Evaluation of Drought Tolerance of Selected Provenances of Taxodium
Geoffrey C. Denny*1 and Michael A. Arnold2)

Abstract: Screening studies of open-pollinated families in a greenhouse suggest a geographic component to variation in drought tolerance of Taxodium distichum. The open-pollinated families from mesic eastern localities were less tolerant of drought than open-pollinated families from more xeric western populations. Further drought screenings suggest that T. distichum seems to avoid drought by limiting water loss from the shoots and tolerate drought tolerance by osmotic adjustment. The field screening supported the conclusions of the greenhouse-based studies that western populations of Taxodium distichum are generally more drought tolerant than eastern populations. Field performance under xeric conditions improved as populations were sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitation. The implication is that when choosing Taxodium for use in more xeric conditions, care should be taken to select western genotypes.

Key Words: Baldcypress, Montezuma cypress, Pondcypress, Taxodium distichum, Water relations

1. Introduction

Taxodium distichum (L.) Rich. is a widely adaptable tree species for landscape use, tolerating both wet and dry soils, and air pollution (Cox and Leslie, 1988; Wasowski and Wasowski, 1997). Watson (1983) reports tolerance to varying nutrient availability conditions, a wide range of soil aeration levels, and somewhat extreme pH levels. It is fast growing, has reliable feathery foliage, and a nice form (Arnold, 2002; Cox and Leslie, 1988). Two varieties, var. distichum (baldcypress) and var. imbricarium (Nutt.) Croom (pondcypress), have fairly good fall color some years, while var. mexicana Gordon (Montezuma cypress) remains semi-evergreen (Arnold, 2002). It is an extremely long-lived tree, with a life span of up to 700 years possible (Cox and Leslie, 1988). All of these factors allow T. distichum to tolerate many environmental stresses, making it a promising choice for urban landscapes. However, there are a few limitations to this species. While it is tolerant of substantial soil salts, it tends to defoliate when leaves come into contact with salty irrigation water, tends to develop chlorosis on sites with high pH, and has a tendency to “brown out” in periods of extended or severe drought (Arnold, 2002).

Urban surfaces and compacted soils frequently decrease the amount of water that infiltrates into the root zone of trees; moreover, trees must compete with turf and other vegetation for the available water (Zwack and Graves, 1998). Therefore, water deficit situations can be common in urban areas. Zwack and Graves (1998) also point out a need for “tree taxa that maintain landscape function during episodes of variable and adverse soil moisture”. St. Hillaire and Graves (2001) suggested that a strategy for selecting ornamentals with “superior resistance to drought stress” was to select from populations native to relatively xeric habitats. The purpose of this study was to determine if there is a geographic basis for drought tolerance in Taxodium and to evaluate selected provenances in an effort to select those which yield individuals that are most adaptable/tolerant to this environmental stress.

2. Materials and Methods

Open-pollinated family identity was coded with four alphanumeric characters. The first two letters signify the general geographic origin of the mother tree. ‘MX’ signifies south Texas and Mexico, ‘TX’ signifies central Texas, and ‘EP’ denotes the southeastern U.S. The numeral is unique to an open-pollinated family from a given geographic area. The final letter indicates the taxonomic variety. ‘M’ indicates that the open-pollinated family belongs to the variety mexicanum, ‘D’ indicates var. distichum, and ‘I’ indicates var. imbricarium.

