8	2.2	-0.1	0.2	1.1	OA
M143T872_NI_Organic_acid	-0.2	-0.3	-0.4	-1.6	-1.1	-0.1	0.2	0.5	-0.7	-0.9	0.3	0.5	OA
M292T912_NI_Organic_acid	0.2	0.3	-0.2	-0.2	0.3	0.5	0.4	0.5	0.0	0.2	0.6	0.3	OA
M267T921_NI_Organic_acid	0.3	-1.2	-0.6	0.7	-0.3	0.5	-0.1	0.2	-0.4	-0.2	0.1	-0.5	OA
M299T1098_NI_Organic_acid	-0.9	0.9	-0.2	-0.5	0.3	0.8	-0.1	1.2	0.8	-0.1	0.1	-0.5	OA
M355T1102_NI_Organic_acid	0.4	-2.1	-0.9	0.9	-0.2	0.2	0.7	-0.2	0.0	-0.3	0.1	-0.6	OA
M249T1110_NI_Organic_acid	0.7	-1.7	-1.9	0.7	-0.2	0.3	0.6	-0.1	0.4	-0.3	0.0	-1.0	OA
Rhamnose (1)	0.1	0.2	0.3	-0.1	0.3	-0.4	0.1	0.0	-0.2	0.2	-0.1	0.2	C
Rhamnose (2)	0.4	0.2	0.3	-0.1	0.3	-0.4	0.1	0.0	-0.1	0.2	-0.1	0.2	C
Fructose (1)	0.0	-1.0	-1.0	0.6	0.2	0.1	0.7	0.4	-0.2	0.4	0.5	-0.3	C
Fructose (2)	0.0	-1.0	-1.0	0.7	0.2	0.1	0.7	0.4	-0.2	0.4	0.5	-0.3	C
Glucose (1)	0.2	-0.7	-0.4	0.3	0.1	0.1	0.5	0.3	-0.4	0.2	0.2	-0.1	C
Glucose (2)	0.3	-0.7	-0.6	0.5	0.1	0.3	0.6	0.4	-0.3	0.2	0.4	-0.1	C
Glucose (3)	0.2	-0.7	-0.9	0.6	-0.1	0.4	0.6	0.4	-0.1	0.2	0.6	-0.1	C
Sucrose	-0.4	0.7	1.4	-0.6	0.2	-0.2	-0.3	-0.5	-0.2	-0.5	-1.4	0.2	C
Digalactosyl glycerol	-0.3	-1.8	-2.7	1.0	-0.2	0.0	-0.3	-0.9	0.5	0.3	-0.4	-0.8	C
Raffinose	0.8	1.6	1.6	1.2	1.6	1.7	2.0	2.0	1.1	1.3	1.6	1.0	C
M218T1072_NI_Carbohydrate	1.4	0.9	1.1	0.4	0.6	0.4	0.0	0.5	0.1	0.3	0.3	0.9	C
M217T1249_NI_Carbohydrate	0.2	-0.6	-1.1	0.9	0.2	0.3	0.7	0.1	0.3	0.1	0.7	-0.3	C
M73T1267_NI_Carbohydrate	0.2	-4.5	-5.1	1.3	0.7	2.9	0.5	-0.3	-2.2	0.1	0.5	-0.6	C

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M129T1364_NI_Carbohydrate	0.5	1.0	0.1	0.3	0.7	0.4	0.5	0.7	0.8	0.4	0.7	0.6	C
M217T1519_NI_Carbohydrate	-0.8	-1.7	-0.8	0.2	-0.4	0.5	-0.4	0.0	0.1	-0.7	0.0	-0.3	C
M204T1672_NI_Carbohydrate	0.2	-0.3	-0.2	0.4	0.3	0.4	-0.2	0.0	-0.2	-0.3	0.0	0.2	C
M361T1744_NI_Carbohydrate	0.5	-0.3	-0.8	1.6	-0.2	0.5	0.4	0.1	0.2	0.1	0.1	0.6	C
M169T1816_NI_Carbohydrate	0.3	0.6	0.6	0.3	0.5	0.6	-0.2	0.7	0.3	-0.1	0.7	0.7	C
myo-Inositol	0.3	0.0	0.6	0.2	0.4	-0.1	0.3	-0.1	0.0	0.2	0.0	0.2	SA
Galactinol	1.4	1.3	1.2	1.5	1.5	0.8	1.5	1.2	0.6	2.1	2.3	1.2	SA
Catechol	0.1	-0.4	0.0	0.1	0.1	0.1	-0.3	-0.4	-0.4	0.0	0.0	-0.3	P
Salicyl alcohol	0.1	-1.3	-0.4	0.6	-0.7	0.2	0.0	-0.1	-0.1	-0.1	-0.3	-1.2	P
Salicin	0.3	1.4	2.6	-0.5	0.4	-0.2	-0.3	0.5	-0.1	0.0	0.0	0.3	P
Catechin	1.6	3.3	3.1	-0.3	2.1	0.7	-0.8	2.0	3.2	0.5	1.3	1.9	P
Kaempferol	-0.9	1.0	0.8	-0.8	1.0	1.8	-0.7	1.5	0.7	-0.8	0.3	1.2	P
Populin	0.3	0.3	0.1	1.0	0.5	0.0	-1.8	-0.7	-0.4	0.1	0.4	0.0	P
Adenosine	-1.4	-1.2	-1.2	-0.4	-1.0	0.0	-0.7	-0.3	-1.1	-0.7	-0.7	-0.3	N
M84T891_NI	-0.6	-0.8	-0.2	-1.9	1.3	0.5	-0.2	-0.6	0.0	0.0	0.1	1.2	NI
M155T904_NI	0.5	-1.7	-1.3	2.0	-0.1	1.2	-0.8	-0.5	-0.8	0.1	0.1	-0.8	NI
M239T957_NI	0.2	0.4	0.9	1.0	0.2	0.2	0.7	0.1	0.3	0.0	-0.2	0.1	NI
M591T994_NI	0.0	1.0	0.4	0.0	0.1	0.1	0.2	-0.1	-0.3	-0.1	-0.4	0.0	NI
M179T1022_NI	-0.1	-0.8	-0.1	0.4	-0.2	0.4	0.1	0.3	-0.4	-0.6	-1.2	-2.1	NI
M180T1035_NI	-0.3	-1.6	-1.4	-0.2	-1.4	0.4	-0.7	-0.6	-0.4	-0.8	-0.1	-3.1	NI
M436T1117_NI	-0.4	1.2	0.8	0.2	0.6	-0.1	0.4	0.5	1.1	0.1	0.0	0.5	NI
M282T1120_NI	1.1	4.3	2.6	4.0	3.4	2.9	3.9	5.1	2.6	4.3	5.2	3.8	NI
M244T1236_NI	3.1	4.3	4.0	-1.0	0.4	-0.4	-0.1	-1.4	2.0	0.5	-0.2	2.6	NI
M374T1242_NI	1.3	2.5	2.1	-1.2	-0.1	0.1	-0.4	-0.2	3.1	0.0	-0.3	3.3	NI
M447T1278_NI	-0.4	-0.6	1.2	0.0	-0.5	0.8	0.1	-1.0	-0.7	-0.8	-0.6	0.4	NI
M182T1285_NI	-1.1	0.4	0.4	0.0	0.5	-0.1	-0.3	-1.8	-0.7	-1.5	-1.8	-0.6	NI
M108T1288_NI	-0.2	-0.3	-0.3	0.6	-0.5	0.2	-0.3	1.0	-0.2	0.0	-0.3	-0.4	NI

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M80T1314_NI	-0.2	0.3	0.0	0.6	-0.6	0.3	-0.5	0.9	-0.3	-0.1	-0.5	-0.5	NI
M179T1386_NI	0.7	-0.2	-0.5	0.9	0.1	1.0	-0.1	1.4	0.4	-0.3	0.3	0.2	NI
M444T1401_NI	1.1	-1.0	-0.7	2.1	3.1	2.1	3.6	4.8	1.8	1.1	3.0	0.9	NI
M204T1410_NI	0.9	1.0	-0.2	0.4	0.6	-0.4	-0.1	0.0	0.4	-0.1	0.1	-0.3	NI
M357T1414_NI	0.5	1.9	2.3	-1.1	1.8	0.4	1.3	2.2	1.2	1.3	1.1	0.9	NI
M457T1450_NI	1.0	2.0	1.0	4.0	4.5	2.1	5.2	5.2	3.1	4.4	5.9	1.7	NI
M373T1461_NI	1.4	1.3	0.6	2.9	4.1	1.7	4.3	4.4	2.3	3.5	4.6	1.3	NI
M471T1463_NI	0.8	2.4	-1.9	3.1	4.0	-1.4	4.0	4.8	1.5	4.0	5.1	1.0	NI
M424T1467_NI	-0.7	0.2	-0.2	1.2	2.2	0.9	1.2	1.5	1.1	-0.3	0.5	0.1	NI
M290T1475_NI	0.1	0.9	0.5	0.1	0.1	1.2	-0.5	1.3	0.6	-0.8	0.7	0.7	NI
M471T1484_NI	2.3	2.2	1.8	3.6	4.9	1.9	4.7	4.6	2.9	3.9	5.9	2.0	NI
M204T1494_NI	-1.1	-2.6	-2.8	1.1	-0.6	0.3	0.1	-1.0	0.2	-0.1	-0.4	-1.0	NI
M98T1498_NI	3.0	2.1	2.1	4.1	5.1	3.5	5.5	5.9	3.8	4.4	5.8	1.8	NI
M180T1509_NI	1.5	0.5	2.5	0.6	1.1	-0.4	-1.4	-0.1	0.4	0.5	0.5	1.3	NI
M204T1529_NI	0.5	0.4	0.3	1.0	0.9	-0.2	-0.1	0.4	0.8	-0.1	0.3	0.9	NI
M531T1536_NI	-0.2	0.6	1.9	2.0	4.2	2.1	2.7	3.9	2.3	3.5	4.1	0.4	NI
M204T1541_NI	0.5	0.7	0.2	0.9	0.9	-0.2	-0.3	0.5	1.0	0.0	0.4	0.9	NI
M179T1545_NI	-0.1	-1.1	-1.0	0.7	-0.3	0.5	0.0	-0.1	-0.4	-0.3	-0.2	0.0	NI
M79T1550_NI	0.3	0.5	0.1	0.9	1.0	-0.3	-0.2	0.6	0.9	0.1	0.2	0.8	NI
M320T1558_NI	0.4	-3.7	0.8	-0.6	-0.2	0.3	0.6	0.2	-0.1	-1.3	-1.6	-0.1	NI
M204T1574_NI	-0.1	-0.2	-0.2	-0.3	0.0	0.1	0.1	0.1	-0.9	-0.1	0.1	-0.1	NI
M184T1585_NI	2.7	-0.4	2.1	0.4	2.1	1.6	0.0	2.0	2.9	-0.7	2.5	0.6	NI
M433T1598_NI	0.3	-2.0	-0.4	1.2	-0.4	1.1	0.1	0.6	-0.8	0.1	-0.2	0.7	NI
M198T1601_NI	-0.3	-1.7	-0.6	0.3	-0.7	0.7	-0.3	0.6	-0.2	-0.4	1.0	-0.2	NI
M321T1608_NI	3.6	3.1	1.5	2.6	1.7	0.0	-0.5	0.7	1.7	0.1	0.5	1.4	NI
M415T1611_NI	-1.3	-2.2	-0.1	0.6	1.0	1.2	1.2	1.0	0.7	0.9	3.0	0.3	NI
M73T1631_NI	0.5	1.6	2.8	-0.5	0.5	0.2	0.0	0.4	-0.1	0.0	0.1	0.2	NI

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M331T1641_NI	-0.3	-1.8	-0.8	0.7	-0.3	0.3	0.0	-0.6	0.3	-0.5	0.5	0.4	NI
M219T1658_NI	0.8	1.1	2.1	1.3	0.9	0.6	-1.2	-0.3	2.8	-0.1	0.7	1.7	NI
M471T1735_NI	-0.3	0.2	0.7	-0.9	0.4	1.2	-0.6	1.1	0.6	-0.5	0.4	1.0	NI
M355T1748_NI	0.1	0.9	1.2	-0.4	0.4	0.2	-0.3	0.7	0.1	-0.1	0.0	0.7	NI
M355T1779_NI	-0.1	0.7	0.6	0.0	0.7	0.2	-0.2	0.7	0.0	-0.2	0.1	0.8	NI
M368T1785_NI	6.8	5.6	4.4	2.4	5.7	0.1	8.0	8.3	5.9	9.1	10.8	6.4	NI
M456T1828_NI	0.7	2.3	1.7	-0.4	1.0	0.2	-1.0	0.8	1.0	-0.1	0.0	1.2	NI
M308T1835_NI	0.8	1.8	1.7	-0.6	0.3	0.5	-0.7	0.7	0.1	-0.5	0.4	0.9	NI
M297T1845_NI	0.8	1.3	0.1	0.8	1.4	-0.3	-0.1	0.6	1.1	-0.2	0.4	0.8	NI
M324T1868_NI	0.0	2.8	1.0	2.2	1.9	-0.4	0.5	1.1	2.4	0.2	0.4	1.2	NI
M396T1885_NI	0.8	1.6	2.0	-1.0	1.0	0.0	-0.8	1.5	-0.1	-0.4	0.0	1.2	NI
M108T1948_NI	0.0	-0.2	-0.4	1.0	1.4	1.1	-0.4	0.6	0.0	-0.6	0.4	0.3	NI
M489T1951_NI	0.1	0.8	0.3	-0.6	1.1	0.2	0.0	0.2	-0.3	-0.2	0.2	0.2	NI
M647T1954_NI	-0.5	0.4	0.6	0.0	1.4	1.4	-0.6	1.0	0.2	-0.9	0.2	1.5	NI
M447T1961_NI	-1.8	0.2	1.1	-0.3	-0.3	1.1	0.2	3.1	0.1	-1.1	-0.9	1.8	NI
M108T1971_NI	0.1	-0.1	-0.7	0.8	1.5	2.0	-0.4	0.7	-0.3	-0.3	0.3	-0.2	NI
M193T1977_NI	0.3	-0.2	0.1	1.1	1.1	0.0	-0.1	0.5	-1.3	0.0	0.1	0.1	NI
M271T1989_NI	0.1	-0.2	-0.5	0.4	1.3	1.3	-0.3	0.4	-0.5	-0.3	0.0	-0.1	NI
M194T1993_NI	0.2	0.3	0.7	0.0	0.8	0.4	-0.5	0.4	-0.2	-0.2	0.2	0.2	NI
M461T2008_NI	0.2	0.4	0.7	-0.1	0.6	0.5	-0.5	0.6	0.1	-0.3	0.2	0.6	NI
M219T2042_NI	0.9	0.5	0.3	0.3	0.8	0.7	-0.7	0.6	1.0	-0.1	0.3	0.8	NI
M476T2050_NI	0.5	1.0	1.4	0.4	0.8	0.5	-0.4	0.9	0.5	-0.5	0.5	0.9	NI
M307T2060_NI	0.3	0.6	0.4	0.0	0.5	0.3	-0.2	0.4	0.3	-0.4	0.2	0.4	NI
M219T2071_NI	0.2	1.1	0.8	0.2	0.5	0.7	-0.8	0.8	0.3	-0.4	0.0	0.7	NI
M171T2082_NI	0.5	0.8	0.9	1.0	1.0	0.6	-1.0	0.6	1.0	-0.4	0.2	1.0	NI

Figure 3.4 Heat map of $\log_2$(droughted/well-watered) fold changes for metabolites. Red indicates high relative abundance and green indicates low relative abundance. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD

(H14); amino acid (AA), inorganic acid (IA), organic acid (OA), carbohydrate (C), sugar alcohol (SA), phenolic (P), nucleoside (N), not identified (NI). P-values for each comparison are shown in Table B.1, $n \geq 5$.

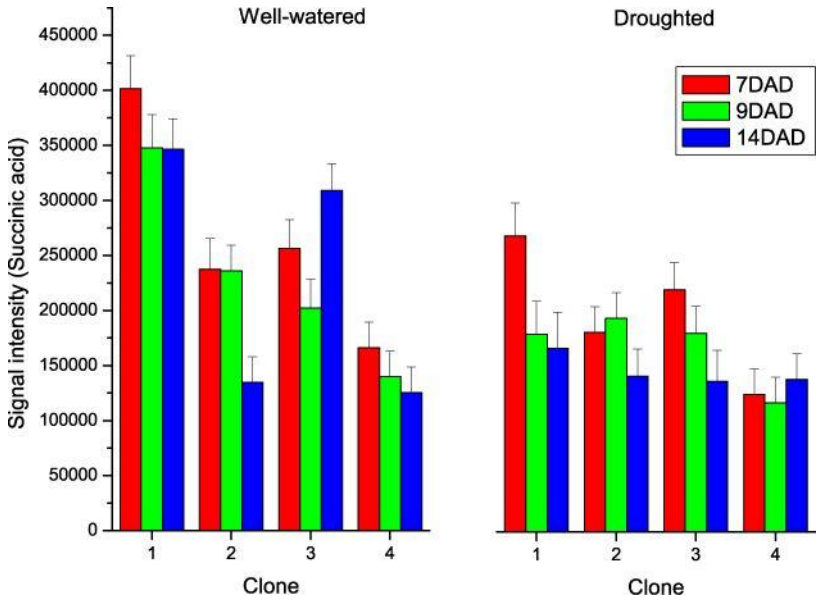

Figure 3.5 Succinic acid showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars represent standard errors of the mean, n ≥ 5. Significant comparisons are shown in Table 3.3 and Table 3.4.

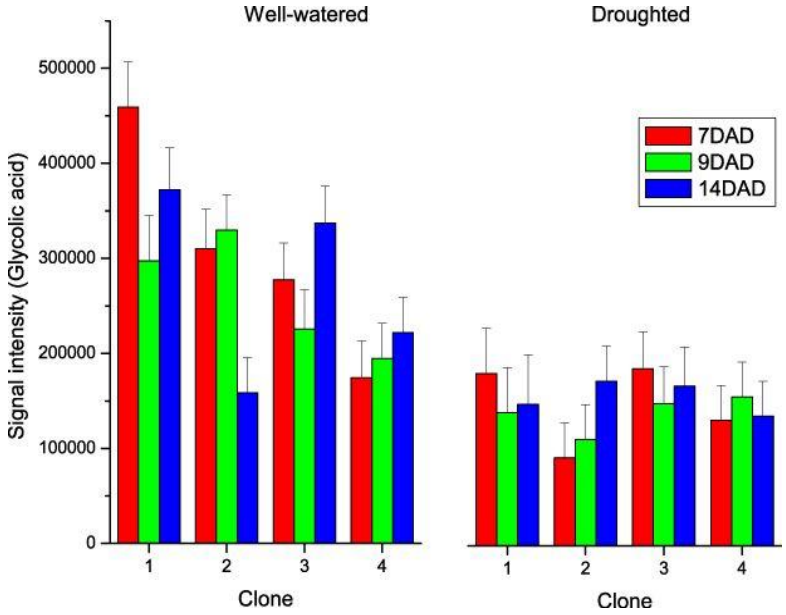

Figure 3.6 Glycolic acid showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars

represent standard errors of the mean, n ≥ 5. Significant comparisons are shown in Table 3.5 and Table 3.6.

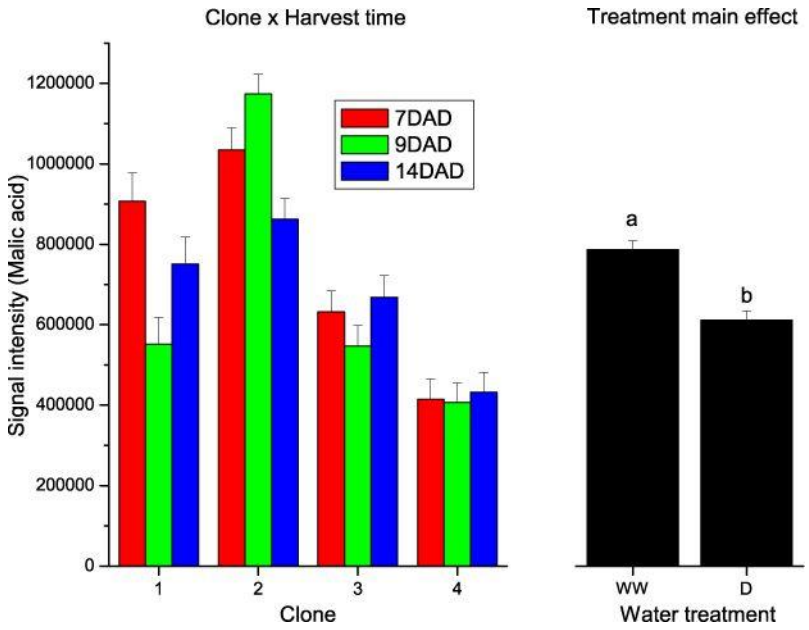

Figure 3.7 Malic acid showing a two-way interaction between clone and harvest time and a treatment main effect. The clone x harvest time interaction shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD. The treatment main effect shows well-watered (WW) and droughted (D) trees. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.05. Significant comparisons for the clone x harvest time interaction are shown in Table 3.7.

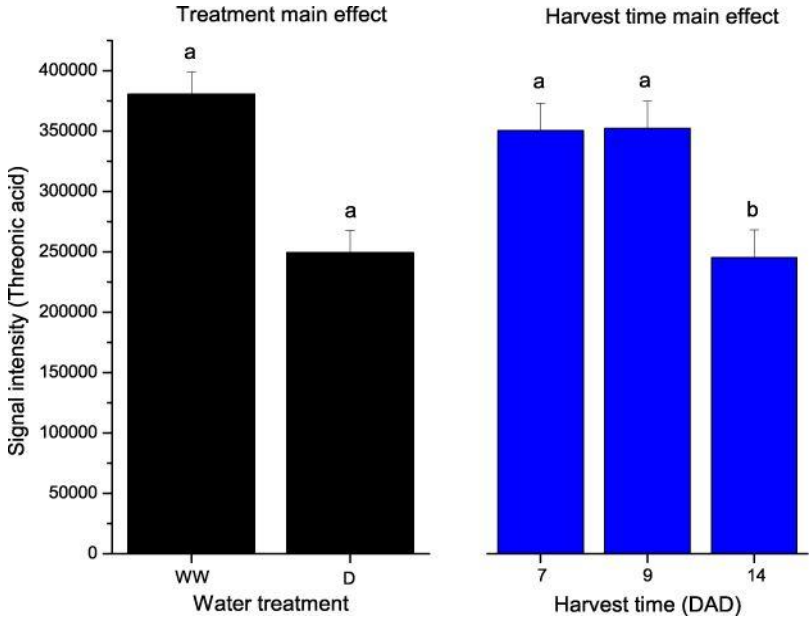

Figure 3.8 Threonic acid showing two main effects, that for treatment and another for harvest time. The treatment main effect shows well-watered (WW) and droughted (D) trees, and the harvest time main effect shows seven days after drought (7DAD), 9DAD, and 14DAD. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.05$ (treatment main effect) or $p \leq 0.0167$ (harvest time main effect).

