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BIOCHEMICAL BASIS OF DROUGHT TOLERANCE IN HYBRID POPULUS GROWN UNDER FIELD PRODUCTION CONDITIONS

CRADA Final Report for CRADA Number ORNL93-0236

T. J. Tschaplinski
G. A. Tuskan
Oak Ridge National Laboratory

C. Wierman
Boise Cascade Corporation

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ABSTRACT

This document is the final report describing the research results of CRADA #93-0236 between Lockheed Martin Energy Research Corp. (formerly Martin Marietta Energy Systems) and Boise Cascade Corporation (BCC). The purpose of this cooperative effort was to assess the use of osmotically active compounds as molecular selection criteria for drought tolerance in *Populus* in a large-scale field trial. It is known that some plant species, and individuals within a plant species, can tolerate increasing stress associated with reduced moisture availability by accumulating solutes. The biochemical matrix of such metabolites varies among species and among individuals. The ability of *Populus* clones to tolerate drought has equal value to other fiber producers, i.e., the wood products industry, where irrigation is used in combination with other cultural treatments to obtain high dry weight yields. The research initially involved an assessment of drought stress under field conditions and characterization of changes in osmotic constitution among the seven clones across the six moisture levels. The near-term goal was to provide a mechanistic basis for clonal differences in productivity under various irrigation treatments over time.

This effort was comprised on two phases. The first phase involved the production of field-grown, drought-stressed *Populus* clones at Wallula, WA, and Boardman, OR. BCC used their expertise in the area of large-scale field production of hybrid *Populus* clones for use in pulp and paper manufacturing to produce up to 4-year-old hybrid *Populus* materials. Two of the clones were omitted from the study due to genetic contamination, as detailed in the progress reports of July 20, 1994, and September 29, 1995. The former report also highlighted focussing effort on three of the most divergent treatments at Wallula, WA (and eliminating two of the irrigation regimes that were determined to not be operationally relevant). Additional effort was focussed on the two larger, older production clones (TD and DN) at the Wallula site, as a severe hailstorm damaged the trees and hampered irrigation control at Boardman, OR (detailed in the report of Sept. 29, 1995; CRADA Amendment A, May 17, 1996).

ORNL coordinated with researchers at BCC to collect leaf tissue samples for biochemical analyses of osmotically active compounds. BCC periodically monitored and recorded plant growth, soil moisture conditions, and plant nutrient status during the growing seasons. The second phase of the study involved the characterization of osmotically active compounds in *Populus*. ORNL staff determined the biochemical profiles, based on changes in soluble carbohydrates, phenolic compounds, organic acids, amino acids, and macro- and micronutrients, from the field-grown leaf samples from two trees per each of the four replicated blocks. Changes in these profiles were related to soil moisture levels and field growth performance in an effort to identify drought tolerant clones. The key findings are summarized in the two reports that follow. In summary, clone and treatment differences in osmotic potential were minimal and, therefore, could not explain clonal differences in drought resistance, but altered carbon allocation below ground during moderate drought stress conferred increased drought resistance (higher growth during drought) on the DN clone, but the TD clone remained the most productive through age 4 due to rapid early growth.

OBJECTIVES

The purpose of this cooperative effort was to assess the use of osmotically active compounds as molecular selection criteria for drought tolerance in *Populus* in a large-scale field trial. The first phase involved the production of field-grown, drought-stressed *Populus* clones at Wallula, WA, and Boardman, OR. The research involved an assessment of the effects of drought stress on the productivity of poplar clones growing under different moisture levels in the field. The second phase of the study involved a characterization of the changes in osmotic constitution of those poplar clones. The near-term goal was to provide a mechanistic basis for clonal differences in productivity under various irrigation treatments over time.

OUTCOME TOWARDS MEETING OBJECTIVES

All the objectives of the CRADA were met. BCC used their expertise in the area of large-scale field production of hybrid *Populus* clones for use in pulp and paper manufacturing to produce up to 4-year-old hybrid *Populus* materials growing under various irrigation regimes, thereby accomplishing the first phase of the study. This phase included BCC periodically determining plant growth and nutritional status, and monitoring soil moisture conditions. Research effort was focussed on three of the most divergent irrigation treatments at Wallula, WA and on the two major production clones (TD and DN) at the Wallula site. The second phase of the study involved the characterization of osmotically active compounds in *Populus*. ORNL coordinated with researchers at BCC to collect field-grown leaf tissue samples for biochemical analyses of osmotically active compounds, and determined the biochemical profiles. Changes in these profiles were related to soil moisture levels and field growth performance in an effort to identify drought tolerant clones.

The research was able to provide a mechanistic basis for clonal differences in productivity under various irrigation treatments over time. For the two production clones, it was determined clone and treatment differences in osmotic potential were minimal and, therefore, could not explain clonal differences in drought resistance, but altered carbon allocation below ground during moderate drought stress conferred increased drought resistance (higher growth during drought) on the DN clone, but the TD clone remained the most productive through age 4 due to rapid early growth. It was concluded that the accumulation of any specific compound could not fully account for drought resistance of poplar clones, but rather a number of traits, in addition to low osmotic potential osmotic adjustment, together determine drought resistance. Therefore, the research determined that use of osmotically active compounds alone as molecular selection criteria for drought tolerance is problematic.

BENEFIT OF CRADA TO BIOFUELS FEEDSTOCK DEVELOPMENT PROGRAM OF DOE

Some plant species, and some individuals within a species, can tolerate increasing water deficit stress by maintaining low osmotic potential at full turgor (π_o) and/or by accumulating solutes in tissues, thereby lowering π_o . Low osmotic potential can enhance the ability of plants to take up water from dry soils, and may be as important as root growth in facilitating water uptake. Such mechanisms can be important to plants in minimizing the impact of drought on productivity, however there are few assessments under field conditions. Furthermore, the accumulation of specific compounds may be used as molecular selection criteria for the rapid screening of clones for drought tolerance. The biochemical basis of these selection criteria was developed in the Environmental Sciences Division at Oak Ridge National Laboratory (ORNL) through previous funding from the DOE's Biofuels Feedstock Development Program (BFDP), as part of an overall effort to develop dedicated feedstocks for the conversion of cellulose to ethanol and other biofuels. The CRADA provided a validation of what had been observed in controlled studies. Specific solutes, such as sucrose accumulated in drought tolerant clones under drought in both controlled and field studies, and thus provided a valuable confirmation of the previous work and a starting point for new research using multiple-generation pedigrees and diverse clones to identify genetic markers for drought tolerance. Some classes of solutes, such as organic acids and phenolic glucosides, that were important in the controlled studies, but were minor in the field, probably the result of different environmental growing conditions and nutrient regimes employed. These results suggest that nutrient regimes may be modified to alter the solute profile and a clone's ability to tolerate drought.