2.1. Greenhouse screening 1

Thirteen open-pollinated families of Taxodium distichum were collected in the late summer and fall of 2003. Seeds were collected off a single mother tree at several locations (Fig. 1) representing the ecophysiological variation throughout the species range. After collection, seeds were stratified for 90 d at 3ºC. Localities representing ‘normal’ seed sources (mesic eastern U.S. sites), as well as sites representing more xeric environmental conditions (western U.S. and Mexican sites) were sampled. Seeds were planted in 36 cm × 51 cm × 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with medium...
vermiculite (Sun Gro Horticulture, Bellevue, Wash.) on 12 Mar. 2004, and germinated before the end of Apr. 2004. On 8-10 Apr. 2004, 200 seedlings of each open-pollinated family were transplanted into 9.6 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with 3 pine bark : 1 coarse perlite (by volume) substrate amended with 6.53 kg m\(^{-3}\) 15N-3.9P-9.9K controlled-release fertilizer (Osmocote\textsuperscript{®} Plus, Scotts Co., Marysville, Ohio), 0.89 kg m\(^{-3}\) micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax\textsuperscript{®}, Scotts Co., Marysville, Ohio), 1.78 kg m\(^{-3}\) CaSO\(_4\) (United States Gypsum Co., Chicago, Ill.) and 4.15 kg m\(^{-3}\) CaMgCO\(_3\) (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown outdoors under 55% light exclusion in a nursery area and irrigated by hand as needed.

Initial drought tolerance evaluations were conducted in a greenhouse beginning 6 June 2005. Containers were arranged in a completely randomized design. Plants were subjected to a regime of decreasing irrigation frequency, beginning with a daily watering, followed by a 2 d interval between irrigations, then a 3 d period, etc. The study was terminated 8 Aug. 2005, after the 10 d interval between irrigations. Plant height and trunk diameter, as well as shoot and root dry mass were taken on the last day of the experiment to evaluate plant growth and biomass partitioning. Height and diameter measurements were taken at the initiation of the experiment and at its end. Pre-dawn xylem water potential was measured just before irrigation at the end of each dry down cycle using a pressure chamber (Model 610, PMS Instrument Company, Albany, Ore.). Volumetric water content of five containers chosen at random was measured hourly using dielectric soil moisture probes (Decagon Devices, Inc., Pullman, Wash.).

Growth and morphology (height, trunk diameter, dry mass, root:shoot ratio and time to mortality), as well as xylem water potential data were analyzed using univariate analysis in the GLM procedure of SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Hierarchical cluster analysis using squared euclidean distance as a measure and the nearest-neighbor method in SPSS utilizing pre-dawn water potentials and mortality was used to generate dendrograms.

### 2.2. Greenhouse screening 2

Four open-pollinated families of \textit{Taxodium distichum} were selected for screening in the spring of 2006 (Fig. 1). Families were selected to represent the ecophysiographic variation between the “Mexican” type populations (extreme south Texas and Mexico, Family MX5M) and those from central Texas (Families TX1D, TX2D, TX5D) because of the superior performance of genotypes from these regions in the initial screening. Cuttings off multiple trees per family from a stock block maintained in the field in College Station, Texas were rooted on 20 March 2006. Cuttings were treated with a 8000 mg L\(^{-1}\) IBA and 4000 mg L\(^{-1}\) NAA dip (Dip ‘n Grow, Inc., Clackamas, Ore.) and were placed in 36 cm × 51 cm × 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture, Bellevue, Wash.). Rooted cuttings were planted on 12 May 2006 into 2.5 L containers (Nursery Supplies, Inc., Kissimmee, Fla.) filled with calcined clay (Oily-Dri Corp. of America, Alpharetta, Ga.) amended with 6.53 kg m\(^{-3}\) of 15N-3.9P-9.9K controlled release fertilizer (Osmocote\textsuperscript{®} Plus, Scotts Co., Marysville, Ohio), 0.89 kg m\(^{-3}\) micronutrient fertilizer 0N-0P-0K-6Ca-3Mg-12S-17Fe (Micromax\textsuperscript{®}, Scotts Co., Marysville, Ohio), 1.78 kg m\(^{-3}\) CaSO\(_4\) (United States Gypsum Co., Chicago, Ill.), 4.15 kg m\(^{-3}\) CaMgCO\(_3\) (Oldcastle Stone Products, Thomasville, Pa.). Plants were grown in a greenhouse with 26.7°C / 23.9°C day/night temperature set points. Typical light levels as measured in mid-afternoon on 30 Aug. 2006 were 702 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PAR.