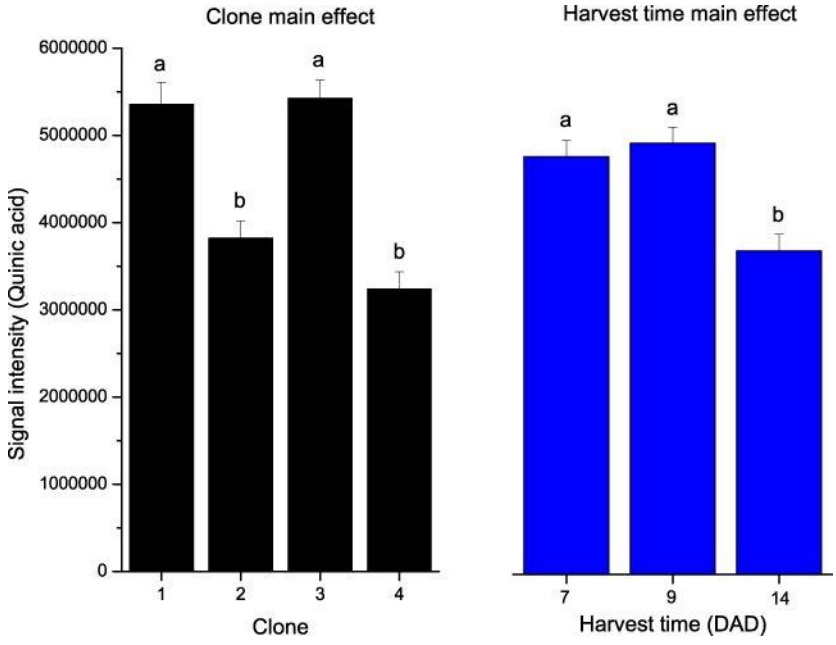

Figure 3.9 Quinic acid showing two main effects, that for clone and another for harvest time. The clone main effect shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees, and the harvest time main effect shows seven days after drought (7DAD), 9DAD, and 14DAD. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same

letter are not significantly different according to Bonferroni's test $p \leq 0.0083$ (clone main effect) or $p \leq 0.0167$ (harvest time main effect).

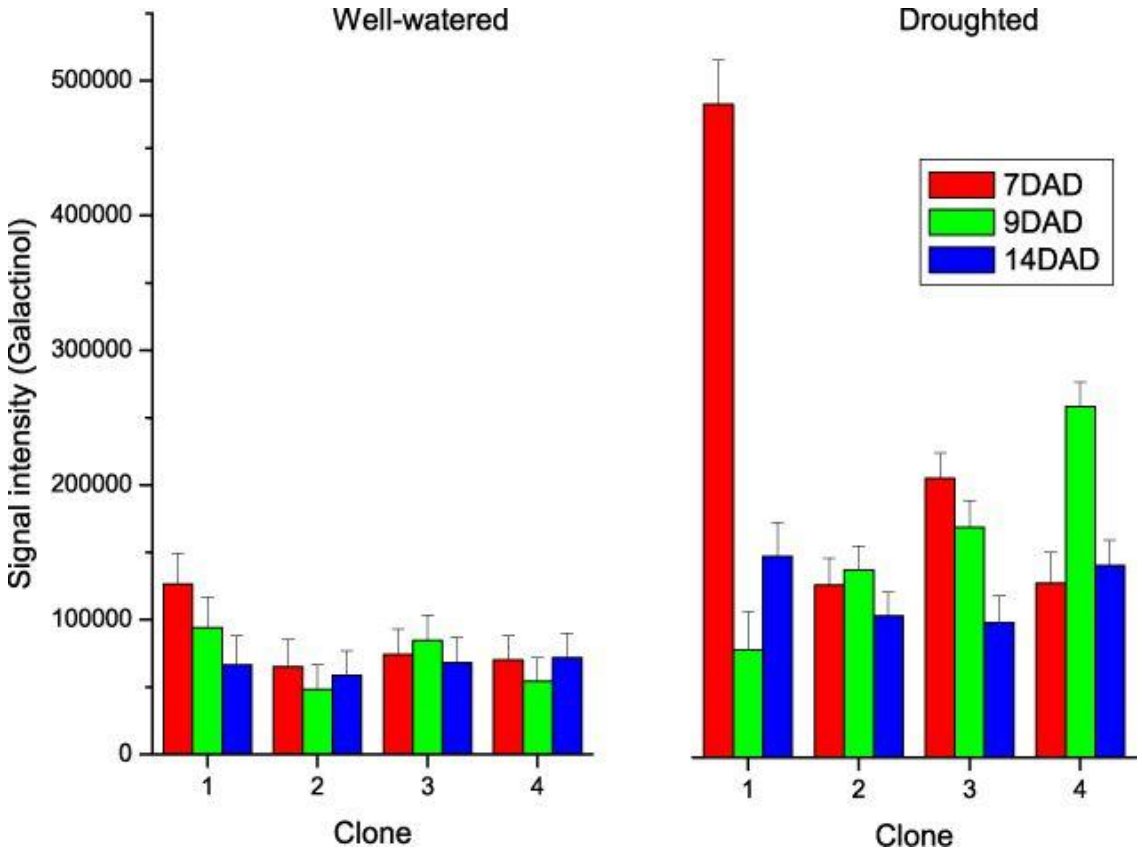

Figure 3.10 Galactinol showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars represent standard errors of the mean, $n \geq 3$. Significant comparisons are shown in Table 3.8 and Table 3.9.

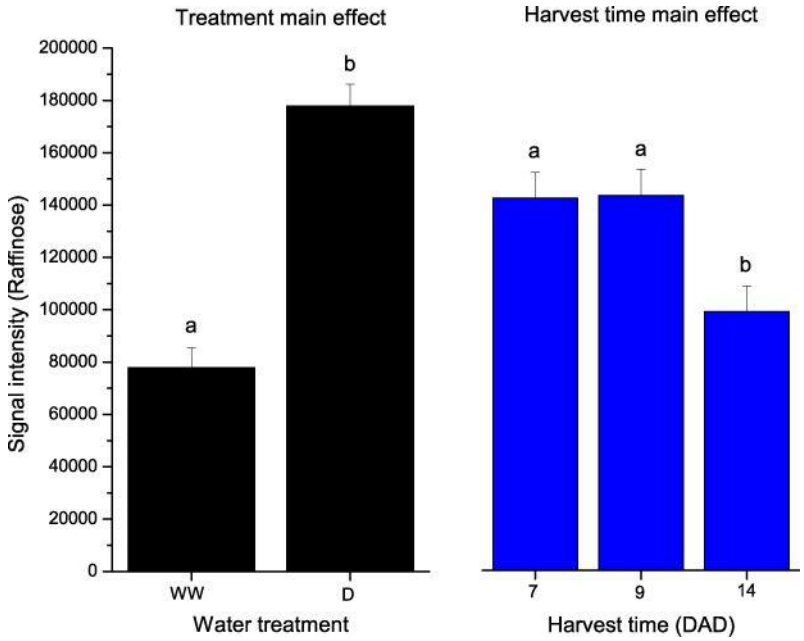

Figure 3.11 Raffinose showing two main effects, that for treatment and another for harvest time. The treatment main effect shows well-watered (WW) and droughted (D) trees, and the harvest time main effect shows seven days after drought (7DAD), 9DAD, and 14DAD. Error bars represent standard errors of the mean, n ≥ 4. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.05$ (treatment main effect) or $p \leq 0.0167$ (harvest time main effect).

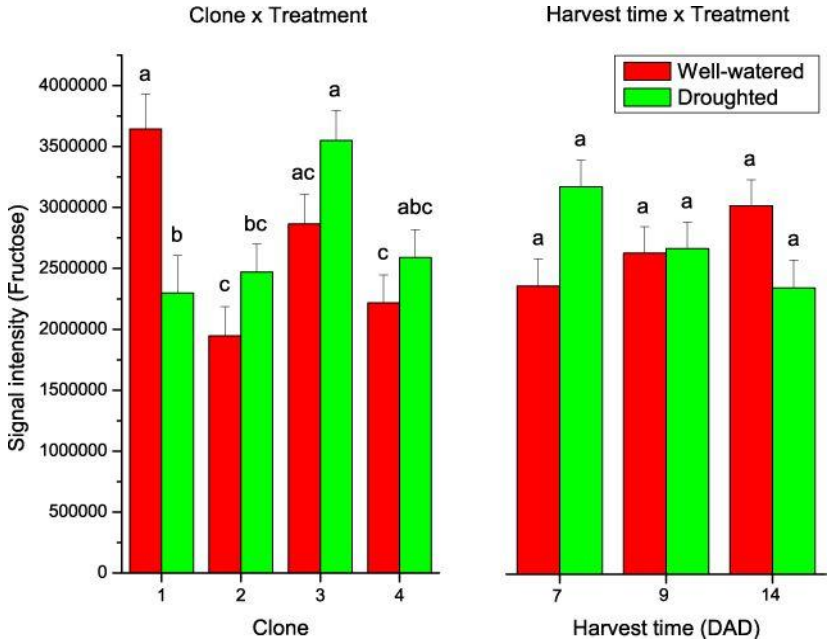

Figure 3.12 Fructose showing two two-way interactions between clone and treatment and between harvest time and treatment. The clone x treatment interaction shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees under well-watered and

droughted conditions. The harvest time x treatment interaction shows harvest times of seven days after drought (7DAD), 9DAD, and 14DAD under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0031 (clone x treatment) or p ≤ 0.0056 (harvest time x treatment).

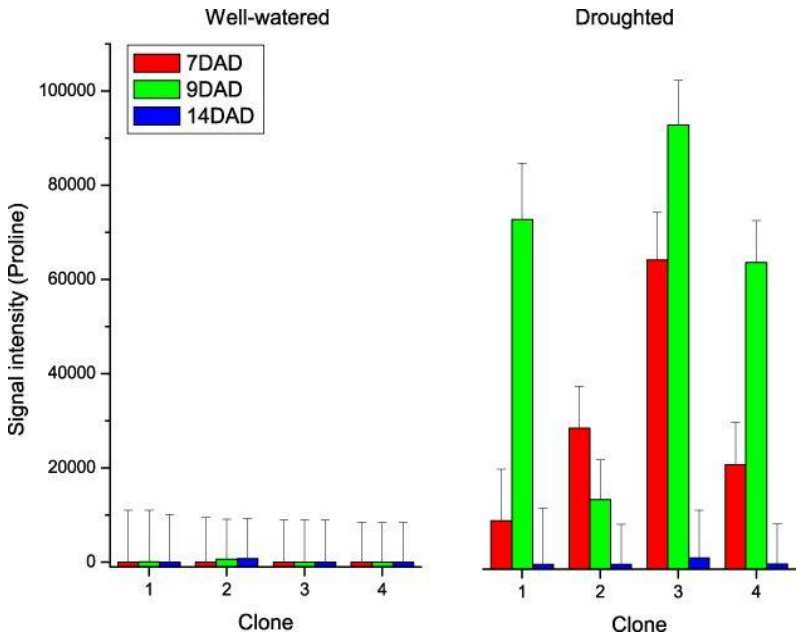

Figure 3.13 Proline showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars represent standard errors of the mean, n ≥ 5. Significant comparisons are shown in Table 3.10 and Table 3.11.

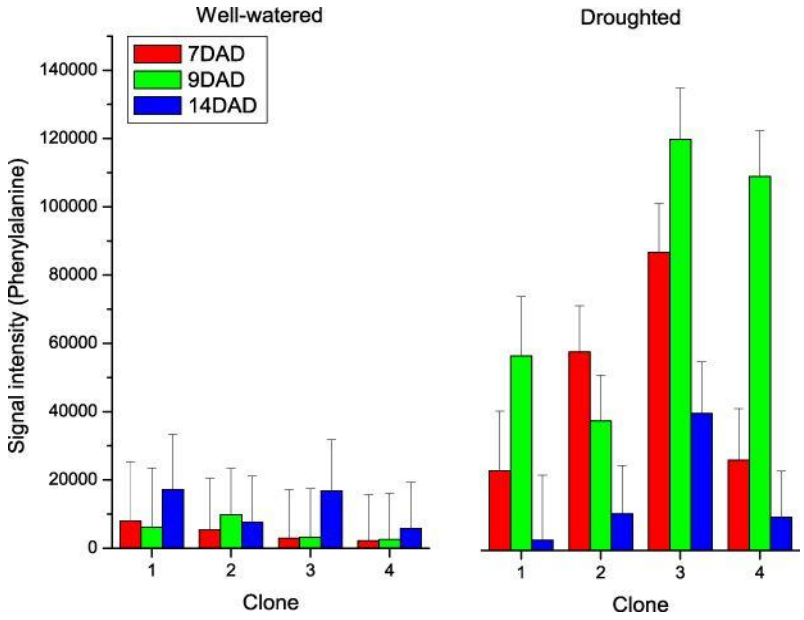

Figure 3.14 Phenylalanine showing a three-way interaction between harvest time, treatment, and clone; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees after seven days after drought (7DAD), 9DAD, and 14DAD for well-watered and droughted trees. Error bars represent standard errors of the mean, n ≥ 5. Significant comparisons are shown in Table 3.12 and Table 3.13.

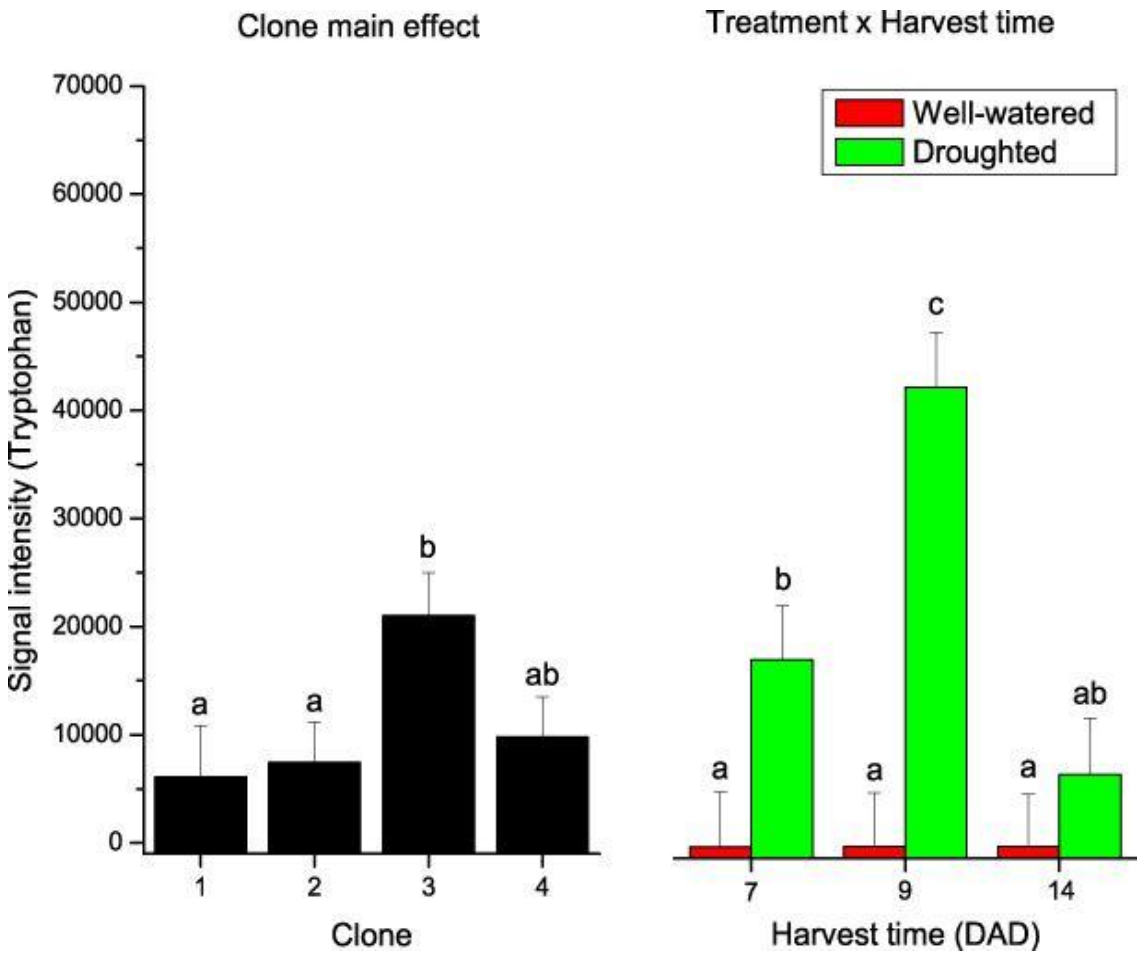

Figure 3.15 Tryptophan showing a clone main effect and a two-way interaction between treatment and harvest time. The clone main effect shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees, and the treatment x harvest time interaction shows harvest times of seven days after drought (7DAD), 9DAD, and 14DAD under well-watered and droughted conditions. Error bars represent standard errors of the mean, $n \geq 5$. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0083$ (clone main effect) or $p \leq 0.0056$ (treatment x harvest time).

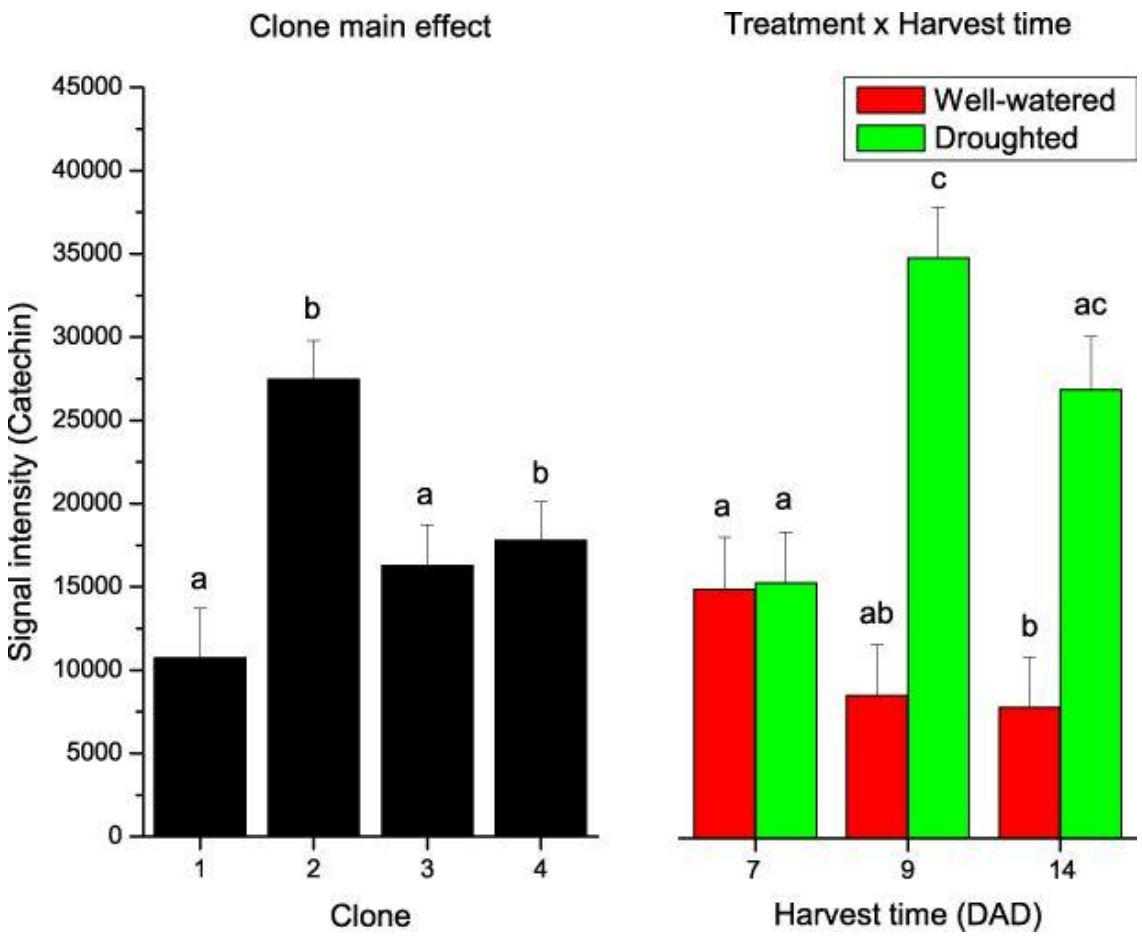

Figure 3.16 Catechin showing a clone main effect and a two-way interaction between treatment and harvest time. The clone main effect shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees, and the treatment x harvest time interaction shows harvest times of seven days after drought (7DAD), 9DAD, and 14DAD under well-watered and droughted conditions. Error bars represent standard errors of the mean, $n \geq 5$. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0083$ (clone main effect) or $p \leq 0.0056$ (treatment x harvest time).

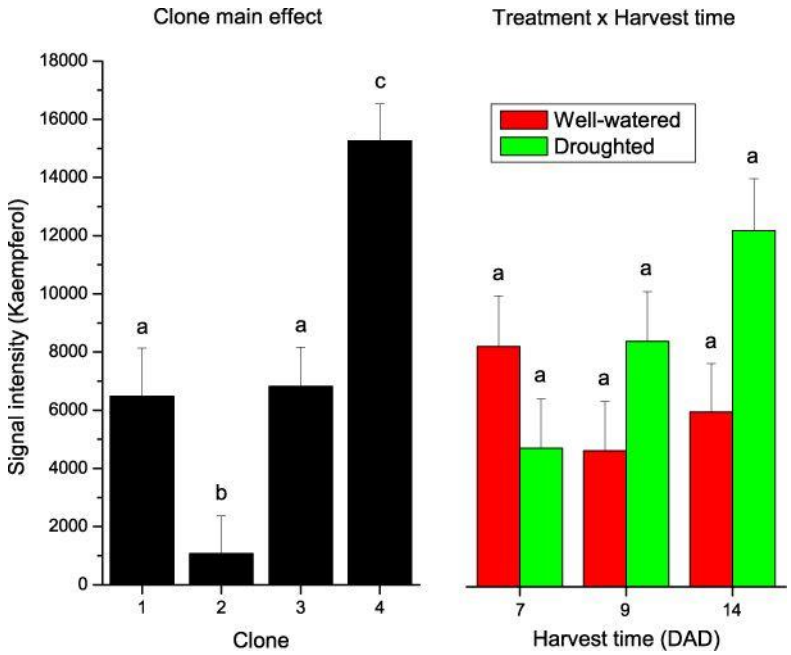

Figure 3.17 Kaempferol showing a clone main effect and a two-way interaction between treatment and harvest time. The clone main effect shows Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees, and the treatment x harvest time interaction shows harvest times of seven days after drought (7DAD), 9DAD, and 14DAD under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0083$ (clone main effect) or $p \leq 0.0056$ (treatment x harvest time).