The ability of *Populus* clones to tolerate drought has value to BFDP and fiber producers, i.e., the wood products industry, where irrigation is used in combination with other cultural treatments to obtain high dry weight yields. The CRADA research suggest that irrigation allotments of the first two growing seasons can be reallocated to the later years to limit the constraints that will be imposed by large trees growing in hot and dry, semi-arid environments. If water availability is restricted, the DN clone may prove to be better suited for growth under such conditions over the latter years of the rotation. The importance of the research to the BFDP lies in the fact that the success of woody biomass crops for renewable fuels in many regions on the country may depend on efficient, timely application of irrigation and prudent clone selection. The research identified clones that will perform better under restricted water availability, the mechanisms involved, and future avenues of productive research to identify drought tolerant poplar clones. The CRADA also demonstrated that other drought responses, such as the timely production of roots as water deficit developed, were of equal or greater importance than osmotic adjustment in determining the overall drought resistance of the poplar clones studied and such mechanisms need to be considered in clonal selection. Although such a response may in hindsight be intuitively obvious, there were few demonstrated examples of such before this CRADA was conducted. The two manuscripts that follow detail the work conducted under the CRADA.

**DROUGHT RESISTANCE OF TWO HYBRID *POPULUS* CLONES
GROWN UNDER IRRIGATION IN LARGE-SCALE
PLANTATIONS**

**Timothy J. Tschaplinski
Gerald A. Tuskan
G. Michael Gebre
Don E. Todd**

Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008,
Oak Ridge, TN, 37831 USA.
Telephone: 423-574-4597, Fax: 423-576-9939, email: t2t@ornl.gov

Running head: Drought resistance plantation-grown hybrid *Populus*

1. DROUGHT RESISTANCE OF TWO HYBRID *POPULUS* CLONES GROWN UNDER IRRIGATION IN LARGE-SCALE PLANTATIONS

1.1 SUMMARY

A large-scale irrigation facility has been established near Wallula, WA, USA, where poplar (*Populus* sp.) trees have been subjected to various degrees of water stress to determine the mechanisms underlying clonal differences in drought resistance and their effects on plant productivity. The site contains a *Populus trichocarpa* x *P. deltoides* (TD) hybrid and a *P. deltoides* x *P. nigra* (DN) hybrid, with trees studied from the end of their second through the fourth growing season. Trees were irrigated with either 46, 76, 107, or 137 cm of supplemental irrigation and all trees received the same fertilization regime. Soil water content at 0.3-, 0.6-, and 0.9-m depths were determined by soil neutron probe analysis. Annual stem volume growth was assessed from the square of stem diameter at breast height times height (D^2H). Predawn and midday leaf water potential were monitored monthly over the third growing season. Osmotic potential at full turgor was determined with a vapor pressure osmometer to assess drought tolerance of clones under the different irrigation treatments.

Stem growth of the DN clone was constrained by the lower irrigation treatments (46 and 76 cm) as early as the second growing season, whereas growth of the TD clone was first limited by the 46- and 76-cm treatments during the third growing season, with trees of both clones in the 46 cm treatment being one-half the size of those in the 137-cm treatment. Aboveground biomass (D^2H) of the TD clone growing with 76 or 137 cm supplemental irrigation remained larger than the DN clone through the end of the third growing season (1994), but relative growth rate (RGR) of the DN clone was greater than the TD clone in all treatments. Although the TD clone had the lowest osmotic potential at full turgor in the 76-cm treatment, clone and treatment differences were minimal and, therefore, could not explain clonal differences in drought resistance. A comparison of both clones subjected to 76 cm of irrigation demonstrated that the DN clone lowered soil water content more than that of the TD clone, with the effect more pronounced as the season progressed. It was hypothesized that the DN clone had a greater root density and thus capacity to dry the soil to a greater extent than the TD clone. Analysis of rooting density confirmed that the greater drought resistance (higher growth rate under drought) of the DN clone was the result of greater carbon allocation to roots during the early stages of drought, as evidenced by its higher root density and root/shoot (R/S) ratio. Additionally, the low R/S ratio for the DN clone growing in the 46 cm treatment suggests that severe moisture limitation restricts the preferential carbon allocation below ground (i.e., both root growth as well as shoot growth are constrained by severe drought).

Keywords: Carbon allocation, leaf water potential, osmotic potential, poplar, root density, soil water content.

1.2 INTRODUCTION

Some plant species, and some individuals within a species, can tolerate increasing water deficit stress by maintaining low osmotic potential at full turgor (π_o) and/or by accumulating solutes in tissues, thereby lowering π_o (Morgan 1984). Tyree and Jarvis (1982) suggested that low osmotic potential should enhance the ability of plants to take up water from dry soils, and may be as important as root growth in facilitating water uptake. Such mechanisms can be important to plants in minimizing the impact of drought on productivity. Other responses, such as stomatal closure, leaf abscission, and leaf rolling are drought avoidance mechanisms that function to maintain high leaf water potential (Ψ_l) (Turner 1979), but may have consequences of reduced productivity depending on the timing of the response. Timely production of roots as water deficit develops may divert carbon from aboveground and limit shoot growth in the near term, but it is a drought avoidance mechanism that results in trees being better able to withstand subsequent drought and maintain a more favorable water balance and productivity. Although such a response may be intuitively obvious, there are few demonstrated examples.

Boise Cascade Corp. has established a drought stress facility at Wallula, WA, USA, where poplar (*Populus* sp.) trees are being subjected to various irrigation regimes to determine which drought resistance mechanisms are occurring in specific clones and their effects on plant productivity. Previous studies suggest that there is clonal variation in drought resistance within *Populus* sp. (Strong and Hansen 1991) and hybrids (Pallardy and Kozlowski 1981, Tschaplinski et al. 1994, Tschaplinski and Tuskan 1994). Given the large number of responses that can occur, it is difficult to determine which mechanisms confer drought resistance to poplar clones growing in the field over multiple growing seasons. Recent studies have demonstrated that a considerable degree of variability exists in π_o (Tyree et al. 1979) and osmotic adjustment to drought in *Populus* (Tschaplinski and Blake 1989b, Gebre et al. 1994, Tschaplinski et al. 1994). The objectives of this study were to assess the role of π_o in determining drought tolerance of two high-production poplar clones and to determine the mechanistic basis for clonal differences in growth under four irrigation regimes. A related manuscript addresses clonal differences in π_o and osmotic adjustment in response to irrigation across different sites and with age, including characterization of the solutes that constitute π_o and osmotic adjustment to low water availability (Gebre et al. 1997).

1.3 MATERIALS AND METHODS

In the spring of 1992 unrooted cuttings of two poplar clones were planted at a 2.4 x 3-m spacing on a site near Wallula, WA. The site typically receives 18–20 cm of precipitation annually, with most of it occurring as snowfall. Trees were irrigated with either 46, 76, 107, or 137 cm of supplemental irrigation with all treatments receiving the same nutrient addition of fertilizer, co-injected into the irrigation lines. Plants were established in four replicated blocks of 11 x 11 trees, including an F1 hybrid *Populus trichocarpa* x *P. deltoides* (TD clone) and an F1 hybrid *P. deltoides* x *P. nigra* (DN clone). The soil classification is predominately active dune land (Ac), consisting of low, rounded, or dune-shaped hills and long, narrow ridges of eolian sand. The active dune land is associated with the Quincy (Qd) soil series, consisting of excessively drained and

moderately well drained, coarse-textured soils derived from reworked eolian sand (USDA 1964).