Drought tolerance evaluations were conducted in the greenhouse beginning on 4 September 2006. Containers were arranged in a completely randomized design. Plants were subjected to an acute drought stress by withholding water. Plants within a family were harvested when at least half of the treated plants in that family showed foliar death. Plant height and trunk diameter, as well as shoot and root fresh and dry mass, were measured at the end of the experiment to evaluate plant growth and biomass partitioning. Pre-dawn xylem water potential was measured at harvest using a pressure chamber. Volumetric water content at harvest of all the
containers was calculated from fresh and dry masses and volume measurements of the substrate. The mass of the water present in the containers was calculated as the difference between the fresh and dry mass of the substrate. The density of water was assumed to be 1.0, allowing the easy conversion from mass to volume. The volumetric water content of the substrate was then calculated with the following formula: 

\[ V_\% = \left( \frac{volumewater}{volumesubstrate} \right) \times 100 \]

Growth and morphology (height, trunk diameters, dry masses, root:shoot ratios, shoot and substrate water contents, and days to mortality), as well as xylem water potential data, were analyzed using univariate analysis in the GLM procedure of SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.).

2.3. Pressure-volume curves

In spring 2006, three open-pollinated families of *Taxodium distichum* were selected which represent the ecophysio- graphic variation throughout the tested species’ range (Fig. 1). The genotypes used represented seed sources from the southeastern U.S. (family EP8D, Vidalia, La.), central Texas (family TX6D, Atascosa River, Texas), and the Rio Grande Valley of Texas (family MX5M, Progresso, Texas). Plants were grown as described in section 2.2. Containers were arranged in a completely randomized design on a single bench during growth and were irrigated as needed.

On 18 Sept. 2006, three rooted cuttings from each of the three selected families were used to perform a pressure-volume analysis as described by Turner (1988). Care was taken to ensure that shoots had comparable amounts of foliage per shoot, as is suggested by Neufeld and Teskey (1986). Shoots were cut and allowed to rehydrate to full turgor in distilled water for 18 h in the dark at 5.5°C. Fresh mass (FW) of each cutting was measured followed immediately by its xylem water potential beginning at the end of the rehydration period and then every 30 min thereafter until xylem water potential reached -4.0 MPa. The initial fresh mass is referred to from here on as TW. Dry mass (DW) of each cutting was also measured. Relative water content (RWC) of the cuttings was calculated using the following formula:

\[ RWC = \frac{FW - DW}{TW - DW} \times 100 \]

Water contents, fresh mass to dry mass ratios, and xylem water potential data were analyzed and parameter estimates generated using univariate analysis in the GLM procedure in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.).

2.4. Field screening

Open-pollinated families of *Taxodium distichum* were collected in the late summer and fall of 2003. Seeds from a single mother tree at all the locations on Figure 1, representing the ecophysiological variation throughout the species’ range were collected and stratified (90 d at 2°C). Localities representing “normal” seed sources (mesic, acidic eastern U.S. sites), as well as sites representing more extreme environmental conditions (more xeric, alkaline western U.S. and Mexican sites) were sampled. Plants were grown as described in section 2.1.

The field site was located at the Texas A&M Research and Extension Center at Overton (USDA hardiness zone 8a). The soil at the site is a Bowie very fine sandy loam, 1% to 4% slopes and has a pH of approximately 6.5. The trees were irrigated as needed the first year only. Seedlings were planted on 29 June 2004. Plants were arranged in a randomized complete block design with 13 families in 20 blocks containing 2 replications of each family per block. Tree heights and trunk diameters were measured at the time of planting and again in the next three Decembers. Growth indices for both height and trunk diameter were calculated as follows: growth index = (new measure – previous measure)/previous measure. This is analogous to relative growth rate calculations, except it is based on non-destructive measures rather than dry masses (Arnold et al., 2007). Growth data were analyzed using univariate analysis in the GLM procedure and hierarchical cluster analysis in SPSS (version 12.0.2 for Windows, SPSS Inc., Chicago, Ill.). Survival data were analyzed with the Chi-square procedure in SPSS.