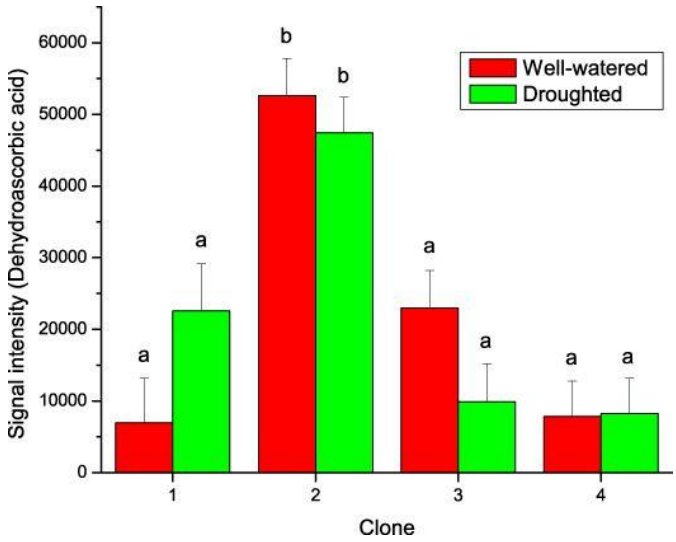

Figure 3.18 Dehydroascorbic acid showing a clone x treatment interaction; Walker (1), Okanese (2), WP-86V-86 (3), and Katepwa (4) hybrid poplar trees under well-watered and droughted

conditions. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0031.

3.5.2 Experiment #2 figures

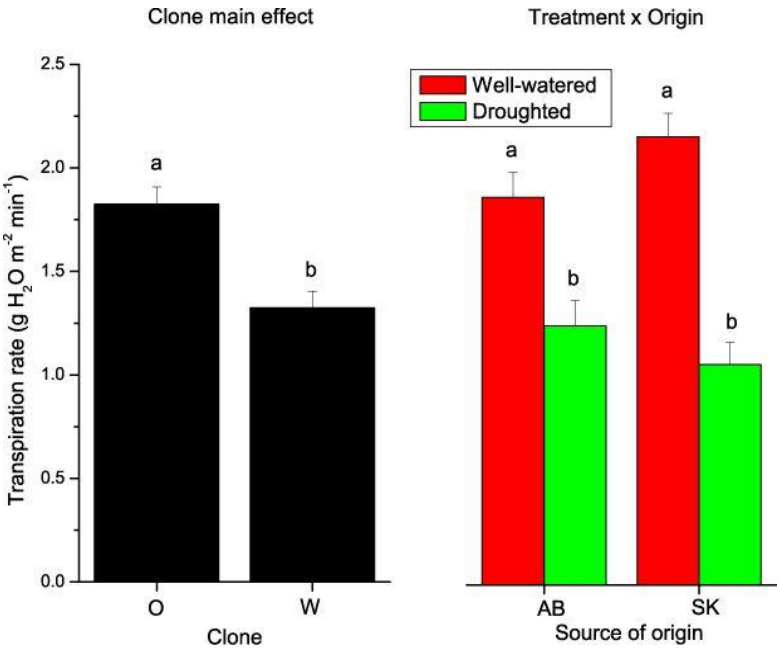

Figure 3.19 Transpiration rate (g H_20 m^{-2} min^{-1}) showing a clone main effect and a two-way interaction between treatment and origin. The clone main effect shows Okanese (O) and Walker (W) hybrid poplar trees and the treatment x origin interaction shows trees sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.05 (clone main effect) or p ≤ 0.0125 (treatment x origin).

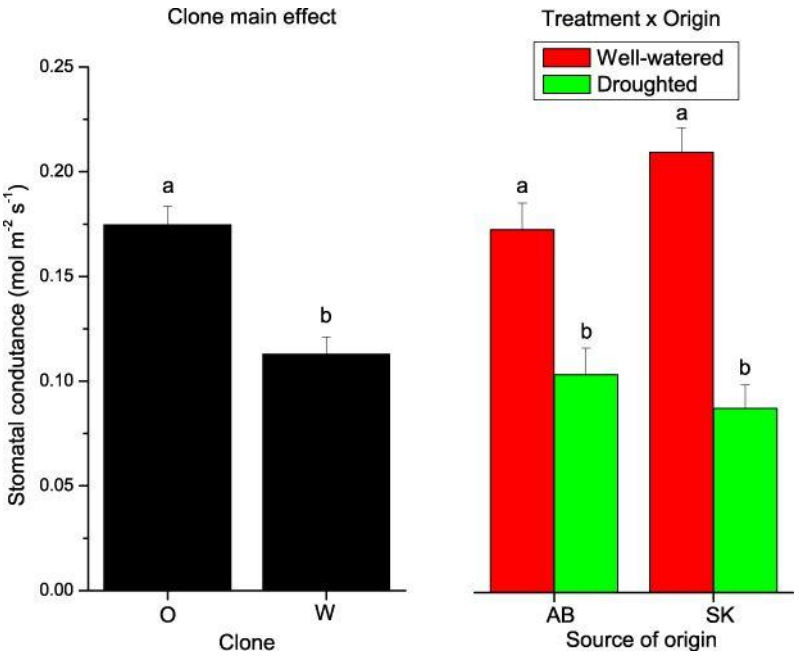

Figure 3.20 Stomatal conductance (mol m^{-2} s^{-1}) showing a clone main effect and a two-way interaction between treatment and origin. The clone main effect shows Okanese (O) and Walker (W) hybrid poplar trees and the treatment x origin interaction shows trees sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 5. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.05 (clone main effect) or p ≤ 0.0125 (treatment x origin).

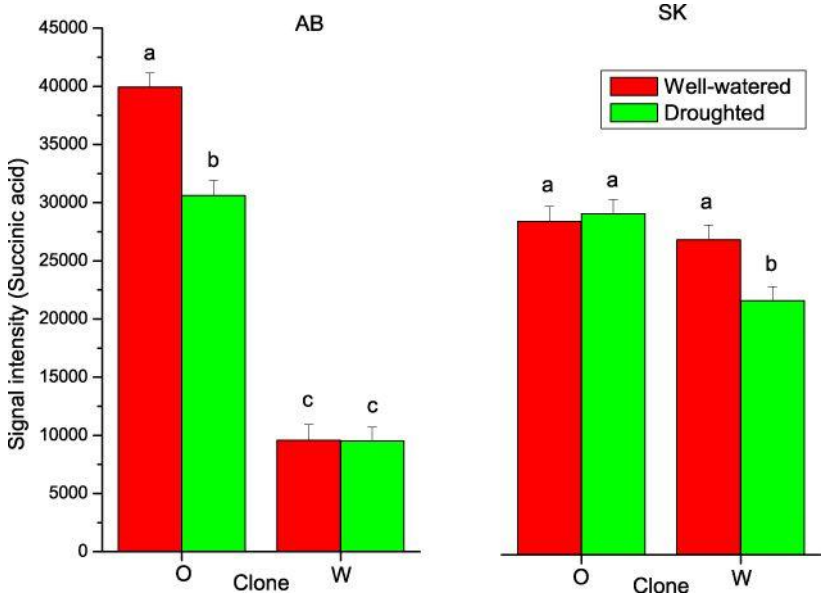

Figure 3.21 Succinic acid showing a three-way interaction between clone, treatment, and origin; Okanese (O) and Walker (W) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 7. Bars

within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0063$.

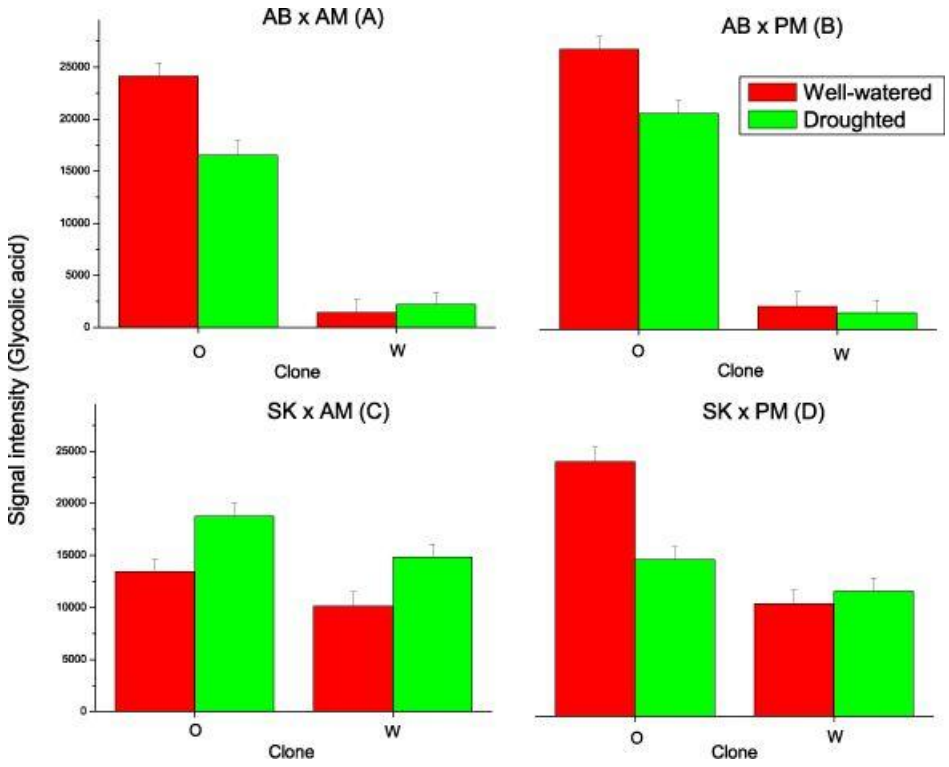

Figure 3.22 Glycolic acid showing a four-way interaction between clone, treatment, origin, and harvest time; Okanese (O) and Walker (W) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, $n \geq 7$. Significant comparisons are shown in Table 3.14.

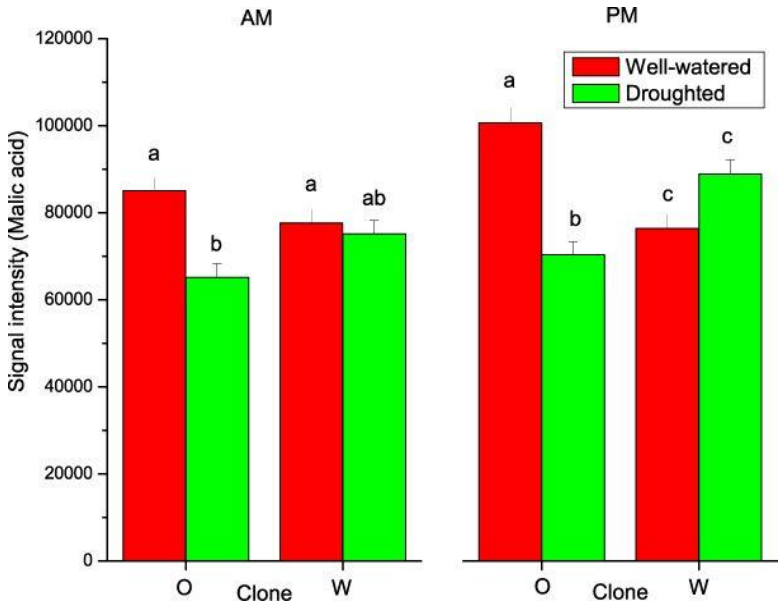

Figure 3.23 Malic acid showing a three-way interaction between clone, treatment, and harvest time; Okanese (O) and Walker (W) under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 7. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0063.

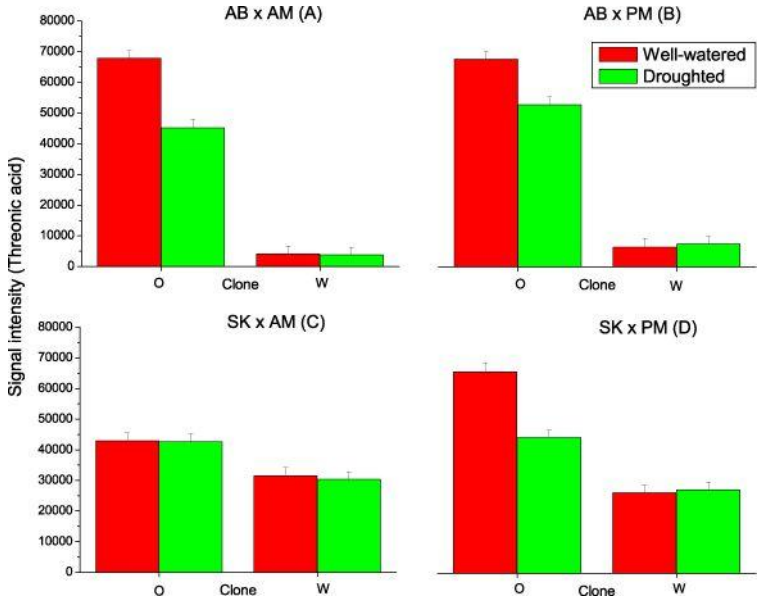

Figure 3.24 Threonic acid showing a four-way interaction between clone, treatment, origin, and harvest time; Okanese (O) and Walker (W) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 7. Significant comparisons are shown in Table 3.15.

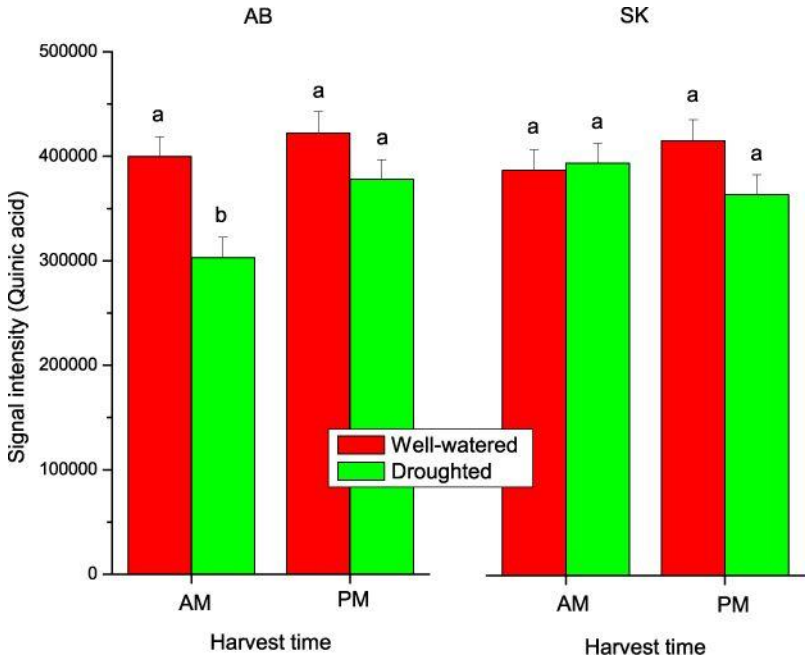

Figure 3.25 Quinic acid showing a three-way interaction between origin, treatment, and harvest time; trees sourced from Alberta (AB) and Saskatchewan (SK), treated under well-watered and droughted conditions, and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 7. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0042.

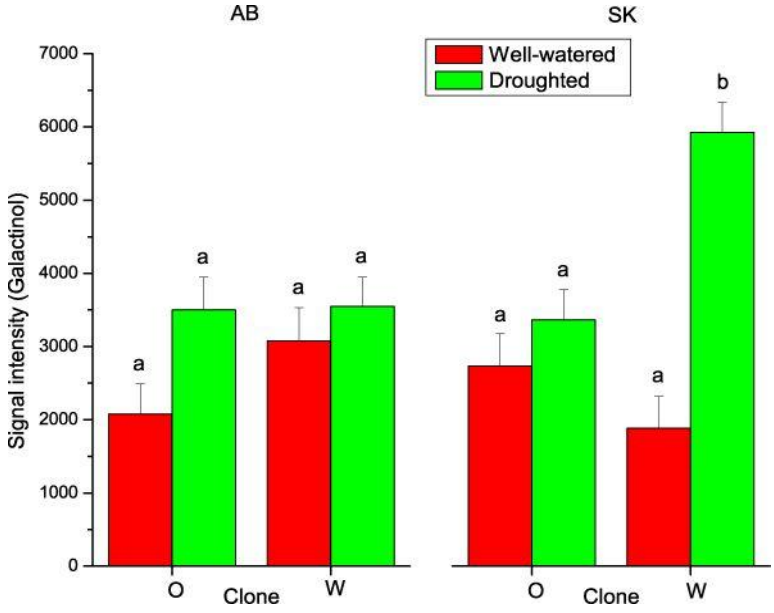

Figure 3.26 Galactinol showing a three-way interaction between clone, treatment, and origin; Okanese (O) and Walker (W) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 7. Bars

within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0063$.

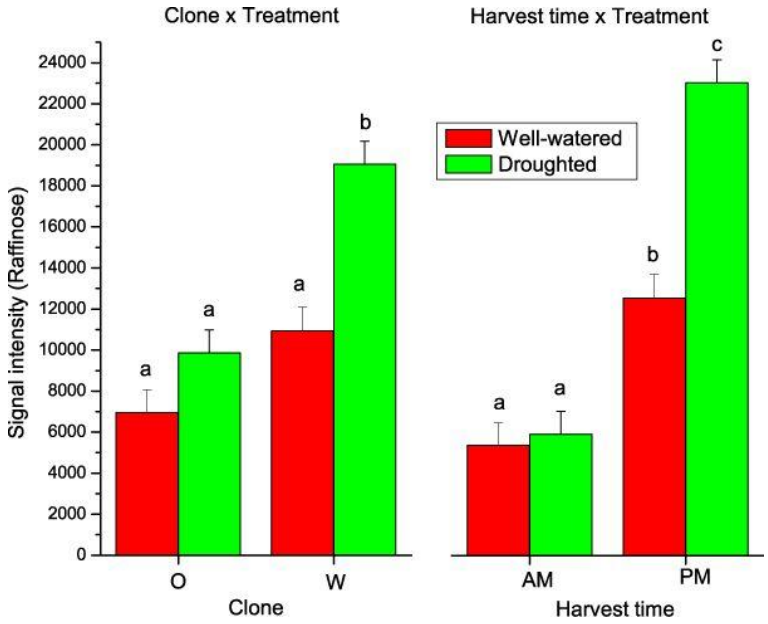

Figure 3.27 Raffinose showing two two-way interactions for clone and treatment, and another for harvest time and treatment. The clone x treatment interaction shows Okanese (O) and Walker (W) under well-watered and droughted conditions. The harvest time x treatment interaction shows trees harvested at predawn (AM) and midday (PM) under well-watered and droughted conditions. Error bars represent standard errors of the mean, $n \geq 7$. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0125$ for both interactions.

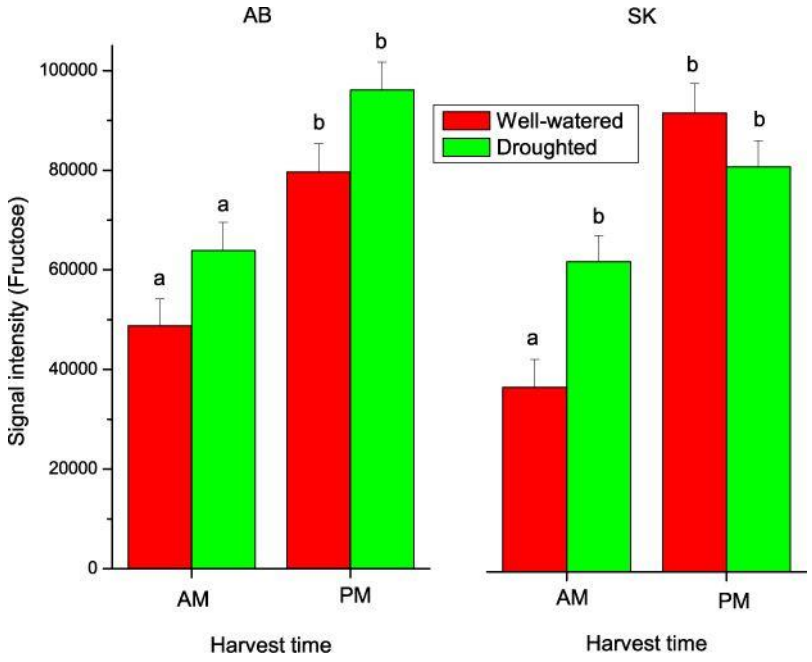

Figure 3.28 Fructose showing a three-way interaction between origin, treatment, and harvest time; trees sourced from Alberta (AB) and Saskatchewan (SK), treated under well-watered and droughted conditions, and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, $n \geq 6$. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.0042$.

3.5.3 Experiment #3 figures

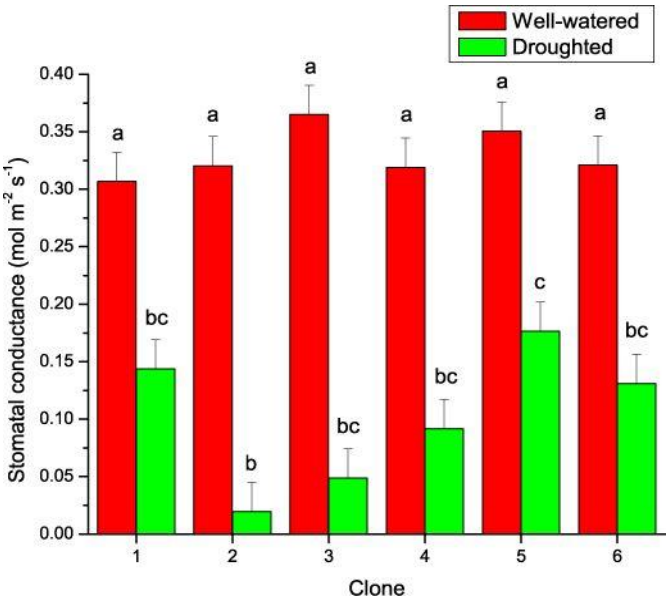

Figure 3.29 Stomatal conductance (mol m^{-2} s^{-1}) showing a clone x treatment interaction; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions. Error bars represent standard errors of the mean, n = 3. Bars within axes with the same letter are not significantly different according to Bonferroni's test $p \leq 0.001$.

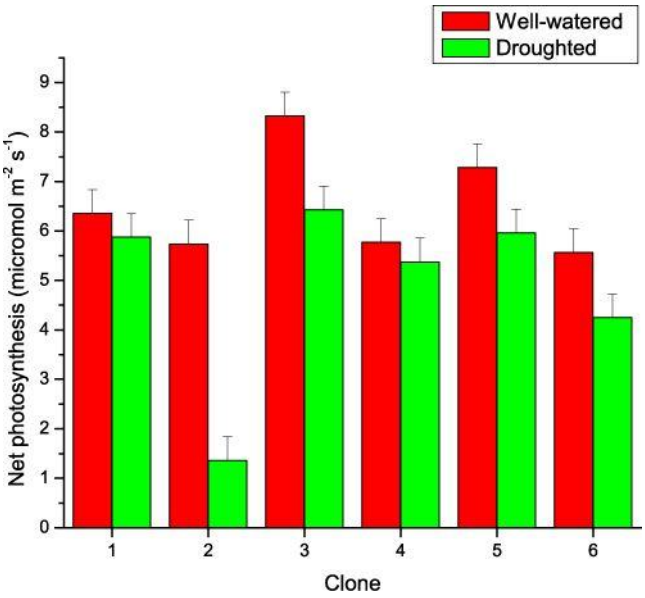

Figure 3.30 Net photosynthetic rate (µmol m^{-2} s^{-1}) showing a two-way interaction between clone and treatment; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions. Error bars represent standard errors of the mean, n = 3. Significant comparisons are shown in Table 3.16.