At the initiation of the intensive physiological studies, trees were completing their second (1993) growing season. Soil water content was determined weekly by soil neutron probe analysis during the third (1994) growing season. There were two probes for each clone and treatment combination. The measured output in inches per foot were converted to % (v/v). Soil water content determined at three depths (0.3 m, 0.6 m, 0.9 m) was averaged to obtain a single value for each probe. Stem volume (square of stem diameter at breast height times height; D^2H) and mean relative growth rate (RGR) of stem volume over the year were determined for the TD and DN clone at the end of the second and third growing seasons (1993 and 1994, respectively). Six trees in the center of each plot (adjacent to the soil neutron, if present) for a total of 24 trees for each clone/treatment combination were used to assess shoot growth. RGR was calculated as the difference of natural log of D^2H at the end of 1994 from that at the end of 1993, divided by the 365 day interval. The growth data was then related to soil water contents over the growing season.

During the 1994 growing season, predawn and midday Ψ_1 of both clones were sampled with a Scholander pressure cylinder (PMS Instruments, Corvallis, OR) to determine whether a plant-based variable could be used to assess drought resulting from low soil water content. Leaf samples were collected from two of the four blocks (corresponding to where the soil neutron probes were located) for a total of two replicates per clone per treatment. Leaf osmotic potential at full turgor (π_o) was determined with a Wescor vapor pressure osmometer (Wescor Inc, Logan, UT) to assess drought tolerance of both clones and the degree of osmotic adjustment (i.e., lowering of π_o in response to drought). During the first sampling (June 29, 1994), only two blocks were sampled for a total of six replicates per clone per treatment. During the second sampling period (July 28, 1994), leaf samples were collected from all four blocks (2 plants per block for a total of 8 replicates per clone per treatment). One leaf (leaf plastochron index 9–11) per plant was collected from a fully exposed side shoot to determine π_o . The shoots were rehydrated overnight in distilled water, after which, one leaf per shoot was removed, frozen in dry ice, thawed, and π_o of the extracted sap measured.

Soil pits, with a volume of ca. 0.28 m^3 (one ft^3) each, were excavated at the center of each plot for the two clones studied. Pits were dug along the irrigation lines, but in between the emitters, to determine root density without the confounding influence of the emitters. A pair of pits were dug in each of the four replicate blocks of the 46-, 76-, and 137-cm treatment plots on July 17–27, 1995, for a total of 48 soil pits. The excavated soil was sieved and the roots (fine and coarse) were collected, dried for over 48h in an oven at 80°C , and weighed to determine rooting density (kg/m^3 soil) in the different irrigation treatments. Root/shoot (R/S) ratio was estimated using stem volume determined before the start of the 1995 growing season.

Data were analyzed using a one-way ANOVA to test for significant ($p \leq 0.10$) irrigation treatment differences in soil water content. A 2×4 factorial experiment in a complete randomized block design was used to determine clonal and irrigation differences ($p \leq 0.05$) in growth and water relations variables. The individual tree was considered the experimental unit. The PDIFF option of least squares means was used to obtain t-tests for mean comparisons (SAS Institute Inc., Cary, NC).

1.4 RESULTS

1.4.1 Soil Water Content and Leaf Water Potential of Hybrid Poplar Clones in 1994

Predawn Ψ_1 of the DN clone tended to decline below -0.8 MPa, corresponding to soil water contents below 14% (Fig. 1.1), both of which can be considered thresholds for stress initiation (at least for the DN clone). However, predawn Ψ_1 of the TD clone, was not related to soil water content (data not shown). The TD clone tended to have leaf bronzing, marginal necrosis, and leaf abscission to a greater extent and sooner than the DN clone, indicating the greater degree of stress experienced by the TD clone. The irrigation treatments of 46, 76, and 137 cm, each had distinguishable effects on soil water content of both clones (Fig. 1.2). The 46 cm irrigation treatment reduced the soil water content of both clones below the 14% stress threshold for most of the third growing season. The 76 cm treatment reduced the soil water content of both clones below 14% for about one-half of the third growing season. Furthermore, a comparison of both clones subjected to 76 cm of irrigation demonstrated that the DN clone lowered its soil water content more than that of the TD clone in the latter one-half of the third growing season (day 222 through day 243) (Fig. 1.2). The same trend was evident in the 46 cm and 137 cm treatments but the differences were not significant. Soil water content for 107 cm treatment was intermediate between the 76 cm and 137 cm treatments, but was not shown to highlight differences between the extreme treatments.

During the middle of the third growing season, these treatment effects were reflected in the predawn Ψ_1 which declined to the lowest levels between days 208 and 215, but with no difference between clones (Fig. 1.3). The 46 cm treatment produced the lowest predawn Ψ_1 . In contrast with predawn Ψ_1 , where there were few clonal differences, there were large clonal differences in midday Ψ_1 . The DN clone maintained midday Ψ_1 above -2.0 MPa in all treatments over the whole growing season, and above -1.8 MPa in the lowest irrigation treatments (Fig. 1.4). In contrast, Ψ_1 of the TD clone declined to well below -2.0 MPa in all treatments over the growing season, to as low as -2.5 MPa. There was a temporal effect evident in the TD clone, where the lower irrigation regimes induced lower midday Ψ_1 ca. 21 d sooner than the higher irrigation treatments. However, the midday Ψ_1 of the lowest irrigation regime recovered over the growing season, coupled with the substantial leaf abscission. Together these responses result in the lack of a relationship between midday leaf Ψ_1 and soil water content for either clone (data not shown).

1.4.2 Osmotic Potential of Hybrid Poplar Clones in 1994

There were clonal differences in π_o with the TD clone, having the lowest π_o in the 76 cm treatment during the first sampling period, which was significantly lower than that of the 137 cm treatment (Table 1.1). There were no treatment differences for the DN clone in either sampling date. At the end of July (second sampling), when midday temperatures reached 40°C , the TD clone displayed a small (0.08 MPa) but significant adjustment of π_o in response to drought in the 46 cm irrigation treatment, relative to the 137 cm treatment (Table 1.1). The absence of a significant difference in π_o of trees of the 76 cm and 137 cm irrigation treatments during a dry and hot period, indicates that osmotic adjustment in leaves was not readily inducible in either clone. The low leaf Ψ_1 observed

Fig. 1.1. Predawn leaf water potential of a DN clone, at various soil water contents, determined for the 46, 76, and 137 cm irrigation treatments from day 187-243 of the third growing season (1994).

Fig. 1.2. Comparison of soil water content, as determined by neutron probe analysis, of a TD clone versus a DN clone in the 46 cm, 76 cm, and 137 cm irrigation treatments over the third growing season (1994). * denotes significant ($p \leq 0.10$) clonal difference.

Fig. 1.3. Predawn leaf water potential of a) TD clone and b) DN clone, grown under different irrigation treatments, over the third growing season (1994).

Fig. 1.4. Midday leaf water potential of a) TD and DN clones, growing under lower (46 and 76 cm) irrigation regimes and b) TD and DN clones, growing under higher (107 and 137 cm) irrigation regimes, over the third growing season (1994).

Table 1.1. Leaf osmotic potential at full saturation for poplar clones in their third growing season at Wallula, WA, under different irrigation regimes (TRT), sampled on June 29 and July 28, 1994

Different letters in a column indicate significant treatment effects ($p \leq 0.05$). Standard error of mean shown in parentheses ($n = 6$ in June and $n = 8$ in July)

TRT (cm)	June 29, 1994		July 28, 1994	
	TD Clone	DN Clone	TD Clone	DN Clone
46	-1.64 ± 0.05 ab	-1.57 ± 0.08 a	-1.67 ± 0.02 b	-1.57 ± 0.03 a ^a
76	-1.76 ± 0.04 b	-1.58 ± 0.05 a ^a	-1.60 ± 0.03 a	-1.58 ± 0.02 a
137	-1.58 ± 0.04 a	-1.51 ± 0.05 a	-1.59 ± 0.02 a	-1.51 ± 0.02 a ^a

^aSignificant ($p \leq 0.05$) difference between clones within a sampling date.

for the TD clone growing under 137 cm of irrigation may have induced the lower π_o observed, relative to the DN clone.