3. Results and Discussion

3.1. Greenhouse screening I

There were significant differences in pre-dawn water potentials among families after 5, 6, 7, 8 and 10 d of imposed drought \((P \leq 0.05)\) (Fig. 2). Additionally, there was a significant difference in the mean survivable water deficit among families \((P \leq 0.05)\) (Fig. 3). A hierarchical cluster analysis of the families based on pre-dawn xylem water potentials from the 5, 6 and 7 d drought periods (Fig. 2) and the survivable drought period of each family (Fig. 3) generated a dendrogram showing the relationship among families based on their performance in this screening (Fig. 4). The parameters utilized in this analysis were selected because the open-pollinated families showed the most separation during the 5, 6 and 7 d drought periods. After the 7 d drought period, some of the individuals exhibited canopy death, leading to a less negative pre-dawn xylem water potential measurement.

Time to canopy death was selected as a parameter in an effort to account for this phenomenon. The dendrogram divides the families into two main groups. The eastern populations all fall into one group (Families EP2D, EP4D, EP5I, EP6D and EP8D) and the Texas (Families MX5M, TX2D and TX8D) and Mexican (Families MX3M and
MX4M populations fall into the other. The exception is the open-pollinated family (TX6D) from Poteet, TX, which clusters with the eastern populations. Within the cluster of eastern populations there are two groups. The first includes populations that all belong to the variety distichum, and the second includes families from both variety distichum (TX6D, Poteet, TX) and var. imbricarium (EP51, Fowl River, AL and EP6D, Biloxi, MS). No significant differences in root to shoot ratios were found ($P = 0.372$).

The clustering of the families suggests that there is a geographic component to variation in drought tolerance of Taxodium distichum. The observed geographic pattern is what might be expected. The open-pollinated families from eastern localities were less tolerant of drought than open-pollinated families from western populations. This is likely due to a general trend in decreasing rainfall as we move from east to west in the southern U.S.

The implication is that when selecting genotypes for more xeric situations, an effort should be made to obtain genotypes from central Texas or Mexico. Additionally, open-pollinated families from south Texas and Mexico appeared less stressed at times of xylem water potential measurement, although no data were taken on general appearance because of its subjective nature.

### 3.2. Greenhouse screening 2

There were significant ($P \leq 0.05$) treatment effects related to water deficits among the families in all the parameters measured. Well-irrigated control plants did not differ significantly among open-pollinated families. Treatment plants from family MX5M had 50% survival after 11 d without irrigation, while families TX1D, TX2D and TX7D had 50% survival after only 8 d. Volumetric water content of the
substrate at the time of harvest was significantly lower \((P \leq 0.01)\) for family MX5M than the other families, which did not differ significantly from each other (Fig. 5).

Shoot water content at harvest was significantly lower \((P \leq 0.01)\) for family MX5M than the other families (Fig. 6). Shoot water content of family TX1D was significantly higher \((P \leq 0.05)\) than all other families (Fig. 6). Families TX2D and TX5D did not differ from each other, but did differ from families MX5M and TX1D.

An estimated substrate water depletion rate was calculated by dividing the difference between the mean substrate volumetric water content for the well watered control plants and the observed substrate volumetric water content for the treatment plants by the number of days to harvest. Family MX5M showed a lower estimated water depletion rate than the other families \((P \leq 0.01)\) (Fig. 7). No significant difference was found between the root to shoot ratios of the families \((P = 0.11)\) (data not shown).

These results support the observation in the initial screenings that the Mexican families appeared less water stressed compared to the central Texas families. After similar drought periods, the Mexican genotypes had higher water contents per unit dry mass. They were able to withstand longer droughts than central Texas families because they were able to survive at lower substrate volumetric water contents. They also removed water from the substrate at a lower rate (Fig. 7), implying that they are better at controlling water loss from their shoots. The Mexican genotype was also able to extract more water from the substrate (Fig. 5). This suggests that *Taxodium* may utilize both drought tolerance and drought avoidance as mechanisms for resisting drought stress.