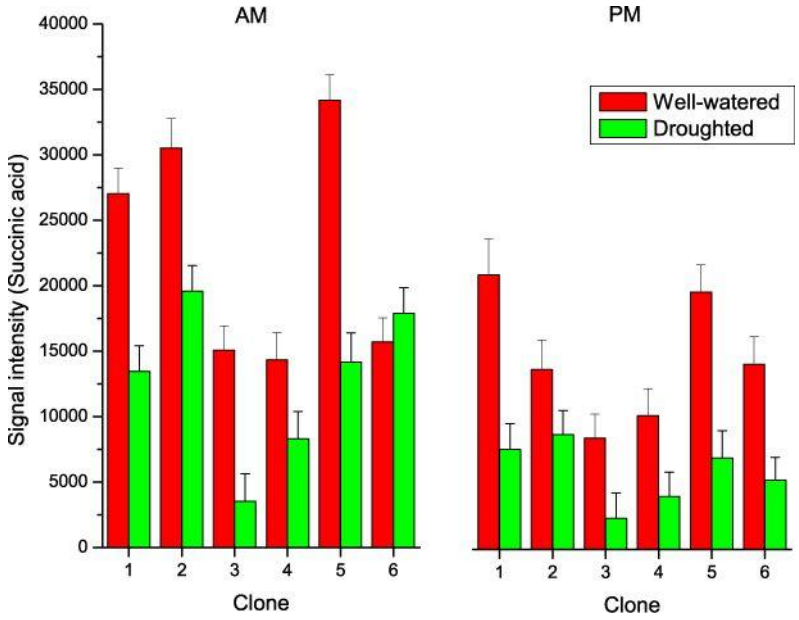

Figure 3.31 Succinic acid showing a three-way interaction between clone, harvest time, and treatment; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 4. Significant comparisons are shown in Table 3.17 and Table 3.18.

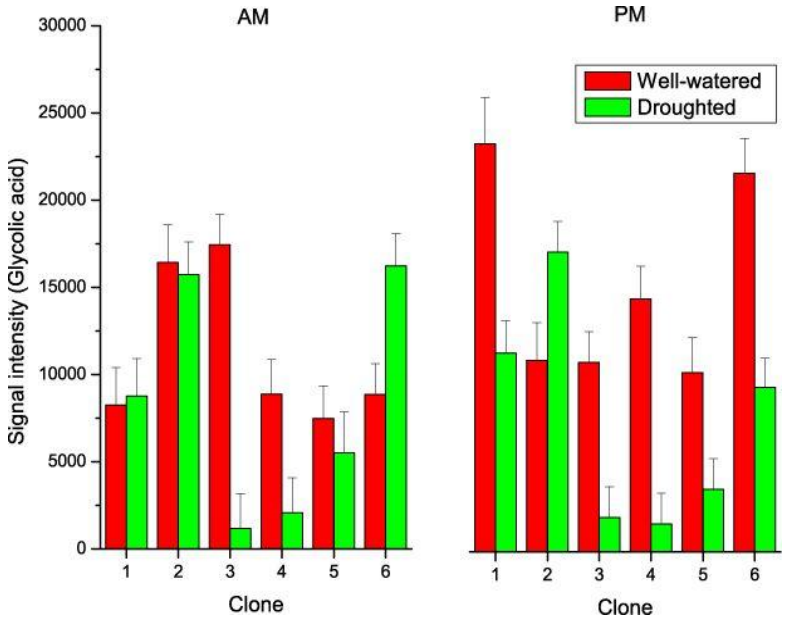

Figure 3.32 Glycolic acid showing a three-way interaction between clone, harvest time, and treatment; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 4. Significant comparisons are shown in Table 3.19 and Table 3.20.

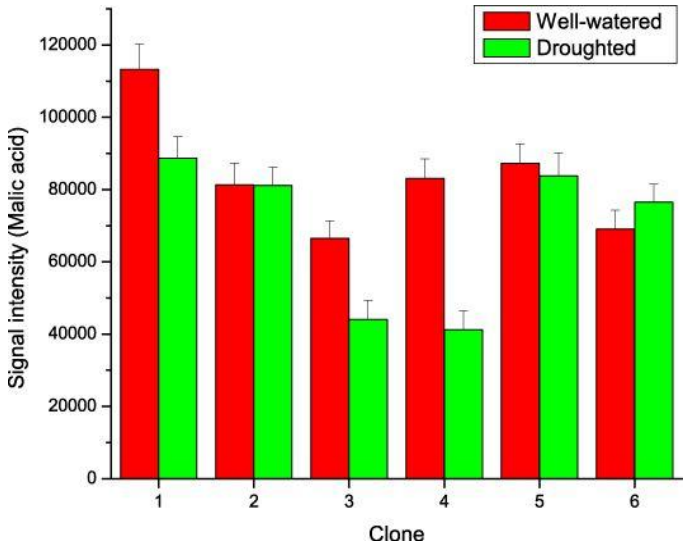

Figure 3.33 Malic acid showing a two-way interaction between clone and treatment; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 4. Significant comparisons are shown in Table 3.21.

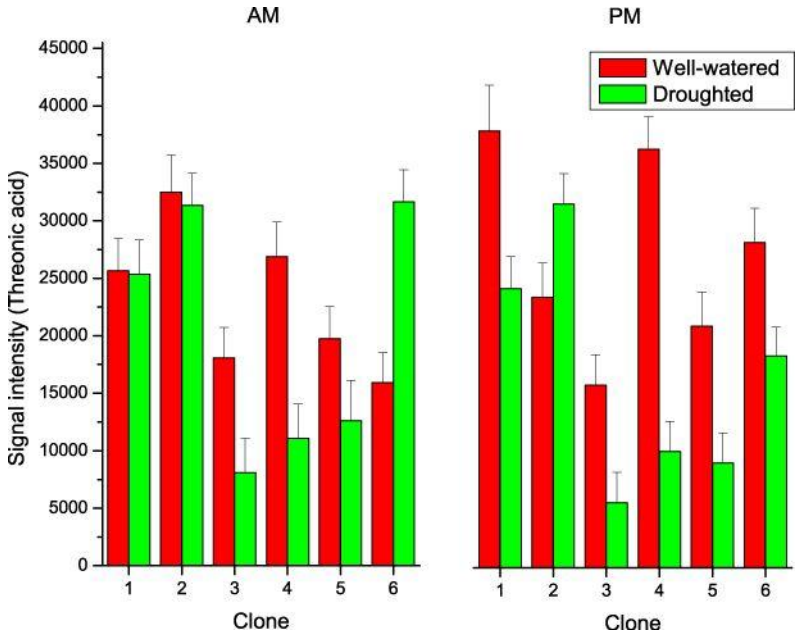

Figure 3.34 Threonic acid showing a three-way interaction between clone, harvest time, and treatment; AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions and harvested at predawn (AM) and midday (PM). Error bars represent standard errors of the mean, n ≥ 4. Significant comparisons are shown in Table 3.22 and Table 3.23.

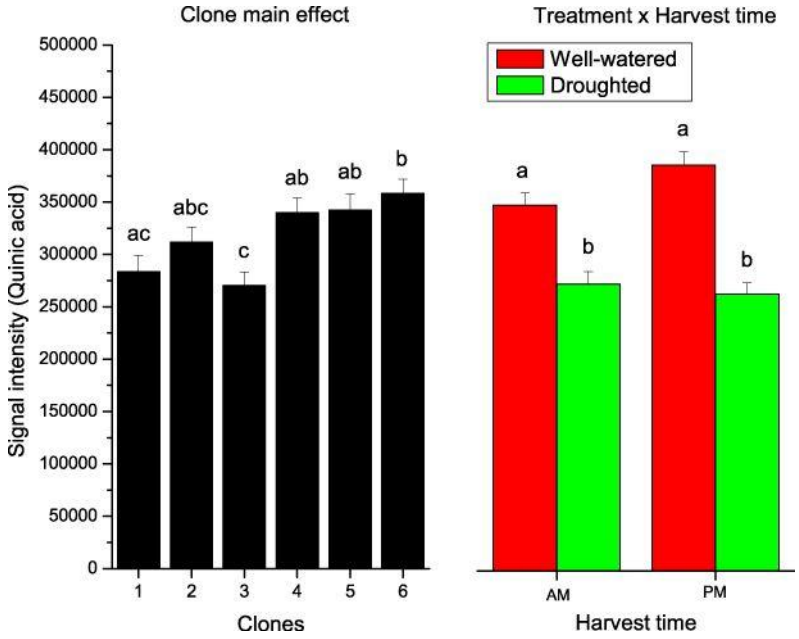

Figure 3.35 Quinic acid showing a clone main effect and a two-way interaction between treatment and harvest time. The clone main effect shows AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees. The treatment x harvest time interaction shows trees harvested at predawn (AM) and midday (PM) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 4. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0017 (clone main effect) or p ≤ 0.0125 (treatment x harvest time).

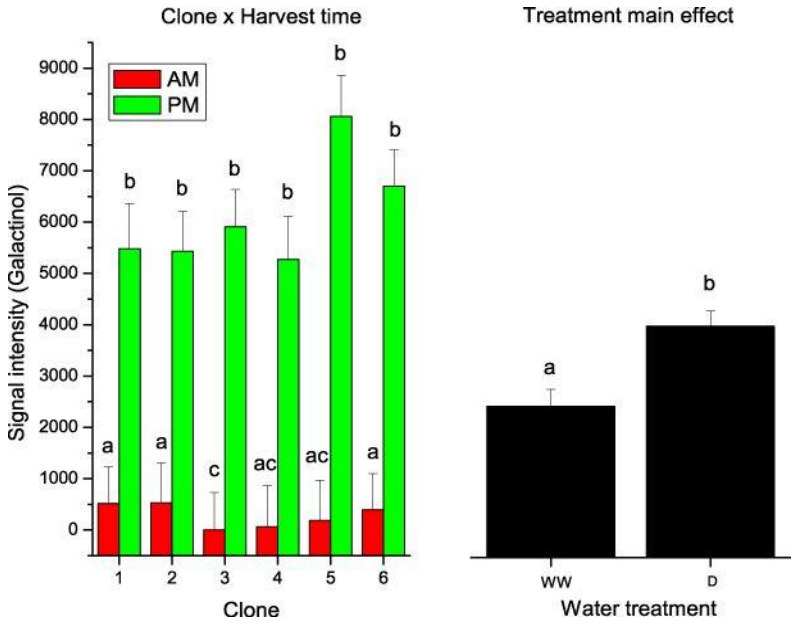

Figure 3.36 Galactinol showing a two-way interaction between clone and harvest time, and a treatment main effect. The clone x harvest time interaction shows AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees harvested at predawn (AM)

and midday (PM). The treatment main effect shows trees under well-watered (WW) and droughted (D) conditions. Error bars represent standard errors of the mean, n ≥ 4. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0014 (clone x harvest time) or p ≤ 0.05 (treatment main effect).

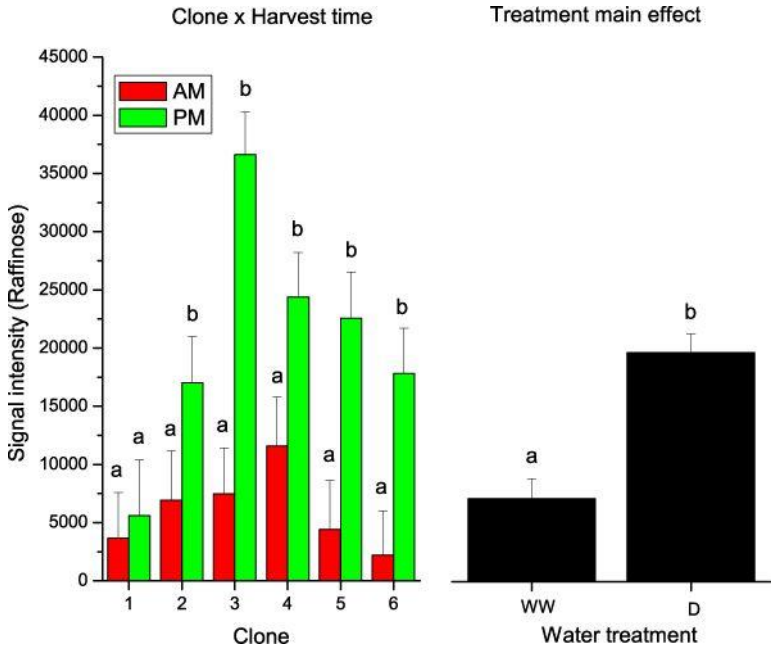

Figure 3.37 Raffinose showing a two-way interaction between clone and harvest time, and a treatment main effect. The clone x harvest time interaction shows AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees harvested at predawn (AM) and midday (PM). The treatment main effect shows trees under well-watered (WW) and droughted (D) conditions. Error bars represent standard errors of the mean, n ≥ 4. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0014 (clone x harvest time) or p ≤ 0.05 (treatment main effect).

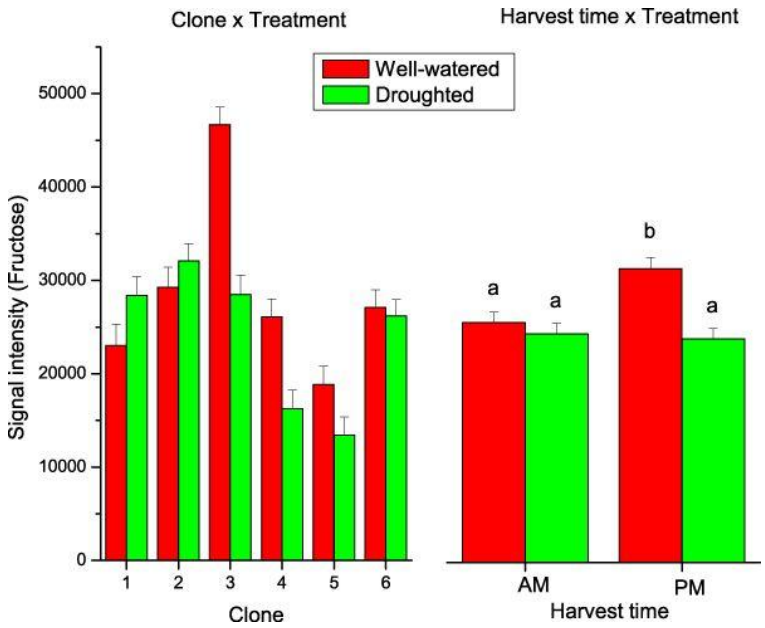

Figure 3.38 Fructose showing two two-way interactions for clone and treatment, and another for harvest time and treatment. The clone x treatment interaction shows AP947 (1), AP1006 (2), AP1005 (3), AP2278 (4), AP2298 (5), and AP2300 (6) poplar trees under well-watered and droughted conditions. The harvest time x treatment interaction shows trees harvested at predawn (AM) and midday (PM) under well-watered and droughted conditions. Error bars represent standard errors of the mean, n ≥ 4. Bars within axes with the same letter are not significantly different according to Bonferroni's test p ≤ 0.0125. Significant comparisons for the clone x treatment interaction are shown in Table 3.24.

3.6 Tables

3.6.1 Experiment #1 tables

Table 3.1 Leaf water potential (MPa) showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test p ≤ 0.0007.

Average leaf water potential (Mpa)	
Well-watered	Droughted
C1H7 vs C1H9	**C1H7 vs C1H9 ***
C1H7 vs C1H14	C1H7 vs C1H14
C1H9 vs C1H14	**C1H9 vs C1H14 ***
C2H7 vs C2H9	C2H7 vs C2H9
C2H7 vs C2H14	C2H7 vs C2H14
C2H9 vs C2H14	C2H9 vs C2H14
C3H7 vs C3H9	**C3H7 vs C3H9 ***
C3H7 vs C3H14	**C3H7 vs C3H14 ***
C3H9 vs C3H14	**C3H9 vs C3H14 ***

Average leaf water potential (Mpa)	
Well-watered	Droughted
C4H7 vs C4H9	**C4H7 vs C4H9 ***
C4H7 vs C4H14	**C4H7 vs C4H14 ***
C4H9 vs C4H14	**C4H9 vs C4H14 ***
C1H7 vs C2H7	C1H7 vs C2H7
C1H7 vs C3H7	C1H7 vs C3H7
C1H7 vs C4H7	C1H7 vs C4H7
C2H7 vs C3H7	C2H7 vs C3H7
C2H7 vs C4H7	C2H7 vs C4H7
C3H7 vs C4H7	C3H7 vs C4H7
C1H9 vs C2H9	**C1H9 vs C2H9 ***
C1H9 vs C3H9	C1H9 vs C3H9
C1H9 vs C4H9	C1H9 vs C4H9
C2H9 vs C3H9	**C2H9 vs C3H9 ***
C2H9 vs C4H9	**C2H9 vs C4H9 ***
C3H9 vs C4H9	C3H9 vs C4H9
C1H14 vs C2H14	**C1H14 vs C2H14 ***
C1H14 vs C3H14	C1H14 vs C3H14
C1H14 vs C4H14	**C1H14 vs C4H14 ***
C2H14 vs C3H14	C2H14 vs C3H14
C2H14 vs C4H14	C2H14 vs C4H14
C3H14 vs C4H14	C3H14 vs C4H14

Table 3.2 Leaf water potential (MPa) showing relevant between treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average leaf water potential (Mpa)
Well-watered vs Droughted
C1H7 vs C1H7
C2H7 vs C2H7
C3H7 vs C3H7 *
C4H7 vs C4H7 *
C1H9 vs C1H9 *
C2H9 vs C2H9
C3H9 vs C3H9 *
C4H9 vs C4H9 *
C1H14 vs C1H14
C2H14 vs C2H14
C3H14 vs C3H14
C4H14 vs C4H14

Table 3.3 Succinic acid showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Succinic acid)	
Well-watered	Droughted
C1H7 vs C1H9	C1H7 vs C1H9
C1H7 vs C1H14	C1H7 vs C1H14
C1H9 vs C1H14	C1H9 vs C1H14
C2H7 vs C2H9	C2H7 vs C2H9
C2H7 vs C2H14	C2H7 vs C2H14
C2H9 vs C2H14	C2H9 vs C2H14
C3H7 vs C3H9	C3H7 vs C3H9
C3H7 vs C3H14	C3H7 vs C3H14
C3H9 vs C3H14	C3H9 vs C3H14
C4H7 vs C4H9	C4H7 vs C4H9
C4H7 vs C4H14	C4H7 vs C4H14
C4H9 vs C4H14	C4H9 vs C4H14
C1H7 vs C2H7 *	C1H7 vs C2H7
C1H7 vs C3H7 *	C1H7 vs C3H7
C1H7 vs C4H7 *	**C1H7 vs C4H7 ***
C2H7 vs C3H7	C2H7 vs C3H7
C2H7 vs C4H7	C2H7 vs C4H7
C3H7 vs C4H7	C3H7 vs C4H7
C1H9 vs C2H9	C1H9 vs C2H9
C1H9 vs C3H9 *	C1H9 vs C3H9
C1H9 vs C4H9 *	C1H9 vs C4H9
C2H9 vs C3H9	C2H9 vs C3H9
C2H9 vs C4H9	C2H9 vs C4H9
C3H9 vs C4H9	C3H9 vs C4H9
C1H14 vs C2H14 *	C1H14 vs C2H14
C1H14 vs C3H14	C1H14 vs C3H14
C1H14 vs C4H14 *	C1H14 vs C4H14
C2H14 vs C3H14 *	C2H14 vs C3H14
C2H14 vs C4H14	C2H14 vs C4H14
C3H14 vs C4H14 *	C3H14 vs C4H14

Table 3.4 Succinic acid showing relevant between treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Succinic acid)
Well-watered vs Droughted
C1H7 vs C1H7
C2H7 vs C2H7
C3H7 vs C3H7
C4H7 vs C4H7
C1H9 vs C1H9 *

Average signal intensity (Succinic acid)
Well-watered vs Droughted
C2H9 vs C2H9
C3H9 vs C3H9
C4H9 vs C4H9
C1H14 vs C1H14 *
C2H14 vs C2H14
C3H14 vs C3H14 *
C4H14 vs C4H14

Table 3.5 Glycolic acid showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Glycolic acid)	
Well-watered	Droughted
C1H7 vs C1H9	C1H7 vs C1H9
C1H7 vs C1H14	C1H7 vs C1H14
C1H9 vs C1H14	C1H9 vs C1H14
C2H7 vs C2H9	C2H7 vs C2H9
C2H7 vs C2H14	C2H7 vs C2H14
C2H9 vs C2H14	C2H9 vs C2H14
C3H7 vs C3H9	C3H7 vs C3H9
C3H7 vs C3H14	C3H7 vs C3H14
C3H9 vs C3H14	C3H9 vs C3H14
C4H7 vs C4H9	C4H7 vs C4H9
C4H7 vs C4H14	C4H7 vs C4H14
C4H9 vs C4H14	C4H9 vs C4H14
C1H7 vs C2H7	C1H7 vs C2H7
C1H7 vs C3H7	C1H7 vs C3H7
C1H7 vs C4H7 *	C1H7 vs C4H7
C2H7 vs C3H7	C2H7 vs C3H7
C2H7 vs C4H7	C2H7 vs C4H7
C3H7 vs C4H7	C3H7 vs C4H7
C1H9 vs C2H9	C1H9 vs C2H9
C1H9 vs C3H9	C1H9 vs C3H9
C1H9 vs C4H9	C1H9 vs C4H9
C2H9 vs C3H9	C2H9 vs C3H9
C2H9 vs C4H9	C2H9 vs C4H9
C3H9 vs C4H9	C3H9 vs C4H9
C1H14 vs C2H14 *	C1H14 vs C2H14
C1H14 vs C3H14	C1H14 vs C3H14
C1H14 vs C4H14	C1H14 vs C4H14
C2H14 vs C3H14	C2H14 vs C3H14
C2H14 vs C4H14	C2H14 vs C4H14
C3H14 vs C4H14	C3H14 vs C4H14

Table 3.6 Glycolic acid showing relevant between treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Glycolic acid)
Well-watered vs Droughted
C1H7 vs C1H7 *
C2H7 vs C2H7 *
C3H7 vs C3H7
C4H7 vs C4H7
C1H9 vs C1H9
C2H9 vs C2H9 *
C3H9 vs C3H9
C4H9 vs C4H9
C1H14 vs C1H14
C2H14 vs C2H14
C3H14 vs C3H14
C4H14 vs C4H14

Table 3.7 Malic acid showing relevant clone x harvest time interaction comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0017$.