1.4.3 Growth of Two Hybrid Poplar Clones in 1994

Stem volume (stem diameter squared times height; D^2H) of trees was determined at the end of 1993 and 1994 to assess the effect of irrigation. Stem volume for trees of the DN clone in the high irrigation treatment was 37 to 78% greater than those of the two lowest irrigation regimes as early as the second year of the study. In contrast, stem volume for trees of the TD clone was similar in all irrigation treatments through the second growing season, but was constrained during the third growing season, with trees (of both clones) growing in the 46-cm treatment one-half the size of those in the 137-cm treatment (Table 1.2). Growth analyses demonstrated that aboveground stem volume of the TD clone growing in the 76- and 137-cm treatments, remained larger than the DN clone through the end of the third growing season (1994), but relative growth rate (RGR) of the DN clone was greater than the TD clone in all treatments. RGR of the DN clone was only constrained (-19%) in the 46 cm treatment during the third growing season. Relative growth rate of the TD clone growing in the 137 cm treatment was 25–43% greater than the two lowest irrigation regimes, indicating growth suppression had occurred over the third growing season.

Given that the DN clone reduced its soil water content below that of the TD clone in the 76-cm treatment, the DN clone had to have considerably more roots per soil volume (i.e., greater root density) or higher rates of water use to dry the soil more than the TD clone. Analysis of rooting density confirmed that the DN clone, allocated a greater proportion of fixed carbon to roots during the early stages of drought, as evidenced by higher root density and root/shoot (R/S) ratio in the 76-cm treatment

Table 1.2. Stem diameter squared times height (D^2H) and relative growth rate (RGR) for a TD and DN clone at the end of the second and third growing seasons (1993 and 1994 respectively) at Wallula, WA, with trees grown under 46, 76, or 137 cm of supplemental irrigation

1993 (year 2) Stem D^2H (dm^3)		
	TD	DN
46 cm	23.1 ± 2.5 a	14.7 ± 1.7 a ^a
76 cm	27.8 ± 2.6 a	11.3 ± 1.8 a ^a
137 cm	28.1 ± 2.4 a	20.1 ± 2.5 b ^a
1994 (year 3) Stem D^2H (dm^3)		
	TD	DN
46 cm	82 ± 6 a	75 ± 7 a
76 cm	118 ± 7 b	79 ± 7 a ^a
137 cm	174 ± 9 c	150 ± 11 b ^a
1993-94 RGR (D^2H) (%/d)		
	TD	DN
46 cm	0.363 ± 0.016 a	0.475 ± 0.026 a ^a
76 cm	0.416 ± 0.019 b	0.629 ± 0.037 b ^a
137 cm	0.518 ± 0.024 c	0.595 ± 0.027 b ^a

^aSignificant ($p \leq 0.05$) difference between clones.

(Table 1.3). In contrast, R/S ratio of TD clone was only increased in the lowest irrigation treatment to the same value observed for the DN clone.

1.5 DISCUSSION

1.5.1 Drought Resistance Mechanisms

Analysis of rooting density confirmed the hypothesis that the greater drought resistance (higher RGR under drought) of the DN clone, during the third growing season, was due to greater carbon allocation to roots during the early stages of drought. The ability of the DN clone to alter carbon allocation patterns during drought to favor root growth increases the plants' ability to occupy a greater soil volume and reestablish a favorable water balance (Pallardy and Kozlowski 1979, Tschaplinski and Blake 1989a). The DN clone displayed a greater plasticity of response to low water availability than did the TD clone. However, the low R/S ratio for the DN clone growing in the 46 cm treatment suggests that severe moisture limitation restricts its preferential carbon allocation below ground that was evident at intermediate moisture availability (i.e., both root growth as well as shoot growth are constrained by severe drought).

Patterns of carbon allocation were reported to be highly deterministic (under strong genetic control) in *Populus* sp., with *P. tristis* x *P. balsamifera* cv. 'Tristis No. 1' increasing the number and length of fine roots under water stress, but with little response of leaf area and shoot growth to increased water and nutrient availability (Dickmann et al. 1994, Pregitzer et al. 1990). The preferential allocation of dry weight to the stem and root system resulting in greater drought resistance has been reported earlier (Mazzoleni and Dickmann 1988, Michael et al. 1988), and it has been shown that 'Tristis No. 1' maintains a higher predawn Ψ_1 than a contrasting clone, *P. deltoides* x *P. nigra* cv. 'Eugenei' during drought and when well watered (Dickmann et al. 1992). 'Eugenei' readily increased its leaf area and shoot growth in response to increased water and nutrient availability, but it responded to drought primarily by shedding its leaves, while maintaining a constant R/S ratio (Dickmann et al. 1994). Despite the drought-induced loss of leaves in 'Eugenei,' it remained the most productive clone of the two studied (Dickmann et al. 1992). The TD clone in our study exhibited similar responses to drought and also remained the most productive clone through the third growing season, but the increased carbon allocation to roots in the DN clone facilitated a higher relative growth rate of shoot volume during that growing season. If drought stress and respective RGRs of the clones were sustained at those levels through the next growing season, then the DN clone would have a greater stem volume than the TD clone in all irrigation levels. However, the DN clone would not likely maintain those growth rates, because ontogenetic changes and the increased impact of drought on larger trees, would further dry the soil and act to reduce RGR.

1.5.2 Indicators of Drought Stress

The ability to predict when and how much irrigation needs to be applied to avoid drought stress and maintain maximum shoot growth has a number of benefits, not the least of which is minimizing water usage and the costs of production, relative to the productivity benefit derived from irrigation. The difficulty lies in determining what indicator or suite of

Table 1.3. Root density and root/shoot ratio (R/S) of the TD and DN clones in July of the fourth growing season (1995) at Wallula, WA, with trees grown under 46, 76, or 137 cm of supplemental irrigation

Root dry weight was determined in 0.028 m³ (1 ft³) soil pits dug between rows and between emitters. The estimates of R/S ratio used shoot volume values determined before the start of the 1995 growing season.

1995 Root density (kg/m ³ soil)		
	TD	DN
46 cm	1.56 ± 0.34 a	1.38 ± 0.26 a
76 cm	1.55 ± 0.15 a	2.78 ± 0.58 b ^a
137 cm	1.84 ± 0.34 a	2.40 ± 0.42 b
R/S (kg _{dw} /m ³ soil/m ³ wood)		
	TD	DN
46 cm	24.4 ± 4.3 b	24.2 ± 4.2 a
76 cm	14.7 ± 1.4 a	56.0 ± 13.8 b ^a
137 cm	11.6 ± 1.2 a	17.6 ± 1.8 a

^aSignificant ($p \leq 0.05$) difference between clones.

indicators can be used to assess the need for timely and sufficient irrigation to avoid growth suppression. The selection of indicators depends on the site characteristics and plant material under culture. Height and diameter growth of poplar clones and species have been reported to decline with very slight declines in soil matric potential below field capacity (< -0.15 MPa) (Hansen and Phipps 1983, Hansen 1988). Regulation of irrigation by monitoring soil matric potential is possible if the soil is uniform and has high water holding capacity, allowing a somewhat even distribution of irrigation water. Otherwise, a well-drained, porous, sandy soil, as evident in this study, has a high degree of heterogeneity, resulting in high water potential (or content) at or near the emitters and little or no available water away from the emitters. At such a site, the average soil water content may not reflect the amount of available water at any given location, even though irrigation treatments can be distinguished. Therefore, a plant-based measure may become a better integrator of soil water availability.