Average signal intensity (Malic acid)
C1H7 vs C1H9 *
C1H7 vs C1H14
C1H9 vs C1H14
C2H7 vs C2H9
C2H7 vs C2H14
C2H9 vs C2H14
C3H7 vs C3H9
C3H7 vs C3H14
C3H9 vs C3H14
C4H7 vs C4H9
C4H7 vs C4H14
C4H9 vs C4H14
C1H7 vs C2H7
C1H7 vs C3H7
C1H7 vs C4H7 *
C2H7 vs C3H7 *
C2H7 vs C4H7 *
C3H7 vs C4H7 *
C1H9 vs C2H9 *
C1H9 vs C3H9
C1H9 vs C4H9
C2H9 vs C3H9 *
C2H9 vs C4H9 *

Average signal intensity (Malic acid)
C3H9 vs C4H9
C1H14 vs C2H14
C1H14 vs C3H14
C1H14 vs C4H14 *
C2H14 vs C3H14
C2H14 vs C4H14 *
C3H14 vs C4H14 *

Table 3.8 Galactinol showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Galactinol)	
Well-watered	Droughted
C1H7 vs C1H9	**C1H7 vs C1H9 ***
C1H7 vs C1H14	C1H7 vs C1H14
C1H9 vs C1H14	C1H9 vs C1H14
C2H7 vs C2H9	C2H7 vs C2H9
C2H7 vs C2H14	C2H7 vs C2H14
C2H9 vs C2H14	C2H9 vs C2H14
C3H7 vs C3H9	C3H7 vs C3H9
C3H7 vs C3H14	C3H7 vs C3H14
C3H9 vs C3H14	C3H9 vs C3H14
C4H7 vs C4H9	C4H7 vs C4H9
C4H7 vs C4H14	C4H7 vs C4H14
C4H9 vs C4H14	C4H9 vs C4H14
C1H7 vs C2H7	**C1H7 vs C2H7 ***
C1H7 vs C3H7	C1H7 vs C3H7
C1H7 vs C4H7	**C1H7 vs C4H7 ***
C2H7 vs C3H7	C2H7 vs C3H7
C2H7 vs C4H7	C2H7 vs C4H7
C3H7 vs C4H7	C3H7 vs C4H7
C1H9 vs C2H9	C1H9 vs C2H9
C1H9 vs C3H9	C1H9 vs C3H9
C1H9 vs C4H9	**C1H9 vs C4H9 ***
C2H9 vs C3H9	C2H9 vs C3H9
C2H9 vs C4H9	C2H9 vs C4H9
C3H9 vs C4H9	**C3H9 vs C4H9 ***
C1H14 vs C2H14	C1H14 vs C2H14
C1H14 vs C3H14	C1H14 vs C3H14
C1H14 vs C4H14	C1H14 vs C4H14
C2H14 vs C3H14	C2H14 vs C3H14
C2H14 vs C4H14	C2H14 vs C4H14
C3H14 vs C4H14	C3H14 vs C4H14

Table 3.9 Galactinol showing relevant between treatment comparisons for galactinol. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Galactinol)
Well-watered vs Droughted
C1H7 vs C1H7 *
C2H7 vs C2H7
C3H7 vs C3H7 *
C4H7 vs C4H7
C1H9 vs C1H9
C2H9 vs C2H9 *
C3H9 vs C3H9
C4H9 vs C4H9 *
C1H14 vs C1H14
C2H14 vs C2H14
C3H14 vs C3H14
C4H14 vs C4H14

Table 3.10 Proline showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Proline)	
Well-watered	Droughted
C1H7 x C1H9	C1H7 x C1H9
C1H7 x C1H14	C1H7 x C1H14
C1H9 x C1H14	**C1H9 x C1H14 ***
C2H7 x C2H9	C2H7 x C2H9
C2H7 x C2H14	**C2H7 x C2H14 ***
C2H9 x C2H14	**C2H9 x C2H14 ***
C3H7 x C3H9	C3H7 x C3H9
C3H7 x C3H14	C3H7 x C3H14
C3H9 x C3H14	**C3H9 x C3H14 ***
C4H7 x C4H9	**C4H7 x C4H9 ***
C4H7 x C4H14	C4H7 x C4H14
C4H9 x C4H14	**C4H9 x C4H14 ***
C1H7 x C2H7	C1H7 x C2H7
C1H7 x C3H7	C1H7 x C3H7
C1H7 x C4H7	C1H7 x C4H7
C2H7 x C3H7	C2H7 x C3H7
C2H7 x C4H7	C2H7 x C4H7
C3H7 x C4H7	C3H7 x C4H7
C1H9 x C2H9	C1H9 x C2H9
C1H9 x C3H9	C1H9 x C3H9
C1H9 x C4H9	C1H9 x C4H9
C2H9 x C3H9	**C2H9 x C3H9 ***

Average signal intensity (Proline)	
Well-watered	Droughted
C2H9 x C4H9	**C2H9 x C4H9 ***
C3H9 x C4H9	C3H9 x C4H9
C1H14 x C2H14	C1H14 x C2H14
C1H14 x C3H14	C1H14 x C3H14
C1H14 x C4H14	C1H14 x C4H14
C2H14 x C3H14	**C2H14 x C3H14 ***
C2H14 x C4H14	C2H14 x C4H14
C3H14 x C4H14	C3H14 x C4H14

Table 3.11 Proline showing relevant between treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Proline)
Well-watered vs Droughted
C1H7 x C1H7
C2H7 x C2H7 *
C3H7 x C3H7 *
C4H7 x C4H7
C1H9 x C1H9
C2H9 x C2H9
C3H9 x C3H9 *
C4H9 x C4H9 *
C1H14 x C1H14
C2H14 x C2H14
C3H14 x C3H14
C4H14 x C4H14

Table 3.12 Phenylalanine showing relevant within treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Phenylalanine)	
Well-watered	Droughted
C1H7 x C1H9	C1H7 x C1H9
C1H7 x C1H14	C1H7 x C1H14
C1H9 x C1H14	C1H9 x C1H14
C2H7 x C2H9	C2H7 x C2H9
C2H7 x C2H14	C2H7 x C2H14
C2H9 x C2H14	C2H9 x C2H14
C3H7 x C3H9	C3H7 x C3H9
C3H7 x C3H14	C3H7 x C3H14
C3H9 x C3H14	C3H9 x C3H14
C4H7 x C4H9	**C4H7 x C4H9 ***
C4H7 x C4H14	**C4H7 x C4H14 ***

Average signal intensity (Phenylalanine)	
Well-watered	Droughted
C4H9 x C4H14	C4H9 x C4H14
C1H7 x C2H7	C1H7 x C2H7
C1H7 x C3H7	C1H7 x C3H7
C1H7 x C4H7	C1H7 x C4H7
C2H7 x C3H7	C2H7 x C3H7
C2H7 x C4H7	C2H7 x C4H7
C3H7 x C4H7	C3H7 x C4H7
C1H9 x C2H9	C1H9 x C2H9
C1H9 x C3H9	C1H9 x C3H9
C1H9 x C4H9	C1H9 x C4H9
C2H9 x C3H9	C2H9 x C3H9
C2H9 x C4H9	C2H9 x C4H9
C3H9 x C4H9	C3H9 x C4H9
C1H14 x C2H14	C1H14 x C2H14
C1H14 x C3H14	C1H14 x C3H14
C1H14 x C4H14	C1H14 x C4H14
C2H14 x C3H14	C2H14 x C3H14
C2H14 x C4H14	C2H14 x C4H14
C3H14 x C4H14	C3H14 x C4H14

Table 3.13 Phenylalanine showing relevant between treatment comparisons. Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0007$.

Average signal intensity (Phenylalanine)
Well-watered vs Droughted
C1H7 x C1H7
C2H7 x C2H7 *
C3H7 x C3H7 *
C4H7 x C4H7
C1H9 x C1H9
C2H9 x C2H9
C3H9 x C3H9 *
C4H9 x C4H9 *
C1H14 x C1H14
C2H14 x C2H14
C3H14 x C3H14
C4H14 x C4H14

3.6.2 Experiment #2 tables

Table 3.14 Glycolic acid showing relevant within origin x harvest time comparisons. Okanese (C1) and Walker (C2) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered (WW) and droughted (D) conditions and harvested at predawn (AM) and midday (PM). Bolded asterisks within columns indicate differences according to Bonferroni's test $p \leq 0.0013$.

Average signal intensity (Glycolic acid)			
AL x AM (A)	AL x PM (B)	SK x AM (C)	SK x PM (D)
C1WW vs C1D *	**C1WW vs C1D ***	C1WW vs C1D	**C1WW vs C1D ***
C2WW vs C2D	C2WW vs C2D	C2WW vs C2D	C2WW vs C2D
C1WW vs C2WW *	**C1WW vs C2WW ***	C1WW vs C2WW	**C1WW vs C2WW ***
C1D vs C2D *	**C1D vs C2D ***	C1D vs C2D	C1D vs C2D

Table 3.15 Threonic acid showing relevant within origin x harvest time comparisons. Okanese (C1) and Walker (C2) sourced from Alberta (AB) and Saskatchewan (SK) under well-watered (WW) and droughted (D) conditions and harvested at predawn (AM) and midday (PM). Bolded asterisks within columns indicate differences according to Bonferroni's test $p \leq 0.0013$.

Average signal intensity (Threonic acid)			
AL x AM (A)	AL x PM (B)	SK x AM (C)	SK x PM (D)
C1WW x C1D *	**C1WW x C1D ***	C1WW x C1D	**C1WW x C1D ***
C2WW x C2D	C2WW x C2D	C2WW x C2D	C2WW x C2D
C1WW x C2WW *	**C1WW x C2WW ***	C1WW x C2WW	**C1WW x C2WW ***
C1D x C2D *	**C1D x C2D ***	**C1D x C2D ***	**C1D x C2D ***

3.6.3 Experiment #3 tables

Table 3.16 Net photosynthesis showing relevant within and between treatment comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions. Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.001$.

Net photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)
Clone x Treatment
C1WW vs C1D
C2WW vs C2D *
C3WW vs C3D
C4WW vs C4D
C5WW vs C5D
C6WW vs C6D
C1WW vs C2WW
C1WW vs C3WW
C1WW vs C4WW
C1WW vs C5WW
C1WW vs C6WW
C2WW vs C3WW *
C2WW vs C4WW

Net photosynthesis (µmol m^{-2} s^{-1})
Clone x Treatment
C2WW vs C5WW
C2WW vs C6WW
C3WW vs C4WW *
C3WW vs C5WW
C3WW vs C6WW *
C4WW vs C5WW
C4WW vs C6WW
C5WW vs C6WW
C1D vs C2D *
C1D vs C3D
C1D vs C4D
C1D vs C5D
C1D vs C6D
C2D vs C3D *
C2D vs C4D *
C2D vs C5D *
C2D vs C6D *
C3D vs C4D
C3D vs C5D
C3D vs C6D
C4D vs C5D
C4D vs C6D
C5D vs C6D

Table 3.17 Succinic acid showing relevant within harvest time comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Succinic acid)	
AM	PM
C1WW vs C2WW	C1WW vs C2WW
C1WW vs C3WW	**C1WW vs C3WW ***
C1WW vs C4WW	C1WW vs C4WW
C1WW vs C5WW	C1WW vs C5WW
C1WW vs C6WW	C1WW vs C6WW
C2WW vs C3WW *	C2WW vs C3WW
C2WW vs C4WW *	C2WW vs C4WW
C2WW vs C5WW	C2WW vs C5WW
C2WW vs C6WW *	C2WW vs C6WW
C3WW vs C4WW	C3WW vs C4WW
C3WW vs C5WW *	**C3WW vs C5WW ***
C3WW vs C6WW	C3WW vs C6WW
C4WW vs C5WW *	C4WW vs C5WW
C4WW vs C6WW	C4WW vs C6WW

Average signal intensity (Succinic acid)	
AM	PM
C5WW vs C6WW *	C5WW vs C6WW
C1D vs C2D	C1D vs C2D
C1D vs C3D *	**C1D vs C3D ***
C1D vs C4D	**C1D vs C4D ***
C1D vs C5D	C1D vs C5D
C1D vs C6D	C1D vs C6D
C2D vs C3D *	**C2D vs C3D ***
C2D vs C4D *	**C2D vs C4D ***
C2D vs C5D	C2D vs C5D
C2D vs C6D	C2D vs C6D
C3D vs C4D *	C3D vs C4D
C3D vs C5D *	**C3D vs C5D ***
C3D vs C6D *	**C3D vs C6D ***
C4D vs C5D	**C4D vs C5D ***
C4D vs C6D *	C4D vs C6D
C5D vs C6D	C5D vs C6D
C1WW vs C1D *	**C1WW vs C1D ***
C2WW vs C2D	C2WW vs C2D
C3WW vs C3D *	**C3WW vs C3D ***
C4WW vs C4D	**C4WW vs C4D ***
C5WW vs C5D *	**C5WW vs C5D ***
C6WW vs C6D	**C6WW vs C6D ***

Table 3.18 Succinic acid showing relevant between harvest time comparisons for galactinol. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Succinic acid)
AM vs PM
C1WW vs C1WW
C2WW vs C2WW *
C3WW vs C3WW
C4WW vs C4WW
C5WW vs C5WW
C6WW vs C6WW
C1D vs C1D
C2D vs C2D *
C3D vs C3D
C4D vs C4D *
C5D vs C5D
C6D vs C6D *

Table 3.19 Glycolic acid showing relevant within harvest time comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Glycolic acid)	
AM	PM
C1WW vs C2WW	**C1WW vs C2WW ***
C1WW vs C3WW	**C1WW vs C3WW ***
C1WW vs C4WW	C1WW vs C4WW
C1WW vs C5WW	**C1WW vs C5WW ***
C1WW vs C6WW	C1WW vs C6WW
C2WW vs C3WW	C2WW vs C3WW
C2WW vs C4WW	C2WW vs C4WW
C2WW vs C5WW	C2WW vs C5WW
C2WW vs C6WW	**C2WW vs C6WW ***
C3WW vs C4WW	C3WW vs C4WW
C3WW vs C5WW *	C3WW vs C5WW
C3WW vs C6WW	**C3WW vs C6WW ***
C4WW vs C5WW	C4WW vs C5WW
C4WW vs C6WW	C4WW vs C6WW
C5WW vs C6WW	**C5WW vs C6WW ***
C1D vs C2D	C1D vs C2D
C1D vs C3D	**C1D vs C3D ***
C1D vs C4D	**C1D vs C4D ***
C1D vs C5D	C1D vs C5D
C1D vs C6D	C1D vs C6D
C2D vs C3D *	**C2D vs C3D ***
C2D vs C4D *	**C2D vs C4D ***
C2D vs C5D	**C2D vs C5D ***
C2D vs C6D	C2D vs C6D
C3D vs C4D	C3D vs C4D
C3D vs C5D	C3D vs C5D
C3D vs C6D *	C3D vs C6D
C4D vs C5D	C4D vs C5D
C4D vs C6D *	C4D vs C6D
C5D vs C6D *	C5D vs C6D
C1WW vs C1D	**C1WW vs C1D ***
C2WW vs C2D	C2WW vs C2D
C3WW vs C3D *	**C3WW vs C3D ***
C4WW vs C4D	**C4WW vs C4D ***
C5WW vs C5D	C5WW vs C5D
C6WW vs C6D	**C6WW vs C6D ***

Table 3.20 Glycolic acid showing relevant between harvest time comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Glycolic acid)
AM vs PM
C1WW vs C1WW *
C2WW vs C2WW
C3WW vs C3WW
C4WW vs C4WW
C5WW vs C5WW
C6WW vs C6WW *
C1D vs C1D
C2D vs C2D
C3D vs C3D
C4D vs C4D
C5D vs C5D
C6D vs C6D

Table 3.21 Malic acid showing relevant between clone and treatment comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions. Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0014$.

Average signal intensity (Malic acid)
Clone x Treatment
C1WW vs C2WW *
C1WW vs C3WW *
C1WW vs C4WW *
C1WW vs C5WW
C1WW vs C6WW *
C2WW vs C3WW
C2WW vs C4WW
C2WW vs C5WW
C2WW vs C6WW
C3WW vs C4WW
C3WW vs C5WW
C3WW vs C6WW
C4WW vs C5WW
C4WW vs C6WW
C5WW vs C6WW
C1D vs C2D
C1D vs C3D *
C1D vs C4D *
C1D vs C5D
C1D vs C6D
C2D vs C3D *
C2D vs C4D *

Average signal intensity (Malic acid)
Clone x Treatment
C2D vs C5D
C2D vs C6D
C3D vs C4D
C3D vs C5D *
C3D vs C6D *
C4D vs C5D *
C4D vs C6D *
C5D vs C6D
C1WW vs C1D
C2WW vs C2D
C3WW vs C3D
C4WW vs C4D *
C5WW vs C5D
C6WW vs C6D

Table 3.22 Threonic acid showing relevant within harvest time comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks within columns indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Threonic acid)	
AM	PM
C1WW vs C2WW	C1WW vs C2WW
C1WW vs C3WW	C1WW vs C3WW *
C1WW vs C4WW	C1WW vs C4WW
C1WW vs C5WW	C1WW vs C5WW
C1WW vs C6WW	C1WW vs C6WW
C2WW vs C3WW	C2WW vs C3WW
C2WW vs C4WW	C2WW vs C4WW
C2WW vs C5WW	C2WW vs C5WW
C2WW vs C6WW *	C2WW vs C6WW
C3WW vs C4WW	**C3WW vs C4WW ***
C3WW vs C5WW	C3WW vs C5WW
C3WW vs C6WW	C3WW vs C6WW
C4WW vs C5WW	**C4WW vs C5WW ***
C4WW vs C6WW	C4WW vs C6WW
C5WW vs C6WW	C5WW vs C6WW
C1D vs C2D	C1D vs C2D
C1D vs C3D *	**C1D vs C3D ***
C1D vs C4D	**C1D vs C4D ***
C1D vs C5D	**C1D vs C5D ***
C1D vs C6D	C1D vs C6D
C2D vs C3D *	**C2D vs C3D ***
C2D vs C4D *	**C2D vs C4D ***
C2D vs C5D *	**C2D vs C5D ***
C2D vs C6D	**C2D vs C6D ***

Average signal intensity (Threonic acid)	
AM	PM
C3D vs C4D	C3D vs C4D
C3D vs C5D	C3D vs C5D
C3D vs C6D *	**C3D vs C6D ***
C4D vs C5D	C4D vs C5D
C4D vs C6D *	C4D vs C6D
C5D vs C6D *	C5D vs C6D
C1WW vs C1D	C1WW vs C1D
C2WW vs C2D	C2WW vs C2D
C3WW vs C3D	C3WW vs C3D
C4WW vs C4D *	**C4WW vs C4D ***
C5WW vs C5D	C5WW vs C5D
C6WW vs C6D *	C6WW vs C6D

Table 3.23 Threonic acid showing relevant between harvest time comparisons. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions, and harvested at predawn (AM) and midday (PM). Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0006$.

Average signal intensity (Threonic acid)
AM vs PM
C1WW vs C1WW
C2WW vs C2WW
C3WW vs C3WW
C4WW vs C4WW
C5WW vs C5WW
C6WW vs C6WW
C1D vs C1D
C2D vs C2D
C3D vs C3D
C4D vs C4D
C5D vs C5D
C6D vs C6D *

Table 3.24 Fructose showing relevant comparisons for clone x treatment interaction. AP947 (C1), AP1006 (C2), AP1005 (C3), AP2278 (C4), AP2298 (C5), and AP2300 (C6) poplar trees under well-watered (WW) and droughted (D) conditions. Bolded asterisks indicate significant differences according to Bonferroni's test $p \leq 0.0014$.

Average signal intensity (Fructose)
Clone x Treatment
C1WW vs C1D
C2WW vs C2D
C3WW vs C3D *
C4WW vs C4D *
C5WW vs C5D
C6WW vs C6D

Average signal intensity (Fructose)
Clone x Treatment
C1WW vs C2WW
C1WW vs C3WW *
C1WW vs C4WW
C1WW vs C5WW
C1WW vs C6WW
C2WW vs C3WW *
C2WW vs C4WW
C2WW vs C5WW *
C2WW vs C6WW
C3WW vs C4WW *
C3WW vs C5WW *
C3WW vs C6WW *
C4WW vs C5WW
C4WW vs C6WW
C5WW vs C6WW
C1D vs C2D
C1D vs C3D
C1D vs C4D *
C1D vs C5D *
C1D vs C6D
C2D vs C3D
C2D vs C4D *
C2D vs C5D *
C2D vs C6D
C3D vs C4D *
C3D vs C5D *
C3D vs C6D
C4D vs C5D
C4D vs C6D *
C5D vs C6D *

CHAPTER 4
Discussion and Conclusions

4.1 Physiological responses to drought

The parameters used to define “drought conditions” for the three independent experiments investigated in this thesis vary. In Experiments #2 and #3, drought conditions were defined as the length of time necessary to produce statistically significant change in midday stomatal conductance for all the clones under study (Hamanishi et al., unpublished manuscript). In contrast, Experiment #1 defined “drought conditions” as the length of time necessary to reduce percent soil water content (% SWC) to approximately 50%, and included an additional drought time point (9DAD) as well as a recovery time point (14DAD). Because of these differences in definition and application of “drought conditions”, it cannot be assumed that the “drought conditions” were experienced by the clones at the same leaf water potentials. The more complex experimental design of Experiment #1 was established in an attempt to identify physiological behaviours and metabolic compounds that show trends across increasing severity of drought conditions, as well as those trends associated with a recovery period.

Overall, stomatal conductance showed significant decreases for drought exposed trees in Experiments #2 and #3 (Figure 3.20, Figure 3.29). In Experiment #3, net photosynthetic rate also decreased under drought conditions, though when compared to stomatal conductance, net photosynthesis seemed to be less affected by drought; this reflects a normal response to osmotic stress and indicates that the trees adjusted to the stress conditions by increasing water use efficiency (Figure 3.30). Experiment #1 showed an overall decrease in leaf water potential for trees grown under drought conditions. However, despite these overall trends, it is interesting to note for all the physiological measurements taken, the clones studied show variations in response to drought stress. For instance, in Experiments #1 and #2, though the physiological measurements taken were not in common, Okanese showed an interesting physiological behaviour that may be associated with improved resistance to drought relative to the other clones studied; Okanese displayed higher leaf water potential and stomatal conductance under drought conditions in Experiments #1 and #2,

respectively, the significance and interpretation of which will be discussed in more detail below (Section 4.3).

The clones employed in Experiment #3 all exhibited higher stomatal conductances under well-watered conditions than the clones employed in Experiment #2. When under drought conditions, however, clones of Experiment #3 displayed sharp decreases in stomatal conductance, in some cases well below the stomatal conductances observed for the clones studied in Experiment #2. One explanation for the observed differences in stomatal conductance and leaf water potential observed for clones from each of the three experiments is the differences of their geoclimatic origins, with the clones employed in Experiment #3 and some of the clones of Experiments #1 and #2 (e.g. Walker and Katepwa) showing greater contribution from their riparian heritage, and Okanese showing characteristics more common of its upland heritage (Silim et al. 2009). Another possible explanation for these differences, however, may be due to differences in maximum photosynthetic photon flux density (PPFD) between the two experiments.