Predawn Ψ_p , which reflects the degree of recovery, was the best indicator of the degree of stress experienced, but the use of such a variable to regulate irrigation is problematic from an operational perspective. If a strong relationship can be established

between soil water content and predawn Ψ_1 for a given clone, then soil water content can be used to assess the degree of stress experienced, at least for the clone that was used to establish the regression. Midday Ψ_1 does not necessarily reflect the degree of stress experienced. It is influenced by water availability, inherent clonal differences in stomatal regulation, previous stress history of plants, and current environmental conditions, that together, result in a poor correlation with soil water content or predawn Ψ_1 . Similarly, the use of π_o as an indicator of the degree of stress experienced is problematic, because it is also dependent on inherent clonal differences in the baseline π_o , the previous stress history of the plant, and the clone's capacity for osmotic adjustment to drought.

Furthermore, the utility of osmotic adjustment in turgor maintenance may be offset by a decline in hydraulic conductivity (Rieger 1995). The π_o observed for these clones was typical of that reported for field-grown or outdoor-grown poplars (Tschaplinski and Blake 1989b, Gebre et al. 1994, Tschaplinski et al. 1994). The degree of adjustment was less than what has been observed, but it is not unusual for poplar clones to not display osmotic adjustment to drought (Tschaplinski et al. 1994). Unexpectedly, the clone with the lowest π_o , and the greatest degree of osmotic adjustment, displayed the greatest degree of leaf abscission. The TD clone experienced considerably lower midday Ψ_1 regardless of irrigation regime. This highly-productive clone evidently functions up to and beyond the limits of Ψ_1 that trigger leaf abscission. Leaf shedding occurred in red alder (*Alnus rubra*) with high π_o , when low Ψ_1 was achieved, which contrasted with black cottonwood (*Populus trichocarpa*), which had lower π_o , but maintained higher Ψ_1 under water stress (Pezeshki and Hinckley, 1988). The common response between the two studies is that tolerance of excessively low Ψ_1 results in leaf abscission, and factors other than π_o , such as root density and distribution, control the degree of stress experienced.

1.5.3 Clonal Selection

Previous studies on *Populus* have suggested that clones that maintain the highest Ψ_1 tended to be the most productive (Pallardy and Kozlowski 1981, Tschaplinski and Blake 1989a). At first glance this does not seem to be the case in the present study, given that the TD clone had the lowest midday Ψ_1 , but remained larger over the 3 years. However, the DN clone, which maintained higher midday Ψ_1 also had higher growth rate over the final year of the study, narrowing the difference in stem volume between the clones. These results have implications for clonal selection, given the constraints of limited water availability. If irrigation water is not limited, then the TD clone will likely be the most productive over a six year rotation. If the availability of irrigation water is restricted, then the inherent growth advantage of this TD clone will not be fully achieved. Without the necessary increases in irrigation with the increasing size of the trees, early, extensive leaf abscission will be common in this clone, restricting productivity. Irrigation allotments of the first two growing seasons can be reallocated to the later years to limit the impact of the constraints that will be imposed by large trees growing in hot and dry, semi-arid environments, as that observed in the Pacific Northwest, east of the Cascade Mountains. If water availability is restricted, the DN clone may prove to be better suited for growth under such conditions over the latter years of the rotation. Given the restrictions on water supply for irrigation, water shortages are not likely to be alleviated as the trees grow. Under such conditions, the increased respiratory costs associated with increased

carbon allocation to roots will be offset by the improved water relations achieved by such clones.

It has been shown that the productivity advantage of the highly productive F_1 TD clones versus the parental species, is not associated with a parallel increase in small and fine root production (Heilman et al. 1994). Under the growing conditions of the moist coastal regions, such as in the delta of the Columbia River, where nutrient availability is also high, there are few negative consequences of the altered carbon allocation pattern, with the exception of increased susceptibility to windthrow of some of the highly productive hybrids with low root/shoot ratio (Harrington and DeBell 1996). In such a coastal area, predawn Ψ_1 did not differ between irrigated and nonirrigated black cottonwood, eastern cottonwood, and hybrid poplar clones (Schulte et al. 1987). Modified stomatal activity and leaf abscission were sufficient to restore midday Ψ_1 . In the hot and dry semi-arid areas, water deficit stress may develop even when growing under irrigation. The trade off for the TD clone is an inability to adjust carbon allocation that is necessary under prolonged daily water deficits.

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**CLONAL AND SEASONAL DIFFERENCES IN LEAF OSMOTIC
POTENTIALS AND ORGANIC SOLUTES OF TWO HYBRID
POPLAR CLONES GROWN UNDER FIELD CONDITIONS**

**G. MICHAEL GEBRE
TIMOTHY J. TSCHAPLINSKI
GERALD A. TUSKAN
DONALD E. TODD**

Environmental Sciences Division, Oak Ridge National Laboratory, P.O.Box 2008, Oak Ridge, TN 37831-6422, USA. Telephone 423-574-8107, fax # 423-576-9939, e-mail gv4@ornl.gov

2. CLONAL AND SEASONAL DIFFERENCES IN LEAF OSMOTIC POTENTIALS AND ORGANIC SOLUTES OF TWO HYBRID POPLAR CLONES GROWN UNDER FIELD CONDITIONS

2.1 SUMMARY

Osmotic potential at full turgor and the major solutes that contribute to osmotic potential were characterized four times over a two-year period in two hybrid poplar clones; *Populus trichocarpa* Torr. & Gray x *P. deltoides* Bartr. (TD) and *P. deltoides* x *P. nigra* L. (DN), grown under field conditions at Wallula, Washington, USA. Trees were subjected to three levels of supplemental drip irrigation; 46 cm, 76 cm, and 137 cm. The TD clone generally had lower leaf osmotic potentials than the DN clone during the 1994 and 1995 sampling periods when trees were in their third and fourth growing season, respectively. The TD clone also exhibited a significant treatment effect with plants from the 46-cm treatment having a lower leaf osmotic potential than those from the 76-cm treatment during a hot and dry period in July 1994. These differences in leaf osmotic potential were not, however, reflected in the organic and inorganic solutes measured. Although the total concentration of free primary amino acids in the TD clone was 28% higher under 46-cm versus 137-cm conditions, amino acids constituted only a small fraction of the total solute pool. About 62% of total solutes in the TD clone was comprised of inorganic ions compared to 52% in the DN clone, with potassium as the main ion constituting about 30% of total solutes and 50% of total ions. Unlike 1994, the osmotic adjustment observed in the TD clone in 1995 was accounted for by an increase in organic solutes, largely the result of an increase in sucrose, which constituted 70% of total organic solutes. However, clonal difference in osmotic potential was not fully accounted for by the difference in solute concentration. Osmotic potential declined over the growing season and with age particularly in the TD clone. Given that the level of osmotic adjustment exhibited by these clones was small, other drought resistance mechanisms are likely contributing to the differences in clonal field performance.

Key words: osmotic adjustment, *Populus*, irrigation, free primary amino acids, sugars, carbohydrate, organic acid, potassium

2.2 INTRODUCTION

Plants differ in their ability to survive and grow under water stress. Plants with dehydration tolerance mechanisms are able to survive low water potentials by one or more of the following methods: low baseline osmotic potential, lowering their osmotic potentials (i.e., active solute accumulation), or by protoplasmic resistance (Jones et al. 1981). Tree species growing in xeric sites generally have low baseline osmotic potentials (Abrams 1988). The lowering of osmotic potential due to solute accumulation, known as osmotic adjustment, has been shown to benefit plants in survival of and recovery from short, dry

periods (Meyer and Boyer 1981, Bennett and Sullivan 1981). Although osmotic adjustment has been suggested to compete with productivity (Munns 1988), controlled studies with tree species show that trees with faster growth performance had more organic solute accumulation (Tscharplinski and Blake 1989, Tan et al. 1992). Such a response may provide an advantage for trees growing in short rotation plantations.

Variation among tree species (and varieties within a species) in osmotic potential or the level of osmotic adjustment may be a useful selection criteria for drought tolerance. Several poplar clones have been shown to osmotically adjust mainly due to carbohydrate accumulation (Tscharplinski and Blake 1989, Gebre et al. 1994) as well as inorganic ions (Tscharplinski and Tuskan 1994). These studies, however, were conducted under controlled environments with seedlings and saplings grown in containers. Although such studies demonstrate mechanisms of drought tolerance, they do not adequately represent trees growing in the field with unlimited rooting volume, high solar radiation, variable wind speed, and fluctuating humidity. How trees respond to controlled water stress under field growing conditions and how that response is affected by season and age has rarely been reported.

In this study, the drought tolerance characteristics of two highly-productive hybrid poplar clones, *Populus trichocarpa* Torr. & Gray x *P. deltoides* Bartr. (TD) and *P. deltoides* x *P. nigra* L. (DN), were investigated under field conditions where water status has been modified by drip irrigation. The site has an annual rainfall of less than 20 cm with much of it occurring outside the growing season. The selected TD clone is considered to have better growth performance but susceptible to drought and wind damage whereas the selected DN clone is more adaptable to a windy and dry environment but grows slower. However, to our knowledge, no previous study on water relations of these productive, commercially important clones has been conducted. The purpose of this study, therefore, was to investigate clonal differences in osmotic potential and osmotic adjustment in trees growing in the field under different irrigation regimes and determine which solutes constitute low osmotic potential and osmotic adjustment to low water availability. Among the osmotically active compounds determined were soluble carbohydrates, phenolics, organic acids, amino acids, and inorganic ions.

2.3 MATERIALS AND METHODS

Plants were established in 1992 by Boise Cascade Corp. in a large-scale water stress facility at Wallula, WA, USA with four replicated blocks of 11 x 11 trees and four supplemental irrigation treatments. The site description is detailed elsewhere (Tscharplinski et al. 1997). Leaf samples were collected twice during each growing season in 1994 and 1995 from trees growing with 46 cm, 76 cm, and 137 cm of supplemental irrigation. A fourth treatment, 107 cm of supplemental irrigation, was not included in this study. On June 29, 1994, three trees from two blocks were sampled for a total of six trees per clone per treatment and on July 28, 1994, samples were collected from all four blocks (two trees per block for a total of eight per clone per treatment). In the 1995 sampling, three plants per block from all blocks were sampled during both periods (June 28 and July 14) except for the 76-cm treatment when only two blocks were sampled in July because of partial interruption of irrigation.

During each sampling, one fully exposed lateral branch was cut in the field (10:00–16:00 h local time) and one leaf (leaf plastochron index (LPI) 10 ± 1) per shoot

was immediately removed and frozen on dry ice for metabolite analyses. The shoot was then recut under water and rehydrated overnight in distilled water after which one leaf ($\text{LPI } 10 \pm 1$, the next leaf to that sampled for metabolite analyses) was removed, placed in a zip-lock bag and frozen on dry ice. Samples were then thawed for 18-20 min while in the plastic bags and tissue sap extracted using a leaf press containing a filter paper disc to collect the sap (Markhart and Lin 1985). Once the sample was disrupted, the wet paper disc was removed and immediately placed in a Wescor vapor pressure osmometer (model 5500C, Wescor Inc., Logan UT, USA) for a reading. The readings in mmol kg^{-1} ($= \text{mol m}^{-3}$) were converted to MPa units of osmotic potential at full saturation (ψ_s) using the van' Hoff relation at 25°C ($\psi_s = -8.314 \times 10^{-6} \text{ m}^3 \text{ MPa mol}^{-1} \text{ K}^{-1} \times 298 \text{ K} \times \text{reading}$). The calibration of the osmometer was checked (and calibrated when needed) with standards obtained from Wescor Inc. Osmotic adjustment was calculated from the difference in ψ_s between the 46 cm and the 137 cm treatments.

The frozen leaf samples collected for metabolite analyses were lyophilized and ground before extraction. The extraction procedure for solutes followed that of Tschaplinski and Tuskan (1994). Briefly, 50 mg of ground leaf sample was twice extracted with 5 ml of 80% ethanol and the extracts combined prior to analyses. After air drying 5 ml of the 10 ml extract, soluble carbohydrates, organic acids, and phenolic compounds were analyzed by gas chromatography-mass spectrometry (GC-MS) as trimethylsilyl derivatives (Tschaplinski et al. 1993). Extracts were silylated with 1 ml of Tri-Sil 'Z' (Pierce Chemical Co., Rockford, IL, USA), heated for 45 min at 70°C , and 1 μL injected the following day into an HP 5890 Series II GC and 5972 MS (Hewlett-Packard Co., Avondale, PA, USA) fitted with an HP-5MS (crosslinked 5% PH ME Siloxane) 30 m x 0.25 mm x 0.25 μm film thickness capillary column. Gas (helium) flow rate was set at 0.6 ml per min with the injection port configured in the splitless mode. The injection port temperature was set at 250°C and the detector temperature at 300°C . The oven temperature was programmed to increase at 8°C per min from 100°C (held for 4 min) to 300°C . Peaks were quantified by area integration and concentration determined based on external standards for the 1994 samples, whereas sorbitol was used as an internal standard to develop response factors of all identified compounds in 1995. Concentrations of some unknown compounds were estimated in glucose equivalents. For analyses of the 1995 samples, the Tri-Sil 'Z' was replaced by 0.5 ml of a stronger silylation reagent *N*-Methyl-*N*-trimethylsilyltri-fluoroacetamide (MSTFA) + 1% trimethyl-chlorosilane (TMCS) and 0.5 ml acetonitrile (Pierce Chemical Co.).