Average daily growth rate also showed clear patterns in response to drought in Experiment #1. Under drought conditions, growth rates significantly decreased for all clones at all three harvest times, 7DAD, 9DAD, and 14DAD (Figure 3.3). Growth rate measured at the recovery time point (14DAD) showed evidence of an increasing trend, as would be expected with re-application of well-watered conditions. Significant differences in clones, regardless of water treatment, were observed, with Okanese and Katepwa displaying the lower growth rates and Walker displaying the highest growth rate.

Despite the leaf water potential measurements of Experiment #1, %SWC did not display a clone effect, and instead displayed a treatment x harvest time effect. This indicates that although Okanese showed higher leaf water potential (Experiment #1), this could not have been the result of a higher soil water content (Figure 3.1).

4.2 Responses of the metabolic profile to drought

One approach to understanding changes in physiological processes is to track the behaviour of soluble metabolites as they are perturbed by a change in environmental conditions. In the studies described in this thesis, metabolites that showed significant differences between treatment and clone comparisons, and were common to all three experiments, were chosen from the metabolite profile for more in depth analysis into the metabolic response to drought stress for trees of *Populus* spp. This group of metabolites includes representatives from the citric acid cycle (succinic acid and malic acid), photorespiration (glycolic acid), redox reactions (threonic acid), and diverse compounds that are thought to play key roles in osmotic adjustment or as compatible solutes (quinic acid, galactinol, raffinose, and fructose). In addition, three amino acid species (which may also function as compatible osmotica), two phenolic species, and an additional ascorbic acid derivative all of which showed interesting fold change patterns under drought stress were also examined further. These additional metabolites include phenylalanine, tryptophan, proline, catechin, kaempferol, and dehydroascorbic acid.

All of the metabolites that were chosen for in depth analysis via analysis of variance showed distinct fold change patterns when comparing well-watered conditions to drought conditions (Figure 3.4). The heat map constructed for metabolites identified in Experiment #1 showed many sharp increases in amino acids (e.g. phenylalanine, tryptophan, and proline), some of which decreased to near or below well-watered levels upon recovery (at 14DAD). Other metabolite species showing a less pronounced, though consistent, increase in response to drought stress were the carbohydrates raffinose and galactinol, noted compatible solutes, and the antioxidants, catechin and kaempferol. General decreases in metabolite level between well-watered and drought conditions were exhibited by all other metabolite species examined. The following discussion will focus on the metabolites chosen for in depth analysis using analysis of variance, and will attempt to describe their possible contribution to the ability of a clone to withstand drought stress. This information will also be discussed in comparison to the

metabolic behaviour of the pure *Populus balsamifera* clones employed in Experiment #3.

The fact that differences in metabolite levels of the aforementioned compounds were found to be significantly represented throughout treatment and clone comparisons in each of the three independent experiments, itself indicates the prominence that these biochemical pathways have in plant response to drought stress. Altered photorespiration is an expected response for plants experiencing stress, particularly water deficit. This metabolic pathway presents an effective way for plants to eliminate excess energy (in the form of ATP and NADPH) that cannot be used in CO_2 fixation due to the decrease in CO_2 concentration in the substomatal space caused by stomatal closure (e.g. Noctor et al. 2002, Biehler and Fock 1996). Elimination of excess energy is important in protecting the integrity of the photosynthetic electron transport chain. In parallel, chemical species involved in regulating the redox state of the cell are also important. These compounds accept electrons from oxygen free radicals that can be produced as a result of electron transfer processes or auto-oxidation. Therefore, with increases in photorespiratory rates relative to net CO_2 fixation under drought stress, one might also expect increases in reactive oxygen species production (through photorespiratory activity, the Mehler reaction, and, if stress is severe enough, through direct transfer to O_2 from photosystem II) and a concomitant need for antioxidants to quench their production (Noctor et al. 2002). In the present study, drought stress in all three experiments showed a decreasing trend in glycolic acid concentrations (Figure 3.6, Figure 3.22, Figure 3.32). This result concurs with previous studies, showing that though relative rates of photorespiration in comparison to net CO_2 fixation increase, the absolute rate of photorespiration actually decreases under drought stress conditions (Noctor et al. 2002, Haupt-Herting and Fock 2002, Biehler and Fock 1996). If this is in fact the case, it might follow that under drought stress there is a reduced need or greater metabolism of antioxidants such as ascorbic acid and its derivatives (such as threonic acid and dehydroascorbic acid) which are commonly ascribed to scavenging reactive oxygen species (such as in the Halliwell-Asada pathway)

(DeBolt et al. 2007, Helsper and Loewus 1982, Robinson and Bunce 2000). Our current findings concur with this theory, as threonic acid shows a decreasing trend under drought stress conditions in all three independent experiments (Figure 3.8, Figure 3.24, Figure 3.34).

Drought stress is also known to affect the metabolic rate of the citric acid cycle (Rodriguez-Calcerrada et al. 2009). It is thought that because of decreases in carbohydrate synthesis resulting from reduced CO_2 fixation, mitochondrial respiration is also depressed under drought conditions. Though metabolite levels cannot be attributed to differences in pathway flux in the studies presented in this thesis, citric acid cycle intermediates (e.g., succinic acid, malic acid) overall display decreased levels under drought conditions (Figure 3.5, Figure 3.7), which may correlate to substrate limitations as would occur under drought stress (Rodriguez-Calcerrada et al. 2009). Succinic acid exhibits variability between clones, harvest times, and water treatments for all three experiments (Figure 3.5, Figure 3.21, Figure 3.31). A potential complication in assessing the impact that drought stress might have on levels of succinic acid arises from the fact that, though succinic acid is a constituent of the citric acid cycle, it also plays a role in γ-aminobutyric acid (GABA) metabolism, a pathway that has been proposed to be up-regulated in response to a variety of abiotic stresses, including drought (Allan et al. 2008).

Malic acid showed an interesting pattern: though it is significantly decreased under drought stress, when comparing the levels in the individual clones of Experiment #1, malic acid showed an increase in what might be considered the more drought resistant clone, Okanese (Figure 3.7). Patterns of increased and decreased abundance of malic acid have previously been reported in the literature (Koussevitzky et al. 2008, Tschaplinski and Tuskan 1994). It has been proposed that tree species having a greater capacity to resist the deleterious effects associated with drought stress accumulate malic acid as an osmotic adjustment mechanism (Tschaplinski and Tuskan 1994, Peltier et al. 1997). However, when comparing the general metabolic effects of drought stress across several clones, malic acid levels decrease (Koussevitzky et al. 2008,

Passarinho et al. 2006). Malic acid is probably involved in a number of different cellular responses to drought stress and is likely not restricted to its roles as a member of the citric acid cycle, or in osmotic adjustment. Malic acid is the most abundant organic acid produced in plants (MacLennan 1963, Passarinho et al. 2006), and is involved in a variety of roles ranging from cellular pH buffering to being an intermediate in biochemical pathways (e.g. citric acid cycle and stomatal response to ABA) and participating in translocation transactions in mitochondria and chloroplasts (MacLennan 1963).

Another organic acid thought to be involved in osmotic adjustment is quinic acid. This acid has been found to increase in cork oak under natural drought conditions (Passarinho et al. 2006). Furthermore, it has also been found to increase in *Populus* spp. under applied drought conditions (Gebre et al. 1994). Quinic acid has a molecular structure similar to another metabolite compound, quercitol, which was found to be part of the osmotic adjustment strategy of drought-resistant *Eucalyptus* species (Merchant et al. 2006). Quinic acid is also a key metabolite involved in lignification and can be formed from shikimic acid by quinate hydrolyase. It was found to accumulate in *Abies picea* needles prior to participating in lignification processes, so it may be possible that under growth limiting conditions (such as those shown to occur during drought), quinic acid accumulates due to lack of utilization in other activities such as active growth (Leuschner et al. 1995, Grace and Logan 2000). For the results obtained in the experiments outlined in this thesis, however, quinic acid levels vary: overall, decreases occured for all clones under drought conditions at both midday and predawn harvests (Experiment #2 and #3) (Figure 3.25, Figure 3.35). This may be due to reduced CO_2 fixation under drought stress. Quinic acid does not show a treatment effect in Experiment #1, but does show a clone main effect, with Okanese and Katepwa showing significantly lower levels than those for Walker and WP-86V-86 (Figure 3.9). One interpretation for this result could be that Okanese and Katepwa were able to adjust their growth patterns to the new conditions of reduced photosynthesis and growth better than that of the other two clones.

Fructose, like malic acid, shows a variable response to drought. Though it displayed an increasing trend for Okanese, WP-86V-86, and Katepwa in Experiment #1, Walker showed decreases in fructose content (Figure 3.12). This is in contrast to the results of Experiment #2 which shows increases in fructose level for both Okanese and Walker (Figure 3.28). Experiment #3 displayed overall decreases in fructose levels in response to drought (Figure 3.38). Fructose is a monosaccharide that has been noted in some studies to increase in response to drought conditions. The level accumulated tends to fluctuate with time of harvest (e.g. harvest at predawn vs. midday) (Gebre and Tschaplinski 2002, Sziderics et al. 2010). The results of Experiment #2 and #3 are in agreement with these previous observations. One complication in the analysis and interpretation of fructose levels is the fact that fructose, like malic acid, is an intermediate in several different biochemical pathways and reactions which are involved in the primary metabolism of plant cells (e.g. synthesis and breakdown of sucrose, cell respiration). Roughly speaking, from the heat map constructed for Experiment #1 (Figure 3.4), fructose seems to exhibit an inverse trend in comparison to the trends exhibited by sucrose (i.e. when fructose levels decreased under drought conditions, sucrose levels appeared to increase). Because both fructose and sucrose are important constituents of the primary metabolism of plant cells, it might be possible that fluctuations in their levels depend on specific drought application methods, the time at which harvest took place, and the specific plant species studied.

Raffinose and galactinol, a trisaccharide and sugar alcohol, respectively, showed very clear responses to drought stress for all clones studied, in contrast to fructose. Overall, all clones showed increases in raffinose and galactinol content under drought stress conditions (Experiment #1) (Figure 3.10, Figure 3.11), and also showed significant increases at midday harvests in comparison to predawn harvests (Experiments #2 and #3) (Figure 3.26, Figure 3.27, Figure 3.36, Figure 3.37). Raffinose and galactinol have been suggested to hold a variety of roles in the plant cell under stress, including osmotic adjustment, reactive oxygen species scavenging, and osmoprotection of membranes and

proteins (Sziderics et al. 2010). Different clones, however, did show varying responses in raffinose and galactinol accumulation. In particular, Okanese and Katepwa in Experiment #1 did not significantly accumulate galactinol under stress conditions until nine days after drought. In contrast, the other two clones in this experiment showed significant increases at day seven (Table 3.9). This pattern was supported in Experiment #2 where Okanese also did not show as significant an increase in galactinol as did Walker (Figure 3.26). Experiment #2 also showed that Okanese had an overall lower content of raffinose in comparison to Walker at both predawn and midday (Figure 3.27). Since galactinol is a precursor to the biosynthesis of raffinose, it is possible that Okanese possessed lower flux through this pathway towards raffinose production and accumulation (Sziderics et al. 2010, Taji et al. 2002). It has been suggested that raffinose plays a role in protecting the membrane structure of chloroplasts, which would be beneficial in maintaining their integrity for photosynthetic processes to resume upon recovery (Santarius 1973).

As indicated in the heat map for Experiment #1 (Figure 3.4), most amino acids showed pronounced increases under drought conditions relative to well-watered conditions. Much of the increases in amino acid content in leaf tissue of drought-stressed trees were likely due to decreases in protein synthesis in leaf cells in response to drought stress and curtailment of growth (Lawlor and Cornic 2002). One clear example of amino acid accumulation can be seen in tryptophan, which shows highly increased levels under drought relative to well-watered trees (Figure 3.15). This pattern can be seen across all clones for all harvest times of Experiment #1, with levels of tryptophan dropping to near well-watered levels upon re-watering at 14DAD. Overall, WP-86V-86 accumulated a significantly higher level of tryptophan than any of the other clones of Experiment #1. Tryptophan has not been previously reported in the literature as being a significant part of osmotic adjustment mechanisms or as a compatible solute, and its apparent increases in drought-stressed leaf tissue is most likely due to declines in protein synthesis. However, another possibility involves its role as a precursor in auxin biosynthesis and, in this capacity, tryptophan may be involved

in regulation of root growth and thus ability to collect water from the soil environment. A recent study investigating the effects of mutant *Oryza sativa* plants deficient in the ability to synthesize auxin from tryptophan showed that these plants had decreased turgor pressure and transpiration rates, and lower root/shoot ratios in comparison to wild type plants (Woo et al. 2007). Levels of tryptophan may be indicative of the ability of a plant to adjust allocation of resources through hormonal mediation towards enhanced root growth under drought conditions, though this is speculation and more studies would be required to substantiate this idea.

As noted in the introduction, proline accumulation under stress conditions is considered in many cases to be adaptive (Alvarez et al. 2008, Verbruggen and Hermans 2008). It has been suggested that proline can act as a compatible solute and osmotic adjuster, a radical scavenger, and as a storage molecule for nitrogen and carbon (Verbruggen and Hermans 2008, Yancey et al. 1982). However, it is clear that the seemingly simple relationship between drought tolerance and accumulation of proline is actually not so simple at all. For instance, exogenously supplied proline, at concentrations above 10 mM, has been shown to be toxic to wild-type *Arabidopsis thaliana* plants but not to salt stressed plants (Mani et al. 2002). Furthermore, in tobacco plants engineered to produce up to 14 times the wild type proline content, it was observed that prior to initiation of drought conditions, osmotic potential of the transgenic plants was not significantly different to that of wild type tobacco plants; after implementation of drought conditions, however, osmotic potential of wild type plants dropped significantly (whereas the plants engineered to accumulate proline remained relatively unchanged), and proline content had increased so that the difference in content between wild type and transgenic tobacco plants was only two fold (Kishor et al. 1995). Proline accumulation was pronounced in the results of Experiment #1, indicating that under drought conditions, proline accumulation in poplar hybrids does occur, though to what extent this contributed to osmotic adjustment remains unknown (Figure 3.13).

Phenylalanine also showed increased accumulation during drought stress (Figure 3.14). In fact, the patterns of increase mirrored the patterns observed for proline very well. Phenylalanine, the entry point of the phenylpropanoid pathway, is thought to be involved in osmotic adjustment, as well as being a precursor in the biosynthesis of phenolics (Carmo-Silva et al. 2009, Grace and Logan 2000). Phenolic compounds such as catechin and kaempferol are thought to have anitoxidant roles in scavenging of reactive oxygen species in the cytosol, and to filter radiation and decrease excitation of chlorophyll during conditions that are unfavourable for photosynthesis, such as during drought (Hura et al. 2008). Okanese displayed the highest catechin levels overall, regardless of water treatment, though its levels were not significantly different than the levels exhibited by Katepwa. Catechin levels were significantly lower in both Walker and WP-86V-86 of Experiment #1 (Figure 3.16). In contrast, kaempferol levels were found to be lowest for Okanese and highest for Katepwa (Figure 3.17). Regardless of clone, both catechin and kaempferol levels were increased under drought conditions at 9DAD and 14DAD, but not significantly increased at 7DAD.

4.3 Okanese displays unique drought response pattern

Okanese exhibited some interesting and unique responses to drought as shown in the experiments outlined above. It displayed the highest leaf water potential under drought conditions, values that were not significantly different from well-watered conditions (Figure 3.2, Table 3.2). This is in contrast to the results of Silim et al. (2009), who studied three clones that are in common with Experiment #1: Okanese, Walker, and Katepwa. Okanese also displayed higher stomatal conductance and transpiration rates under the drought conditions of Experiment #2 when compared to Walker (Figure 3.20). In the study carried out by Silim et al. (2009), Okanese showed a very gradual decline in stomatal conductance in relation to leaf water potential, indicating a sensitive control over stomatal conductance in response to physiological water status. Metabolically, in comparison to other clones, Okanese showed the lowest accumulation of osmotica such as proline, raffinose, and galactinol, but also displayed unique patterns among metabolites such as malic acid, glycolic acid, catechin and

dehydroascorbic acid; each of these metabolite species showed high levels regardless of water treatment (dehydroascorbic acid and catechin) or a slight increasing trend under drought conditions (malic and glycolic acids) (Figure 3.6, Figure 3.7, Figure 3.16, Figure 3.18). The apparent trends in these latter metabolites is unique in comparison to the decreasing trends witnessed in other clones, and is suggestive of a better functioning and possibly more conserved (i.e. more resistant) metabolic system under the prevailing growth conditions. Okanese is putatively the most drought-resistant clone studied in Experiments #1 and #2, though did not seem to exhibit the behaviour illustrative of osmotic adjustment in order to lower its leaf water potential in response to drought, as has previously been put forward as a possible mechanism for its drought resistance (Silim et al. 2009). Interestingly, low osmotic adjustment was also observed in another study which investigated the differential drought tolerance mechanisms of two *Populus* spp. clones of different lineage (TD, *P. trichocarpa* x *P. deltoides* and DN, *P. deltoides* x *P. nigra*) (Tschpalinski et al. 1998). Tschaplinski et al. (1998) found that the more drought-resistant clone (DN) exhibited higher leaf water potential and displayed lower osmotic adjustment than the less drought-resistant clone (TD). It was suggested that these differences were due to differential allocation of resources: the more drought-resistant clone (DN) opting to increase root biomass under stress conditions, while the less drought-resistant clone (TD) accumulated osmolytes, lowered leaf water potential, was able to continue growing until the stress became severe, at which time leaf abscission commenced and growth ceased (Tschaplinski et al. 1998).

One possible explanation of the potentially conflicting physiological results observed for Okanese in Experiments #1 and #2 (Okanese in Experiment #2 showing a higher stomatal conductance overall in comparison to Walker, and Okanese in Experiment #1 displaying the highest leaf water potential), is to consider the relative time along the drought stress continuum at which the stomatal conductance and leaf water potential measurements were taken. The physiological behaviour of leaf tissue under drought conditions, as shown by the results of Silim et al. (2009), exhibits a more gradual decline in stomatal

conductance versus leaf water potential for Okanese than for Walker. The stomatal conductance data for Experiment #2 may have been obtained at a leaf water potential corresponding to higher stomatal conductances for Okanese than for Walker (i.e. after the stomatal conductance of Walker “crashes”).

4.4 Conclusions

The experiments outlined in this thesis showed several overall patterns. Biochemically, metabolites involved in the citric acid cycle (succinic acid, malic acid), photorespiration (glycolic acid), and redox regulation (threonic acid) tended to decrease under drought conditions. In contrast, phenolic compounds (catechin, kaempferol) and metabolites involved in osmotic adjustment (raffinose, galactinol, amino acids), generally increased under drought conditions (Figure 3.4).

The clones studied in each of the three experiments described in this thesis displayed evidence of differential physiological and metabolic response to drought stress. Experiments #2 and #3 were discussed in relation to the results obtained for Experiment #1 and the majority of metabolites analyzed in all three experiments exhibited common trends. In general, the metabolite profiling results of Experiments #1 and #2 supported each other, particularly with Walker displaying a greater overall accumulation of compatible solutes in comparison to Okanese, and Okanese displaying greater overall levels of metabolites involved in reactive oxygen species scavenging. In contrast to the metabolite profiling results, the physiological measurements obtained in Experiments #1 and #2 showed the potential for conflicting interpretation. The measurements obtained were not common between experiments: stomatal conductance and transpiration rate were measured in Experiment #2 and leaf water potential, growth rate, and percent soil water content were measured in Experiment #1. It is therefore difficult to ascertain where these two experiments sit in relation to each other on a theoretical continuum of drought stress, and underlines the importance of obtaining an array of common physiological measurements in order to better interpret and qualify the results of biochemical assays such as metabolite profiles. Despite the physiological uncertainty, the metabolite profiles did seem to

indicate that Okanese, putatively the most drought-resistant clone studied in this thesis, does not exhibit osmotic adjustment to the level that is evident in the other clones. The results of a previous study (Silim et al. 2009) suggested that Okanese closes its stomata earlier in response to drought stress than other related hybrid poplars, though another possible explanation for this difference is that it may inherently employ metabolic resources preferentially for the expansion of the root system under drought conditions rather than osmolyte accumulation. Root growth and architecture, however, were not considered in the experiments described in this thesis, and therefore this assertion cannot be validated, though consideration of these physiological and morphological attributes represents an area that could potentially be explored as an aspect for future characterization of this hybrid poplar clone. The other clones studied in Experiment #1, Katepwa and WP-86V-86, exhibited many similar patterns both in physiological and metabolic response to drought as Walker.

As mentioned, the results of Experiment #3 showed similar overall trends in metabolic response to drought stress as those of Experiments #1 and #2. However, the different genetic background of the clones studied in Experiment #3 in comparison with those of Experiments #1 and #2 confounds conclusions that could be made in relation to the results of the other two experiments. Nevertheless, a few of the clones from Experiment #3 displayed notable responses to drought stress. Physiologically, AP1006 displayed the lowest stomatal conductance, whereas AP2298 displayed the highest stomatal conductance under drought conditions. This pattern was reflected for these clones in photosynthetic rates as well as some metabolites such as glycolic and threonic acids, which were uniquely accumulated in AP1006 at midday (Figure 3.32, Figure 3.34). It was noted in a related study (Hamanishi et al., unpublished manuscript) that stomatal conductance of AP1006 was the first to show a statistically significant change in comparison to well-watered trees; it may be that this clone then exhibits the similar gradual decrease in conductance due to drought stress that has been previously attributed to more drought-resistant clones, such as Okanese (Silim et al. 2009).

The objectives set out in the introduction for this thesis were to better understand and characterize the metabolic response to drought stress in *Populus* spp. and to try to relate these responses to the potential for improved drought resistance in select poplar clones. To this end, four different hybrid poplar clones and six different *P. balsamifera* clones were characterized for a set of highly significant metabolites common to all three experiments. As previously mentioned, one particular hybrid poplar, Okanese, stood out from the rest as having a unique response to drought, and this uniqueness was observed on both the physiological and biochemical scales. Thus, Okanese may indeed represent a prototype from which to work from for identifying drought-resistant genotypes that are at present unknown.

4.5 Further research

One advantage of the analytical technique (GC/MS) used in the experiments described in this thesis to investigate metabolic response to drought stress is the capability of producing an enormity of data on large sample sets. This capability provides the opportunity for multiple and flexible approaches to data analysis, from univariate or multivariate statistical analyses to correlation with results of parallel proteomics or transcriptomics studies. For instance, future research directions might use alternative multivariate statistical techniques such as principal components analysis to mine for broader distinctions in the drought response of the poplar clones under study. Another option for further characterization of the drought resistance mechanisms in poplar clones is the coupling of this metabolite profiling information with the information obtained in parallel transcriptomics or proteomics studies; this may uncover correlations between specific metabolite profiles and the underlying genetic/protein background and physiological ability to withstand drought stress.