Primary amino acids were analyzed as *o*-phthalaldehyde (OPA) derivatives by reversed-phase (C_{18}) high performance liquid chromatography (HPLC) and fluorescence detection, according to protocols described earlier (Tschaplinski and Tuskan 1994). Peaks were quantified by area integration and calibrated by injection of an external standard mix of amino acids (Sigma Chemical Co., St. Louis, MO, USA). The concentration of an unidentified amino acid that eluted after phenylalanine was estimated in phenylalanine equivalents. Major inorganic ions were determined by inductively-coupled plasma and carbon-arc spark emission spectroscopy by the Soil Testing and Plant Analysis Laboratory at University of Georgia (Athens, GA, USA). Biochemical analysis for samples collected in 1994 was limited to treatments with significant irrigation or clonal difference in osmotic potential (e.g., 76-cm treatment). In 1995 however, comparisons were either within the 137-cm or between the 46-cm and 137-cm treatments.

Data analysis of osmotic potentials and solutes was based on a one-way analysis of variance (ANOVA) for a randomized complete block design to test clonal differences, and

two-way ANOVA when samples of more than one irrigation treatment were analyzed (i.e., clone and irrigation). Each tree represented the experimental unit. The PDIFF option of least squares means (LSMEANS) was used to obtain *t*-tests for mean comparison (SAS Institute, Inc., Cary, NC). Significant differences were based on $p \leq 0.05$.

2.4 RESULTS

2.4.1 First Sampling Year (1994 Growing Season)

During the first sampling period in 1994 (June 29), the 76-cm treatment had lower ψ_s than the 137-cm treatment in the TD clone (Fig. 2.1a). Despite a lower ψ_s in the TD clone than the DN clone (in June), or no difference between the two clones (in July) in the 76-cm treatment, the TD clone had a lower organic solute concentration than the DN clone during both sampling periods. This was largely the result of higher concentrations of glucose, fructose, and quinic acid in the DN clone than the TD clone (Fig. 2.2). However, there was no difference in the concentration of sucrose which accounted for more than a third of the total carbohydrate and organic acid concentration of these clones (Fig. 2.2). In July, the concentration of water-soluble carbohydrates in the TD clone was less in the 46-cm than the 76-cm treatment despite an osmotic adjustment of 0.07 MPa (Fig. 2.2b). This decline was however offset by an increase ($p = 0.073$) in total inorganic ion concentration in the 46-cm treatment. Potassium (K) and sodium (Na) were the most abundant inorganic ions although there were no significant clonal differences in these ions (Fig. 2.2).

The DN clone had higher total free primary amino acids than the TD clone in June, but clonal differences were not significant in July (Figs. 2.3a, b). Clonal differences in several amino acids were evident, including serine and an unidentified amino acid (UNK1) that eluted between phenylalanine and isoleucine. The total amino acid concentration increased by 28% in the 46-cm over that of 76-cm treatment in the TD clone in July, largely the result of increases in serine and UNK1. In June, aspartic acid, glutamic acid, and glutamine accounted for 81–83% of the total in the DN clone, whereas their contribution was limited to 36–45% in the TD clone. The major amino acid measured in the TD clone, serine, accounted for 32–37% of the total amino acid concentration, whereas it accounted for less than 5% in the DN clone (Fig. 2.3).

2.4.2 Second Sampling Year (1995 Growing Season)

There were significant clonal and treatment differences in ψ_s in both sampling periods (Fig. 2.1). The 76-cm treatment had the lowest ψ_s although the 46-cm treatment was not different from the 137-cm treatment for both clones on June 28, 1995 (Fig. 2.1). During the second sampling period (July 13, 1995), there was an osmotic adjustment of 0.07 MPa (46 cm) and 0.11 MPa (76 cm) for the TD clone. However, this was not observed in the DN clone which had similar values for both the 46-cm and 137-cm treatments. The TD clone maintained generally lower ψ_s than the DN clone within a given treatment (Fig. 2.1).

The clonal difference in organic solutes was less pronounced in 1995 than in 1994. However, the general trend remained similar with the DN clone exhibiting higher organic solute concentration than the TD clone in the 137 cm treatment in June, mainly due to

Fig 2.1. Leaf osmotic potential at saturation for two hybrid *Populus* clones (*P. trichocarpa* x *P. deltoides* (TD) and *P. deltoides* x *P. nigra* (DN) in their third (1994) and fourth (1995) growing seasons at Wallula, WA. Trees were subjected to different supplemental irrigation regimes. (a) Data of the 76 cm treatment are presented separately from (b) the 46 cm and 137 cm treatments for clarity purposes only. Different letters on a particular sampling date indicate significant treatment effects within a clone whereas an asterisk (*) indicates significant clonal difference for that irrigation treatment ($p \leq 0.05$). ($n = 6$ in June 1994, $n = 8$ in July 1994, and $n = 12$ July 1995 except $n = 6$ in the 76 cm treatment).

Fig. 2.2. Concentrations ($\mu\text{mol g}^{-1} \text{ dw}$) of water-soluble organic solutes and inorganic ions for two hybrid *Populus* clones in: (a) June 29, 1994 and (b) July 28, 1994 under different supplemental irrigation treatments. Only samples from the 76 cm treatment were analyzed for solutes in June whereas the 46 cm treatment was included for the TD clone in July. Different letters indicate significant ($p \leq 0.05$) treatment effects for a given solute. An asterisk (*) indicates significant clonal difference for that variable ($n = 6$ in June and $n = 8$ in July 1994).

Fig. 23. Concentrations ($\mu\text{mol g}^{-1} \text{ dw}$) of free primary amino acids for two hybrid *Populus* clones in: (a) June 1994 (b) July 1994 and (c) July 1995 under different supplemental irrigation treatments. An asterisk (*) indicates significant clonal difference for that variable in the 1994 samples ($p \leq 0.05$). Other details as in Figs. 1 and 2.

fructose, glucose and sucrose (Fig. 2.4). Only the TD clone showed a significant increase in sucrose and total soluble carbohydrate concentration due to water stress in July. Although not significant, there was also an 18% increase in sucrose and a 14% increase in total sugar concentration in the DN clone in July. Similar to the 1994 data, sucrose constituted a large fraction of the total organic solute concentration in both clones. Sucrose alone accounted for about 73% of the total water-soluble carbohydrate increase in the 46-cm over the 137-cm treatment in July. The concentration of glucose and fructose in the DN clone was more than double that of the TD clone although these compounds together accounted only for less than 14% of the total organic solute concentration (Fig. 2.4).

The concentration of free primary amino acids was low in 1995, as was evident in 1994. The DN clone had higher concentration than the TD clone for most of the amino acids except serine and the UNK1. The UNK1 amino acid was low or not detected in the DN clone but was higher in the 46-cm versus the 137-cm treatment in the TD clone. Similar to the 1994 data, serine and UNK1 were responsible for the increase in total amino acids in the TD clone (Fig. 2.3).

2.5 DISCUSSION

The TD clone consistently maintained lower ψ_x than the DN clone and osmotic adjustments of 0.07–0.12 MPa (Fig. 2.1). The only osmotic adjustment (0.11 MPa) evident in the DN clone was in the 76-cm treatment in June 1995. Since we had observed some irrigation line interruption in the 76-cm treatment during this period, it is possible that the DN clone was responding to this sudden water stress period. However, there appears a consistent pattern for the 76-cm treatment to exhibit lower ψ_x than the 137-cm in June both in 1994 and 1995 for the TD clone (Fig. 2.1a). When ψ_x data of both years were combined, there was a decline in ψ_x over the sampling season (as well as increasing age and size) in both clones except for the 76-cm treatment which was not consistent (Fig. 2.1a,b). The season-dependent decline coincided with the higher water stress level imposed as the season progressed from June to July. Seasonal declines in osmotic potentials have been reported in some tree species including *P. deltoides* clones (Tyree et al. 1978, Gebre and Kuhns 1991). Because these poplar hybrids also grow rapidly, the demand for water to support the greater size is expected to be higher and such an increase in the water stress level may explain in part the age-dependent decline in ψ_x . The reduction in relative growth rate with age may also reduce the demand of carbohydrates for growth, increasing their availability and thereby lowering ψ_x .