Additional directions for further research should include identification and further investigation of unknown/unidentified metabolites (e.g. some unidentified metabolites that showed increases under drought such as M457T1450, M373T1461, M471T1463, and M368T1785 and decreases under drought such as M155T904 and M204T1494) or broader characterization of the metabolite

profile using alternative analytical platforms such as high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS). One of the major and well-known limitations to both GC/MS and HPLC/MS technologies is a general dearth of plant compounds present in mass spectral search software for use in metabolite identification, putting the onus on individual laboratories to develop their own metabolite libraries for use in identification, though this particular limitation seems to be gradually being addressed in newer versions of mass spectral search software (Anonymous 2005).

CHAPTER 5

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5.1 References

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APPENDIX A

ANOVA Tables

Appendix A.1. Experiment #1

Table A.1 Anova table for percent soil water content (%SWC) for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.1).

Percent soil water content					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	1.98E+04	6.61E+03	26.04	<.0001
T	1	7.45E+05	7.45E+05	2937.46	<.0001
C*T	3	1.02E+04	3.39E+03	13.37	<.0001
R(C*T)	24	8.10E+04	3.38E+03	13.30	<.0001
H	8	5.31E+05	6.64E+04	261.74	<.0001
C*H	24	3.24E+03	1.35E+02	0.53	0.9653
T*H	8	2.84E+05	3.55E+04	139.86	<.0001
C*T*H	24	1.82E+03	7.58E+01	0.30	0.9995

Tests of Hypotheses Using the Type III MS for R(C*T) as an Error Term					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	1.98E+04	6.61E+03	1.96	0.1473
T	1	7.45E+05	7.45E+05	220.83	<.0001
C*T	3	1.02E+04	3.39E+03	1.01	0.4076

Table A.2 Anova table for leaf water potential for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.2).

Average leaf water potential (Mpa)					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	7.26	2.42	33.20	<.0001
T	1	19.85	19.85	272.51	<.0001
H	2	8.20	4.10	56.29	<.0001
C*T	3	2.57	0.86	11.76	<.0001
C*H	6	2.57	0.43	5.87	<.0001
T*H	2	8.38	4.19	57.53	<.0001
C*T*H	6	2.82	0.47	6.45	<.0001

Table A.3 Anova table for average growth rate per day for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.3).

Average growth rate (cm/day)					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	9.37	3.12	20.13	<.0001
T	1	34.13	34.13	220.04	<.0001
H	2	0.91	0.45	2.93	0.0560
C*T	3	0.54	0.18	1.15	0.3289
C*H	6	4.30	0.72	4.62	0.0002

Average growth rate (cm/day)					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
T*H	2	0.00	0.00	0.00	0.9956
C*T*H	6	4.31	0.72	4.63	0.0002

Table A.4 Anova table for succinic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.5).

Succinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	5.30E+11	1.77E+11	32.73	<.0001
T	1	2.45E+11	2.45E+11	45.34	<.0001
H	2	6.76E+10	3.38E+10	6.26	0.0024
C*T	3	1.33E+11	4.43E+10	8.20	<.0001
C*H	6	7.68E+10	1.28E+10	2.37	0.0316
T*H	2	3.46E+09	1.73E+09	0.32	0.7260
C*T*H	6	7.48E+10	1.25E+10	2.31	0.0358

Table A.5 Anova table for glycolic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.6).

Glycolic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	2.32E+11	7.72E+10	5.71	0.0009
T	1	8.58E+11	8.58E+11	63.47	<.0001
H	2	2.21E+10	1.10E+10	0.82	0.4438
C*T	3	1.56E+11	5.20E+10	3.85	0.0106
C*H	6	1.29E+11	2.15E+10	1.59	0.1533
T*H	2	1.61E+10	8.07E+09	0.60	0.5515
C*T*H	6	2.10E+11	3.50E+10	2.59	0.0196

Table A.6 Anova table for malic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.7).

Malic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	23.60	7.87	62.38	<.0001
T	1	2.38	2.38	18.88	<.0001
H	2	0.61	0.31	2.43	0.0909
C*T	3	0.71	0.24	1.87	0.1365
C*H	6	2.87	0.48	3.79	0.0014
T*H	2	0.43	0.21	1.69	0.1868
C*T*H	6	1.07	0.18	1.42	0.2096

Table A.7 Anova table for threonic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.8).

Threonic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	1.79E+11	5.97E+10	1.77	0.1536
T	1	8.52E+11	8.52E+11	25.34	<.0001
H	2	4.89E+11	2.44E+11	7.27	0.0009
C*T	3	1.55E+11	5.17E+10	1.54	0.2061
C*H	6	3.94E+11	6.57E+10	1.96	0.0743
T*H	2	8.34E+09	4.17E+09	0.12	0.8834
C*T*H	6	2.58E+11	4.30E+10	1.28	0.2687

Table A.8 Anova table for quinic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.9).

Quinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	1.88E+14	6.25E+13	27.47	<.0001
T	1	2.31E+12	2.31E+12	1.01	0.3155
H	2	5.88E+13	2.94E+13	12.92	<.0001
C*T	3	7.00E+12	2.33E+12	1.03	0.3826
C*H	6	2.31E+13	3.85E+12	1.69	0.1254
T*H	2	3.38E+12	1.69E+12	0.74	0.4775
C*T*H	6	2.50E+13	4.17E+12	1.83	0.0954

Table A.9 Anova table for galactinol for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.10).

Galactinol					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	5.45	1.82	6.48	0.0004
T	1	23.75	23.75	84.63	<.0001
H	2	3.13	1.56	5.57	0.0045
C*T	3	0.40	0.13	0.47	0.7024
C*H	6	7.09	1.18	4.21	0.0006
T*H	2	0.72	0.36	1.29	0.2778
C*T*H	6	7.11	1.18	4.22	0.0005

Table A.10 Anova table for raffinose for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.11).

Raffinose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	1.14E+10	3.80E+09	0.64	0.5910
T	1	4.59E+11	4.59E+11	77.20	<.0001
H	2	7.91E+10	3.95E+10	6.65	0.0017
C*T	3	1.76E+10	5.87E+09	0.99	0.4001

Raffinose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C*H	6	1.82E+10	3.03E+09	0.51	0.8000
T*H	2	3.10E+10	1.55E+10	2.61	0.0767
C*T*H	6	4.43E+10	7.38E+09	1.24	0.2874

Table A.11 Anova table for fructose for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.12).

Fructose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	3.50E+13	1.17E+13	7.35	0.0001
T	1	1.72E+11	1.72E+11	0.11	0.7424
H	2	4.98E+11	2.49E+11	0.16	0.8550
C*T	3	2.64E+13	8.80E+12	5.55	0.0011
C*H	6	6.20E+12	1.03E+12	0.65	0.6892
T*H	2	1.80E+13	9.01E+12	5.68	0.0040
C*T*H	6	6.51E+12	1.09E+12	0.68	0.6628

Table A.12 Anova table for proline for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.13).

Proline					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	4.97	1.66	2.62	0.0525
T	1	51.40	51.40	81.19	<.0001
H	2	34.34	17.17	27.13	<.0001
C*T	3	9.63	3.21	5.07	0.0022
C*H	6	7.50	1.25	1.97	0.0716
T*H	2	17.19	8.60	13.58	<.0001
C*T*H	6	13.68	2.28	3.60	0.0022

Table A.13 Anova table for phenylalanine for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.14).

Phenylalanine					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	18.57	6.19	4.60	0.0040
T	1	64.69	64.69	48.07	<.0001
H	2	17.40	8.70	6.47	0.0019
C*T	3	19.88	6.63	4.92	0.0026
C*H	6	9.52	1.59	1.18	0.3197
T*H	2	45.05	22.53	16.74	<.0001
C*T*H	6	22.81	3.80	2.83	0.0119

Table A.14 Anova table for tryptophan for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.15).

Tryptophan					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	3	74.40	24.80	4.26	0.0062
T	1	559.17	559.17	96.05	<.0001
H	2	131.75	65.87	11.32	<.0001
C*T	3	21.57	7.19	1.23	0.2985
C*H	6	22.93	3.82	0.66	0.6849
T*H	2	108.12	54.06	9.29	0.0001
C*T*H	6	27.17	4.53	0.78	0.5884

Table A.15 Anova table for catechin for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.16).

Catechin					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	3	56.87	18.96	25.99	<.0001
T	1	56.72	56.72	77.77	<.0001
H	2	5.21	2.60	3.57	0.0302
G*T	3	3.99	1.33	1.82	0.1448
G*H	6	2.15	0.36	0.49	0.8140
T*H	2	18.12	9.06	12.42	<.0001
G*T*H	6	4.23	0.70	0.97	0.4499

Table A.16 Anova table for kaempferol for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.17).

Kaempferol					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	3	237.94	79.31	104.96	<.0001
T	1	0.85	0.85	1.12	0.2905
H	2	0.24	0.12	0.16	0.8524
G*T	3	1.09	0.36	0.48	0.6953
G*H	6	8.46	1.41	1.87	0.0888
T*H	2	9.19	4.60	6.08	0.0028
G*T*H	6	2.14	0.36	0.47	0.8291

Table A.17 Anova table for dehydroascorbic acid for four hybrid poplar clones (C) grown under two watering regimes (T) and measured at three harvest times (H). (Data shown in Figure 3.18).

Dehydroascrobic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	3	5875.79	1958.60	36.93	<.0001
T	1	14.29	14.29	0.27	0.6044
H	2	177.35	88.67	1.67	0.1908
G*T	3	480.31	160.10	3.02	0.0312
G*H	6	323.00	53.83	1.01	0.4170

Dehydroascrobic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
T*H	2	247.43	123.71	2.33	0.0999
G*T*H	6	348.23	58.04	1.09	0.3675

Appendix A.2. Experiment #2

Table A.18 Anova table for stomatal conductance for two hybrid poplar clones (C) grown under two watering regimes (T) and sourced from two regions (O). (Data shown in Figure 3.20).

Stomatal conductance (mol m^{-2} s^{-1})					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	0.04	0.04	25.86	<.0001
O	1	0.00	0.00	0.74	0.3953
T	1	0.10	0.10	62.06	<.0001
C*O	1	0.01	0.01	3.27	0.0787
C*T	1	0.00	0.00	0.19	0.6650
O*T	1	0.01	0.01	4.74	0.0359
C*O*T	1	0.00	0.00	0.11	0.7394

Table A.19 Anova table for transpiration rate for two hybrid poplar clones (C) grown under two watering regimes (T) and sourced from two regions (O). (Data shown in Figure 3.19).

Transpiration rate (g m^{-2} min^{-1})					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	2.75	2.75	18.90	0.0001
O	1	0.03	0.03	0.21	0.6481
T	1	8.01	8.01	54.96	<.0001
C*O	1	0.30	0.30	2.06	0.1600
C*T	1	0.09	0.09	0.62	0.4350
O*T	1	0.62	0.62	4.27	0.0458
C*O*T	1	0.02	0.02	0.16	0.6902

Table A.20 Anova table for succinic acid for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.21).

Succinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	8.18E+09	8.18E+09	290.06	<.0001
O	1	6.55E+08	6.55E+08	23.22	<.0001
T	1	4.32E+08	4.32E+08	15.33	0.0001
H	1	1.87E+09	1.87E+09	66.27	<.0001
C*O	1	4.02E+09	4.02E+09	142.54	<.0001
C*T	1	2.49E+07	2.49E+07	0.88	0.3491
C*H	1	1.02E+07	1.02E+07	0.36	0.5490
O*T	1	5.18E+07	5.18E+07	1.84	0.1777
O*H	1	4.58E+07	4.58E+07	1.63	0.2046
T*H	1	1.66E+07	1.66E+07	0.59	0.4445

Succinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C*O*T	1	5.14E+08	5.14E+08	18.22	<.0001
C*O*H	1	4.50E+08	4.50E+08	15.95	0.0001
O*T*H	1	4.71E+06	4.71E+06	0.17	0.6833
C*T*H	1	2.63E+07	2.63E+07	0.93	0.3355
C*O*T*H	1	3.23E+06	3.23E+06	0.11	0.7354

Table A.21 Anova table for glycolic acid for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.22).

Glycolic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	5.95E+09	5.95E+09	397.72	<.0001
O	1	3.02E+08	3.02E+08	20.18	<.0001
T	1	7.61E+07	7.61E+07	5.09	0.0258
H	1	7.83E+07	7.83E+07	5.23	0.0238
C*O	1	1.76E+09	1.76E+09	117.92	<.0001
C*T	1	3.08E+08	3.08E+08	20.56	<.0001
C*H	1	1.42E+08	1.42E+08	9.49	0.0025
O*T	1	1.29E+08	1.29E+08	8.62	0.0040
O*H	1	2.40E+06	2.40E+06	0.16	0.6895
T*H	1	1.76E+08	1.76E+08	11.79	0.0008
C*O*T	1	8.48E+06	8.48E+06	0.57	0.4528
C*O*H	1	3.61E+06	3.61E+06	0.24	0.6242
O*T*H	1	1.82E+08	1.82E+08	12.13	0.0007
C*T*H	1	3.87E+07	3.87E+07	2.58	0.1104
C*O*T*H	1	1.05E+08	1.05E+08	7.02	0.0091

Table A.22 Anova table for malic acid for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.23).

Malic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	2.15E+07	2.15E+07	0.13	0.7191
O	1	6.99E+08	6.99E+08	4.22	0.0420
T	1	3.43E+09	3.43E+09	20.71	<.0001
H	1	2.36E+09	2.36E+09	14.27	0.0002
C*O	1	6.38E+09	6.38E+09	38.52	<.0001
C*T	1	7.75E+09	7.75E+09	46.79	<.0001
C*H	1	1.48E+08	1.48E+08	0.89	0.3462
O*T	1	3.53E+08	3.53E+08	2.13	0.1466
O*H	1	4.12E+06	4.12E+06	0.02	0.8750
T*H	1	4.64E+07	4.64E+07	0.28	0.5977
C*O*T	1	5.65E+07	5.65E+07	0.34	0.5603
C*O*H	1	4.31E+07	4.31E+07	0.26	0.6108
O*T*H	1	7.19E+08	7.19E+08	4.34	0.0393

Malic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C*T*H	1	1.38E+09	1.38E+09	8.30	0.0047
C*O*T*H	1	1.97E+06	1.97E+06	0.01	0.9133

Table A.23 Anova table for threonic acid for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.24).

Threonic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	4.75E+10	4.75E+10	790.33	<.0001
O	1	1.64E+09	1.64E+09	27.33	<.0001
T	1	1.92E+09	1.92E+09	31.99	<.0001
H	1	4.50E+08	4.50E+08	7.49	0.0071
C*O	1	9.72E+09	9.72E+09	161.62	<.0001
C*T	1	2.00E+09	2.00E+09	33.24	<.0001
C*H	1	6.38E+08	6.38E+08	10.61	0.0014
O*T	1	1.23E+08	1.23E+08	2.05	0.1543
O*H	1	6.78E+05	6.78E+05	0.01	0.9156
T*H	1	5.42E+07	5.42E+07	0.90	0.3441
C*O*T	1	1.59E+08	1.59E+08	2.64	0.1069
C*O*H	1	5.28E+08	5.28E+08	8.78	0.0036
O*T*H	1	4.38E+08	4.38E+08	7.28	0.0079
C*T*H	1	1.52E+08	1.52E+08	2.53	0.1145
C*O*T*H	1	4.82E+08	4.82E+08	8.01	0.0054

Table A.24 Anova table for quinic acid for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.25).

Quinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	6.78E+10	6.78E+10	10.00	0.0020
O	1	7.67E+09	7.67E+09	1.13	0.2894
T	1	7.70E+10	7.70E+10	11.37	0.0010
H	1	2.03E+10	2.03E+10	3.00	0.0857
C*O	1	1.49E+11	1.49E+11	21.95	<.0001
C*T	1	4.46E+10	4.46E+10	6.57	0.0115
C*H	1	4.57E+08	4.57E+08	0.07	0.7955
O*T	1	2.07E+10	2.07E+10	3.05	0.0832
O*H	1	2.19E+10	2.19E+10	3.22	0.0749
T*H	1	6.08E+07	6.08E+07	0.01	0.9247
C*O*T	1	6.93E+07	6.93E+07	0.01	0.9196
C*O*H	1	5.29E+10	5.29E+10	7.80	0.0060
O*T*H	1	2.75E+10	2.75E+10	4.06	0.0459
C*T*H	1	2.12E+10	2.12E+10	3.13	0.0794
C*O*T*H	1	6.37E+09	6.37E+09	0.94	0.3342

Table A.25 Anova table for galactinol for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.26).

Galactinol					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	88.35	88.35	12.93	0.0005
O	1	17.16	17.16	2.51	0.1155
T	1	102.08	102.08	14.94	0.0002
H	1	3339.03	3339.03	488.72	<.0001
C*O	1	5.73	5.73	0.84	0.3614
C*T	1	28.85	28.85	4.22	0.0419
C*H	1	65.47	65.47	9.58	0.0024
O*T	1	19.38	19.38	2.84	0.0946
O*H	1	55.11	55.11	8.07	0.0053
T*H	1	6.53	6.53	0.96	0.3300
C*O*T	1	52.43	52.43	7.67	0.0064
C*O*H	1	9.13	9.13	1.34	0.2498
O*T*H	1	1.27	1.27	0.19	0.6674
C*T*H	1	1.88	1.88	0.27	0.6010
C*O*T*H	1	0.42	0.42	0.06	0.8040

Table A.26 Anova table for raffinose for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.27).

Raffinose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	1.52E+09	1.52E+09	34.13	<.0001
O	1	7.72E+07	7.72E+07	1.74	0.1899
T	1	1.07E+09	1.07E+09	23.98	<.0001
H	1	5.16E+09	5.16E+09	116.24	<.0001
C*O	1	9.93E+07	9.93E+07	2.23	0.1375
C*T	1	2.38E+08	2.38E+08	5.36	0.0222
C*H	1	2.80E+07	2.80E+07	0.63	0.4288
O*T	1	6.60E+06	6.60E+06	0.15	0.7007
O*H	1	6.65E+07	6.65E+07	1.50	0.2233
T*H	1	8.68E+08	8.68E+08	19.53	<.0001
C*O*T	1	7.82E+07	7.82E+07	1.76	0.1869
C*O*H	1	4.46E+07	4.46E+07	1.00	0.3182
O*T*H	1	2.72E+06	2.72E+06	0.06	0.8051
C*T*H	1	1.11E+08	1.11E+08	2.50	0.1164
C*O*T*H	1	2.02E+07	2.02E+07	0.46	0.5009

Table A.27 Anova table for fructose for two hybrid poplar clones (C) grown under two watering regimes (T) harvested at two harvest times (H) and sourced from two regions (O). (Data shown in Figure 3.28).

Fructose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	1	1.15E+11	1.15E+11	220.68	<.0001
O	1	5.77E+08	5.77E+08	1.11	0.2945
T	1	4.47E+09	4.47E+09	8.59	0.0040
H	1	3.97E+10	3.97E+10	76.30	<.0001
C*O	1	8.79E+09	8.79E+09	16.89	<.0001
C*T	1	4.11E+08	4.11E+08	0.79	0.3761
C*H	1	8.18E+09	8.18E+09	15.73	0.0001
O*T	1	6.24E+08	6.24E+08	1.20	0.2755
O*H	1	2.46E+08	2.46E+08	0.47	0.4928
T*H	1	2.52E+09	2.52E+09	4.85	0.0296
C*O*T	1	8.57E+06	8.57E+06	0.02	0.8981
C*O*H	1	5.58E+07	5.58E+07	0.11	0.7439
O*T*H	1	2.94E+09	2.94E+09	5.64	0.0191
C*T*H	1	6.33E+08	6.33E+08	1.22	0.2721
C*O*T*H	1	6.19E+08	6.19E+08	1.19	0.2774

Appendix A.3. Experiment #3

Table A.28 Anova table for stomatal conductance for six pure poplar clones (C) grown under two watering regimes (T). (Data shown in Figure 3.29).

Stomatal conductance (mol m^{-2} s^{-1})					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	0.03	0.01	2.92	0.0337
T	1	0.47	0.47	241.22	<.0001
C*T	5	0.03	0.01	3.34	0.0198

Table A.29 Anova table for net photosynthetic rate for six pure poplar clones (C) grown under two watering regimes (T). (Data shown in Figure 3.30).

Net photosynthetic rate (µmol m^{-2} s^{-1})					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	54.63	10.93	15.87	<.0001
T	1	23.99	23.99	34.84	<.0001
C*T	5	15.97	3.19	4.64	0.0042

Table A.30 Anova table for succinic acid for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.31).

Succinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	28.18	5.64	40.79	<.0001
T	1	28.19	28.19	204.04	<.0001

Succinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
H	1	14.13	14.13	102.23	<.0001
C*T	5	5.95	1.19	8.61	<.0001
C*H	5	0.70	0.14	1.02	0.4090
T*H	1	0.70	0.70	5.09	0.0254
C*T*H	5	2.22	0.44	3.22	0.0085

Table A.31 Anova table for glycolic acid for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.32).

Glycolic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	2.20E+09	4.40E+08	15.79	<.0001
T	1	1.24E+09	1.24E+09	44.53	<.0001
H	1	1.17E+08	1.17E+08	4.20	0.0420
C*T	5	1.13E+09	2.26E+08	8.11	<.0001
C*H	5	5.90E+08	1.18E+08	4.24	0.0012
T*H	1	2.46E+08	2.46E+08	8.84	0.0034
C*T*H	5	1.10E+09	2.19E+08	7.87	<.0001

Table A.32 Anova table for malic acid for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.33).

Malic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	3.59E+10	7.19E+09	16.55	<.0001
T	1	8.36E+09	8.36E+09	19.25	<.0001
H	1	6.66E+08	6.66E+08	1.53	0.2177
C*T	5	1.30E+10	2.60E+09	5.99	<.0001
C*H	5	1.26E+09	2.51E+08	0.58	0.7167
T*H	1	1.59E+06	1.59E+06	0.00	0.9519
C*T*H	5	1.32E+09	2.65E+08	0.61	0.6931

Table A.33 Anova table for threonic acid for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.34).

Threonic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	7.31E+09	1.46E+09	23.37	<.0001
T	1	2.10E+09	2.10E+09	33.51	<.0001
H	1	1.96E+06	1.96E+06	0.03	0.8598
C*T	5	3.26E+09	6.52E+08	10.42	<.0001
C*H	5	5.27E+08	1.05E+08	1.68	0.1413
T*H	1	6.25E+08	6.25E+08	9.99	0.0019
C*T*H	5	1.39E+09	2.77E+08	4.43	0.0008

Table A.34 Anova table for quinic acid for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.35).

Quinic acid					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	1.80E+11	3.59E+10	6.45	<.0001
T	1	3.94E+11	3.94E+11	70.78	<.0001
H	1	8.21E+09	8.21E+09	1.47	0.2268
C*T	5	6.22E+10	1.24E+10	2.23	0.0541
C*H	5	5.84E+10	1.17E+10	2.10	0.0692
T*H	1	2.29E+10	2.29E+10	4.12	0.0443
C*T*H	5	6.23E+10	1.25E+10	2.24	0.0536

Table A.35 Anova table for galactinol for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.36).