The July 1994 sampling occurred during a dry and hot period (40°C), and we had noticed the TD plants in the 46-cm treatment had more leaf necrosis and leaf loss than that of the DN clone. This effect was again observed in 1996 after a high temperature period. It is possible that the level of osmotic adjustment in the TD clone was limited by the absence of or reduction in photosynthesis due to a combination of drought and heat stress. This may also explain the lower carbohydrate concentrations in the 46-cm compared to the 137-cm treatment in the TD clone. In *P. deltoides* clones grown in the greenhouse, a severe stress (10 days without watering) resulted in no osmotic adjustment possibly reflecting the stomatal closure and a lack of photosynthetic carbon assimilation (Gebre and Kuhns 1993). The clones in that study were however capable of osmotic adjustment between 0.2 and 0.5 MPa under moderate stress (Gebre and Kuhns 1993, Gebre et al.

Fig. 2.4. Concentrations ($\mu\text{mol g}^{-1} \text{ dw}$) of water-soluble organic solutes and inorganic ions for two hybrid *Populus* clones in: (a) June 1995 and (b) July 1995 under different supplemental irrigation treatments. Different letters indicate significant ($p \leq 0.05$) treatment effects for a given solute. An asterisk (*) indicates significant clonal difference for that variable in the June 1995 samples ($p \leq 0.05$) ($n = 12$).

1994). In the current study, the lowest predawn leaf water potential of the growing season (-1.2 MPa) also occurred during the July sampling period and the absence of a large osmotic adjustment suggests these clones use dehydration postponement (e.g., stomatal control and leaf abscission) as their drought tolerance mechanism (Kramer 1983). The midday leaf water potentials during the July 1994 sampling period remained between -1.2 MPa and -1.7 MPa for DN and -1.6 MPa to -1.9 MPa for TD for all treatments (data not shown), reflecting that stomatal regulation of water loss maintained relatively high leaf water potentials (Tschaplinski et al. 1997).

Carbohydrate accumulation due to stress was observed only in the 1995 samples with sucrose as the major component in the TD clone. Although the osmotic adjustment during this period was accounted for by the increase in solutes, clonal difference in ψ_{π} was not accounted for by the difference in solute concentration. There were clonal differences in the solute profile with the DN clone containing more fructose, glucose, and quinic acid than the TD clone (Fig. 2.4). The concentration of malic acid in these clones was very low, although concentrations as high or greater than sucrose were reported in greenhouse-grown *P. deltoides* and some TD clones (Gebre et al. 1994, Tschaplinski and Tuskan 1994). It is not clear whether the low malic acid concentrations reflect clonal differences or differences in growing conditions (field versus controlled), because even in the greenhouse conditions, two of four TD clones had negligible malic acid concentration (Tschaplinski and Tuskan 1994).

The low concentration of free primary amino acids in these clones is similar to earlier results on hybrid *Populus* grown in pots (Tschaplinski and Tuskan 1994). In that study, glutamic acid was the major component accounting for about 50% of the total free primary amino acids similar to that observed for the DN clone in the current study. Griffin et al. (1991) also reported high concentrations of glutamic acid in *P. tremuloides* Michx. although this varied by clone. Serine was not identified as a major component of the amino acid pool in any of these studies. A decline in stomatal conductance followed by a likely increase in photorespiration rate may have been a factor in the increased concentration of serine in the TD clone, particularly during the hot July of 1994 when the serine concentration was elevated (Fig. 2.3b). Increased photorespiration rate relative to photosynthetic CO_2 fixation under increasing leaf temperature and water stress was reported in fully exposed cotton leaves growing in the San Joaquin Valley, CA (Björkman and Demming-Adams, 1994). Lawlor and Fock (1977a, b) also reported that photorespiration increased relative to photosynthesis with an increase in the water stress level of sunflower leaves (*Helianthus annuus* L. var Mennonite). This was accompanied by an increase in serine and glycine. Although the concentration of free primary amino acids in our study was too low to appreciably affect osmotic potentials, the significant clonal and treatment differences in specific amino acids may be useful indicators of or selection criteria for drought resistance/sensitivity. For example, if the increase in serine in the TD clone indicates an increase in photorespiration over photosynthesis, then the growth performance of the TD clone may be affected negatively under severe stress more than that of the DN clone. We cannot speculate what the role of the UNK1 in the TD clone and its absence from the DN clone means except that it appears it is unique for the TD clone, with the concentration increasing by about 155% in the 46-cm over the 76-cm or 137-cm treatments.

There was only a small increase in inorganic ion concentration in the TD clone in July 1994 although clonal differences have been reported for several poplars grown in controlled environments (Tschaplinski and Tuskan 1994). High K concentrations have

also been reported in crops (Morgan 1992) and tree species (Ranney et al. 1991). The lack of K accumulation in this study may have been associated with a lack of counter ion availability.

In conclusion, small clonal and treatment differences in ψ_x were observed. However, the organic solutes measured could not fully explain for these differences. Although at low concentration, two free primary amino acids (serine and an UNK1) were associated with low ψ_x and plant stress, further study is required involving many clones to test the significance of these compounds. Given that the degree of osmotic adjustment was limited, it is concluded that these clones do not appreciably utilize osmotic adjustment in leaves to resist drought under low irrigation. It is likely that differences in stomatal conductance and/or root development contributed to the difference in these clones and play a greater role in drought resistance.

2.6 ACKNOWLEDGMENTS

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INVENTIONS MADE OR REPORTED

There were no inventions made or reported as part of this CRADA.

ASSESSMENT OF COMMERCIALIZATION

Principal output of project was information, not a product for commercialization.

PLANS FOR FUTURE COLLABORATION

Talks with ORNL, BCC, and the University of Washington (UW) will be conducted this quarter on follow-on collaborative research in the Pacific northwest. The research will focus on the advanced generation poplar pedigrees and clones from diverse crosses generated by UW and established and managed by BCC at Ice Harbor Fiber Farms, near Pasco, WA. The goals will be to determine (1) the extent of drought tolerance in poplar in currently available germ plasm, (2) genetic markers for drought tolerance, analyzed by using structured multiple-generation pedigrees, and (3) how far can drought tolerance be increased through selective breeding. The work will likely be conducted outside of the CRADA mechanism.

CONCLUSIONS

The CRADA provided access to a well-conducted irrigation experiment in the Pacific Northwest under BCC's management, where large trees were subjected to varying degrees of drought stress under operational growing conditions and over time. The experiment provided a real-world validation of the role that low osmotic potential and osmotic adjustment have in determining drought tolerance of poplar clones. The importance of multiple drought resistance mechanisms in determining a clone's overall drought resistance was highlighted by the research. The role that specific compounds involved in determining low osmotic potential and adjustment was clarified, and will serve to focus future research that will aim to increase the limits in drought tolerance of poplar and maintain productivity on sites that are prone to drought, thereby expanding the acreage suitable for planting short-rotation woody crops.

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