Galactinol					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	132.90	26.58	3.26	0.0079
T	1	221.53	221.53	27.21	<.0001
H	1	4337.36	4337.36	532.66	<.0001
C*T	5	50.11	10.02	1.23	0.2974
C*H	5	99.51	19.90	2.44	0.0367
T*H	1	0.06	0.06	0.01	0.9336
C*T*H	5	64.10	12.82	1.57	0.1706

Table A.36 Anova table for raffinose for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.37).

Raffinose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	1039.36	207.87	5.80	<.0001
T	1	1744.15	1744.15	48.63	<.0001
H	1	3800.13	3800.13	105.95	<.0001
C*T	5	368.10	73.62	2.05	0.0740
C*H	5	682.04	136.41	3.80	0.0028
T*H	1	101.00	101.00	2.82	0.0952
C*T*H	5	191.03	38.21	1.07	0.3816

Table A.37 Anova table for fructose for six pure poplar clones (C) grown under two watering regimes (T) and harvested at two harvest times (H). (Data shown in Figure 3.38).

Fructose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C	5	7.99E+09	1.60E+09	28.57	<.0001
T	1	8.06E+08	8.06E+08	14.43	0.0002
H	1	2.87E+08	2.87E+08	5.13	0.0249

Fructose					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
C*T	5	2.62E+09	5.23E+08	9.36	<.0001
C*H	5	4.66E+08	9.31E+07	1.67	0.1460
T*H	1	4.20E+08	4.20E+08	7.52	0.0068
C*T*H	5	4.51E+08	9.01E+07	1.61	0.1600

APPENDIX B

Experiment #1 T-tests

Appendix B.1. Experiment #1 T-tests

Table B.1 P-values for droughted/well-watered comparisons within harvest times (data shown in Figure 3.4). Walker (C1), Okanese (C2), WP-86V-86 (C3), Katepwa (C4); seven days after drought (DAD) (H7), 9DAD (H9), 14DAD (H14); amino acid (AA), inorganic acid (IA), organic acid (OA), carbohydrate (C), sugar alcohol (SA), phenolic (P), nucleoside (N), not identified (NI). Bold indicates p-values ≤ 0.05.

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
L-Valine	0.143	**0.034**	0.387	**0.029**	0.054	**0.045**	**0.009**	**0.005**	0.443	**0.025**	**0.000**	0.136	AA
L-Leucine	0.270	0.060	0.983	**0.048**	0.100	0.838	**0.025**	**0.005**	0.289	0.053	**0.000**	0.297	AA
L-Alanine	0.619	0.857	0.194	0.610	0.697	0.359	0.192	0.063	0.100	0.146	0.326	0.512	AA
L-Glutamate	0.959	0.867	0.433	0.386	0.381	0.145	0.095	**0.034**	0.715	0.321	**0.001**	0.993	AA
L-Glutamine	0.744	0.406	0.259	0.235	0.235	0.123	0.241	0.193	0.214	0.161	**0.019**	0.555	AA
L-Proline (1)	0.328	0.065	0.536	0.102	0.143	0.344	**0.040**	**0.012**	0.324	0.150	**0.001**	0.164	AA
L-Proline (2)	0.089	**0.036**	0.680	**0.018**	**0.046**	0.100	**0.034**	**0.001**	0.308	0.089	**0.013**	0.075	AA
L-Isoleucine	0.249	**0.048**	0.288	**0.029**	0.051	**0.031**	**0.013**	**0.003**	0.306	0.056	**0.000**	0.138	AA
L-Threonine	0.380	0.057	0.307	**0.048**	0.093	**0.035**	**0.020**	**0.004**	0.523	0.065	**0.000**	0.454	AA
L-Phenylalanine	0.409	0.089	**0.044**	**0.013**	0.139	0.576	**0.020**	**0.004**	0.334	0.081	**0.003**	0.614	AA
L-Tryptophan	0.257	0.069	0.377	0.104	0.125	0.094	**0.023**	0.065	0.165	0.138	**0.005**	0.082	AA
Glycine	0.199	0.268	0.065	0.081	0.185	0.068	**0.033**	**0.002**	0.934	0.447	**0.000**	0.293	AA
L-Serine	0.434	0.087	0.401	0.130	0.425	0.315	**0.041**	**0.032**	0.183	0.124	**0.001**	0.381	AA
Pyroglutamic acid	0.692	0.225	0.510	0.223	0.339	**0.014**	**0.020**	**0.000**	0.594	0.144	**0.000**	0.059	AA
M116T624_NI_Amino_acid	0.827	0.625	0.374	0.409	0.855	0.094	0.636	0.954	0.209	**0.011**	0.414	0.081	AA
M186T636_NI_Amino_acid	0.833	0.773	0.789	0.330	0.628	0.719	0.835	0.241	**0.048**	0.821	0.281	0.962	AA
M142T657_NI_Amino_acid	0.247	0.093	**0.001**	0.264	**0.042**	0.720	0.564	0.599	0.524	0.629	0.390	0.087	AA
M172T842_NI_Amino_acid	0.911	0.325	0.327	0.364	0.541	0.791	0.540	0.251	0.837	0.500	0.658	0.860	AA
Phosphoric acid	0.818	0.179	0.855	**0.001**	0.789	0.187	0.081	0.099	0.104	0.265	0.886	0.659	IA
Citric acid	0.120	0.990	**0.035**	0.066	0.181	0.149	0.147	**0.020**	0.070	0.234	**0.001**	0.106	OA
2-Ketoglutaric acid	0.907	0.141	0.087	0.460	0.551	0.233	0.068	**0.024**	0.966	**0.033**	**0.000**	0.187	OA
Succinic acid	0.147	**0.015**	**0.000**	0.125	0.134	0.365	0.179	0.272	0.088	0.098	0.159	0.614	OA
Fumaric acid	**0.014**	**0.028**	0.688	0.074	**0.001**	0.053	0.182	0.213	0.126	0.077	0.092	0.664	OA

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
Malic acid	0.254	0.844	**0.019**	**0.018**	0.528	0.994	0.623	0.824	0.382	0.412	0.289	0.526	OA
Glycolic acid	**0.025**	**0.007**	**0.000**	**0.006**	**0.000**	0.829	0.252	0.159	**0.001**	0.207	0.261	**0.010**	OA
Dehydroascorbic acid	0.353	0.344	0.255	0.591	0.838	0.716	0.143	0.087	0.385	0.573	0.636	0.140	OA
Threonic acid	0.102	**0.029**	**0.004**	**0.012**	0.050	0.839	0.729	0.874	**0.029**	0.485	0.592	**0.017**	OA
Tartaric acid	0.959	0.966	0.066	**0.044**	0.298	0.088	0.432	0.154	**0.031**	0.714	0.162	0.764	OA
2,3-Dihydroxybenzoic acid	0.567	0.072	0.410	0.146	0.270	0.736	0.671	0.749	0.607	0.798	0.812	0.277	OA
Ribonic acid	0.458	0.997	0.353	0.169	0.139	**0.037**	0.947	0.514	0.220	0.963	0.329	0.230	OA
Shikimic acid	0.707	0.092	0.432	**0.021**	**0.004**	0.310	0.116	0.594	0.573	0.082	0.152	0.245	OA
Quinic acid	0.340	0.822	0.137	**0.038**	0.155	0.378	0.355	0.174	0.993	0.234	0.720	0.282	OA
Caffeic acid	0.918	0.376	0.388	0.228	0.116	0.369	0.754	0.585	0.796	0.519	0.917	0.293	OA
3-Caffeoylquinic acid	0.953	0.172	0.421	0.115	**0.024**	0.443	0.706	0.087	**0.045**	0.732	0.526	0.160	OA
M143T872_NI_Organic_acid	0.440	0.471	0.428	**0.023**	**0.026**	0.896	0.631	0.152	0.086	0.142	0.570	0.253	OA
M292T912_NI_Organic_acid	0.591	0.500	0.438	0.429	0.158	0.081	0.184	0.079	0.886	0.514	**0.007**	0.381	OA
M267T921_NI_Organic_acid	0.541	0.092	0.357	0.115	0.412	0.231	0.721	0.740	0.348	0.763	0.726	0.374	OA
M299T1098_NI_Organic_acid	0.461	0.420	0.539	0.317	0.425	0.122	0.796	0.065	0.390	0.886	0.876	0.443	OA
M355T1102_NI_Organic_acid	0.617	0.064	0.362	0.145	0.767	0.794	0.282	0.799	0.950	0.599	0.757	0.364	OA
M249T1110_NI_Organic_acid	0.304	0.056	**0.006**	0.273	0.794	0.640	0.273	0.906	0.530	0.616	0.941	**0.046**	OA
Rhamnose (1)	0.761	0.236	0.110	0.701	**0.050**	**0.029**	0.483	0.770	0.522	**0.042**	0.556	0.135	C
Rhamnose (2)	0.176	0.267	0.113	0.590	**0.046**	**0.029**	0.403	0.794	0.705	0.170	0.543	0.110	C
Fructose (1)	0.901	0.095	**0.024**	**0.016**	0.432	0.721	**0.044**	0.355	0.470	0.207	0.103	0.278	C
Fructose (2)	0.908	0.098	**0.023**	**0.013**	0.383	0.742	0.051	0.331	0.482	0.218	0.087	0.375	C
Glucose (1)	0.472	0.139	0.157	0.181	0.577	0.676	**0.046**	0.411	0.111	0.313	0.379	0.544	C
Glucose (2)	0.534	0.202	0.055	0.141	0.791	0.446	0.095	0.379	0.335	0.421	0.173	0.560	C
Glucose (3)	0.615	0.327	**0.014**	0.121	0.591	0.274	0.135	0.440	0.784	0.592	0.089	0.593	C
Sucrose	0.441	0.283	0.064	0.091	0.359	0.379	0.152	0.278	0.694	**0.033**	**0.001**	0.156	C
Digalactosyl glycerol	0.764	**0.048**	**0.010**	0.162	0.737	0.929	0.735	0.214	0.552	0.645	0.511	0.222	C
Raffinose	0.194	0.069	0.111	**0.037**	**0.002**	**0.008**	**0.000**	**0.004**	0.230	0.064	**0.000**	0.103	C

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M218T1072_NI_Carbohydrate	**0.000**	**0.000**	0.129	0.097	**0.014**	0.056	0.919	0.091	0.879	0.203	0.160	**0.004**	C
M217T1249_NI_Carbohydrate	0.733	0.382	**0.028**	0.052	0.624	0.479	0.104	0.859	0.495	0.676	0.054	0.422	C
M73T1267_NI_Carbohydrate	0.894	0.181	0.085	0.082	0.092	0.220	0.764	0.698	0.262	0.872	0.317	0.304	C
M129T1364_NI_Carbohydrate	0.097	**0.007**	0.621	0.167	**0.002**	**0.008**	**0.017**	**0.017**	**0.042**	**0.012**	**0.000**	**0.038**	C
M217T1519_NI_Carbohydrate	0.148	**0.001**	0.178	0.577	0.493	0.343	0.545	0.963	0.794	**0.043**	0.911	0.541	C
M204T1672_NI_Carbohydrate	0.639	0.448	0.689	0.187	0.355	0.354	0.561	0.887	0.585	0.281	0.824	0.586	C
M361T1744_NI_Carbohydrate	0.526	0.806	0.121	0.055	0.788	0.478	0.516	0.898	0.716	0.887	0.875	0.323	C
M169T1816_NI_Carbohydrate	0.607	0.313	0.469	0.523	0.215	0.182	0.576	**0.050**	0.533	0.823	0.137	0.088	C
myo-Inositol	0.248	0.883	**0.002**	0.455	**0.008**	0.745	**0.017**	0.458	0.911	0.092	0.951	0.130	SA
Galactinol	0.053	0.231	**0.037**	**0.034**	**0.005**	**0.032**	**0.000**	**0.024**	0.193	**0.023**	**0.000**	**0.026**	SA
Catechol	0.713	0.192	0.957	0.442	0.449	0.686	0.167	0.064	0.127	0.775	0.994	0.128	P
Salicyl alcohol	0.877	0.072	0.569	0.202	0.075	0.627	0.989	0.787	0.875	0.849	0.342	**0.021**	P
Salicin	0.727	**0.034**	**0.047**	0.235	0.085	0.538	0.470	0.285	0.906	0.904	0.905	0.269	P
Catechin	0.218	0.062	0.226	0.709	**0.001**	0.127	0.444	**0.041**	0.074	0.114	**0.001**	**0.018**	P
Kaempferol	0.265	0.373	0.362	0.158	0.110	0.076	0.129	0.064	0.120	0.208	0.407	0.173	P
Populin	0.557	0.365	0.680	**0.009**	0.112	0.902	0.112	0.508	0.758	0.719	0.177	0.910	P
Adenosine	**0.007**	**0.048**	**0.023**	0.333	**0.003**	0.944	0.158	0.452	0.055	**0.034**	0.108	0.491	N
M84T891_NI	0.351	0.280	0.755	0.091	0.237	0.568	0.761	0.548	0.966	0.972	0.866	**0.014**	NI
M155T904_NI	0.432	0.076	0.057	0.178	0.909	0.161	0.220	0.551	0.187	0.932	0.912	0.163	NI
M239T957_NI	0.748	0.662	0.180	0.165	0.802	0.640	0.297	0.926	0.687	0.993	0.717	0.729	NI
M591T994_NI	0.958	0.068	0.190	0.929	0.773	0.748	0.553	0.809	0.171	0.710	0.094	0.854	NI
M179T1022_NI	0.812	0.149	0.879	0.186	0.436	0.176	0.869	0.545	0.259	0.495	0.077	**0.028**	NI
M180T1035_NI	0.676	0.095	0.055	0.808	0.092	0.582	0.346	0.399	0.556	0.266	0.912	**0.010**	NI
M436T1117_NI	0.478	0.201	0.256	0.380	**0.001**	0.741	0.442	0.319	0.084	0.798	0.976	0.238	NI
M282T1120_NI	0.223	0.053	0.287	**0.030**	0.063	**0.004**	**0.015**	**0.012**	0.161	**0.044**	**0.001**	**0.037**	NI
M244T1236_NI	0.224	0.089	0.173	0.206	0.389	0.527	0.958	0.220	0.140	0.699	0.897	0.127	NI
M374T1242_NI	0.051	**0.018**	0.192	0.202	0.669	0.874	0.656	0.850	0.066	0.970	0.837	0.191	NI

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M447T1278_NI	0.539	0.431	0.317	0.986	0.209	0.333	0.831	**0.043**	0.324	0.373	0.351	0.601	NI
M182T1285_NI	0.486	0.713	0.742	0.960	0.418	0.919	0.730	**0.010**	0.290	0.340	0.098	0.502	NI
M108T1288_NI	0.668	0.679	0.717	0.193	0.237	0.551	0.551	0.080	0.569	0.976	0.377	0.298	NI
M80T1314_NI	0.715	0.675	0.983	0.289	0.120	0.472	0.304	0.140	0.503	0.812	0.181	0.220	NI
M179T1386_NI	0.286	0.710	0.452	0.074	0.904	**0.027**	0.847	0.071	0.598	0.261	0.411	0.509	NI
M444T1401_NI	0.270	0.329	0.517	0.099	0.179	0.127	**0.011**	**0.013**	0.360	0.153	**0.022**	0.186	NI
M204T1410_NI	0.107	**0.036**	0.659	0.085	**0.023**	0.074	0.680	0.954	0.424	0.842	0.502	0.321	NI
M357T1414_NI	0.420	0.066	0.232	0.148	**0.020**	0.498	0.050	**0.018**	0.116	**0.043**	0.057	0.194	NI
M457T1450_NI	0.297	0.315	0.351	0.088	0.128	0.293	**0.038**	**0.023**	0.272	0.113	**0.005**	0.209	NI
M373T1461_NI	0.153	0.408	0.514	0.086	0.133	0.235	**0.037**	**0.029**	0.265	0.110	**0.010**	0.166	NI
M471T1463_NI	0.521	0.245	0.157	0.055	0.132	0.436	**0.025**	**0.038**	0.331	0.124	**0.014**	0.196	NI
M424T1467_NI	0.551	0.711	0.781	**0.009**	0.051	**0.030**	0.152	0.107	0.223	0.659	0.385	0.909	NI
M290T1475_NI	0.654	0.252	0.355	0.883	0.841	**0.034**	0.197	**0.016**	0.152	0.062	0.052	0.118	NI
M471T1484_NI	0.072	0.263	0.205	0.084	0.118	0.304	**0.032**	**0.030**	0.223	0.121	**0.008**	0.101	NI
M204T1494_NI	0.435	**0.037**	**0.004**	0.053	0.260	0.428	0.792	0.345	0.829	0.936	0.517	0.104	NI
M98T1498_NI	0.080	0.300	0.161	**0.045**	0.109	0.218	**0.049**	**0.025**	0.178	0.126	**0.009**	0.060	NI
M180T1509_NI	0.233	0.602	0.237	0.306	0.113	0.429	0.298	0.870	0.579	0.528	0.619	0.248	NI
M204T1529_NI	0.275	0.174	0.470	**0.003**	**0.019**	0.509	0.782	0.309	0.085	0.924	0.086	**0.014**	NI
M531T1536_NI	0.897	0.685	0.224	0.169	0.125	0.335	0.095	**0.049**	0.215	0.155	**0.013**	0.671	NI
M204T1541_NI	0.250	0.091	0.678	**0.003**	**0.017**	0.573	0.528	0.232	0.073	0.939	**0.037**	**0.006**	NI
M179T1545_NI	0.806	**0.029**	**0.036**	0.075	0.409	0.150	0.919	0.903	0.191	0.153	0.592	0.991	NI
M79T1550_NI	0.554	0.214	0.777	**0.004**	**0.012**	0.460	0.685	0.213	0.107	0.849	0.485	**0.036**	NI
M320T1558_NI	0.776	0.154	0.615	0.474	0.833	0.715	0.465	0.672	0.938	0.129	0.094	0.843	NI
M204T1574_NI	0.737	0.588	0.499	0.397	0.949	0.706	0.758	0.804	0.082	0.646	0.686	0.721	NI
M184T1585_NI	0.387	0.589	0.373	0.614	0.120	0.275	0.972	0.201	0.136	0.248	0.087	0.368	NI
M433T1598_NI	0.713	0.084	0.635	0.125	0.572	0.155	0.802	0.321	0.264	0.675	0.683	0.459	NI
M198T1601_NI	0.660	0.074	0.392	0.521	0.165	0.257	0.503	0.240	0.639	0.523	0.097	0.797	NI

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M321T1608_NI	0.119	**0.012**	0.126	**0.003**	**0.035**	0.962	0.531	0.334	0.358	0.820	0.262	**0.015**	NI
M415T1611_NI	0.254	**0.022**	0.877	0.397	0.384	0.353	0.138	0.225	0.304	0.327	**0.006**	0.648	NI
M73T1631_NI	0.584	**0.028**	**0.035**	0.320	0.090	0.684	0.948	0.395	0.856	0.945	0.670	0.241	NI
M331T1641_NI	0.614	0.091	0.251	0.203	0.565	0.521	0.927	0.343	0.639	0.454	0.285	0.499	NI
M219T1658_NI	0.478	0.120	0.117	**0.025**	0.129	0.243	0.170	0.662	0.163	0.900	0.289	0.059	NI
M471T1735_NI	0.506	0.634	0.360	0.078	0.411	0.138	0.151	0.050	0.205	0.273	0.187	0.278	NI
M355T1748_NI	0.901	0.074	0.154	0.212	0.064	0.438	0.110	0.059	0.898	0.599	0.944	**0.011**	NI
M355T1779_NI	0.867	0.126	0.239	0.824	**0.016**	0.486	0.216	**0.039**	0.994	0.373	0.532	**0.016**	NI
M368T1785_NI	0.312	0.233	0.390	0.185	0.110	0.924	**0.029**	0.083	0.173	0.102	**0.007**	0.236	NI
M456T1828_NI	0.488	0.122	0.314	0.260	**0.005**	0.479	0.070	0.269	0.119	0.749	0.937	**0.038**	NI
M308T1835_NI	0.429	**0.010**	0.064	0.236	0.423	0.414	0.097	0.098	0.866	0.294	0.376	0.164	NI
M297T1845_NI	0.273	**0.037**	0.761	0.083	**0.032**	0.579	0.812	0.141	0.220	0.710	0.167	0.163	NI
M324T1868_NI	0.997	0.135	0.168	**0.039**	0.078	0.670	0.300	**0.020**	0.073	0.544	0.217	0.123	NI
M396T1885_NI	0.568	0.168	0.430	**0.019**	**0.039**	0.998	0.284	0.063	0.942	0.376	0.948	**0.029**	NI
M108T1948_NI	0.993	0.690	0.497	**0.038**	**0.011**	0.082	0.186	0.222	0.981	0.068	0.258	0.558	NI
M489T1951_NI	0.875	0.477	0.308	0.157	**0.003**	0.635	0.998	0.767	0.689	0.688	0.675	0.655	NI
M647T1954_NI	0.443	0.521	0.459	0.965	0.132	0.068	0.245	0.067	0.636	0.197	0.744	0.091	NI
M447T1961_NI	0.266	0.828	0.283	0.588	0.394	0.171	0.789	**0.049**	0.862	0.341	0.647	0.193	NI
M108T1971_NI	0.845	0.816	0.321	0.238	**0.024**	**0.046**	0.409	0.186	0.551	0.336	0.329	0.641	NI
M193T1977_NI	0.519	0.554	0.900	**0.006**	**0.042**	0.978	0.737	0.144	0.248	0.856	0.781	0.440	NI
M271T1989_NI	0.775	0.558	0.392	0.408	**0.012**	**0.044**	0.404	0.309	0.153	0.224	0.846	0.816	NI
M194T1993_NI	0.629	0.455	0.268	0.973	**0.016**	0.270	**0.024**	0.224	0.592	0.469	0.337	0.478	NI
M461T2008_NI	0.611	0.337	0.173	0.703	0.081	0.244	**0.043**	0.092	0.700	0.329	0.419	0.089	NI
M219T2042_NI	0.144	0.311	0.598	0.326	**0.049**	0.098	0.094	0.199	**0.006**	0.687	0.322	**0.010**	NI
M476T2050_NI	0.529	0.067	0.131	0.231	0.110	0.290	0.287	0.131	0.294	0.439	0.220	0.052	NI
M307T2060_NI	0.630	0.117	0.469	0.957	0.059	0.194	0.589	0.397	0.377	0.337	0.442	0.256	NI
M219T2071_NI	0.735	0.151	0.310	0.628	0.170	0.106	**0.022**	0.164	0.456	0.286	0.989	0.067	NI

Metabolite	C1H7	C1H9	C1H14	C2H7	C2H9	C2H14	C3H7	C3H9	C3H14	C4H7	C4H9	C4H14	Class
M171T2082_NI	0.588	0.182	0.301	0.215	0.249	0.533	0.071	0.483	0.225	0.313	0.721	**0.026**	NI