### 3.3. Pressure-volume curves

Pressure-volume analysis allows many plant-water parameters to be derived including: total water content, turgid/dry mass ratio, relative water content, apoplastic and symplastic water contents, relative symplastic water contents, osmotic pressure at full and zero turgor, relative water content at zero turgor, bulk moduli of elasticity, and tissue moisture release curves (Turner, 1988). It also provides the needed parameters to create a Höfler diagram (Turner, 1988). Pressure-volume curves have been used extensively to examine many aspects of plant-water relations by numerous authors (Fan *et al*., 1994; Roberts *et al*., 1981; White *et al*., 2001). Li (1998) utilized pressure-volume analysis to compare leaf water relations of *Eucalyptus microtheca* F.
Table 1. Analysis of covariance table for the rates of shoot relative water content decrease, xylem water potential decrease, and change in the fresh to dry mass ratio in three open-pollinated families of Taxodium distichum.

<table>
<thead>
<tr>
<th>Measured Characteristic</th>
<th>²</th>
<th>Source</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative water content</td>
<td>0.94</td>
<td>Model</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family</td>
<td>0.958</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family X Time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Xylem water potential</td>
<td>0.83</td>
<td>Model</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RWC</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family X RWC</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fresh: Dry Mass Ratio</td>
<td>0.72</td>
<td>Model</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family X Time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Xylem water potential</td>
<td>0.84</td>
<td>Model</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercept</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family X Time</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Muell. provenances.

Relative water content of each of the samples was calculated for each measurement point. The rate of relative water content (RWC) loss was significantly different among the eastern family (EP8D) and both the central Texas (TX6D) and south Texas (MX5M) families (P ≤ 0.001) (Table 1). The families from Texas did not significantly (P ≥ 0.05) differ in RWC loss rates. The difference in rate of RWC loss among the three families in the study supports the findings of the previous screenings of the open-pollinated families. Eastern families tended to desiccate (decrease in RWC) more rapidly than south Texas families, while families from central Texas tended to be intermediate. The relationship between the plant water potential and RWC is referred to as the water potential isotherm or the moisture release curve for a tissue (Turner, 1988). It has been used to determine the drought resistance characteristics of various species (Jones et al., 1981).

Xylem water potential also differed significantly in response to decreasing RWC among all three families (Table 1). The south Texas family (MX5M) showed the largest decrease in xylem water potential per unit change in RWC, followed by the central Texas family (TX6D) and then the eastern family (EP8D). The south Texas family has the steepest moisture release curve (Table 2), implying that its tissues retain water more tightly than the other families. The eastern family has the shallowest moisture release curve implying that the water in these plants is held the least tightly. The moisture release curve of the central Texas family was intermediate.

Xylem water potential of family MX5M decreases slightly faster than those of either of the other two families (Table 2). The fresh mass to dry mass ratio decreases more rapidly in the eastern family (EP8D) compared to the Texas families (Table 2). The turgid to dry mass ratio has been shown to correlate well with osmotic adjustment in some species (Turner, 1988). This is logical because the higher the ratio is the more water per unit dry mass the plant contains. However, this may be due to higher osmotic potentials or to more elastic cells. Differences in the rate of change in the ratio between fresh mass and dry mass over time can indicate the concentration of osmolytes in the leaf tissue. This may be the case here. As the tissues began to desiccate, the south Texas (MX5M) family shows only a slight decrease in the fresh to dry mass ratio and the slope of the line does not differ significantly from 0 (Table 2). The central Texas family (TX6D) also shows only a slight decrease in fresh to dry mass ratio during desiccation. Although the slope of the regression line for this family does not differ significantly from that of the south Texas family (MX5M), it is less than zero (Table 2). The slope of the linear regression for the eastern family (EP8D) differs from those of both Texas families and is significantly less than zero (Table 2).
This means that as the tissue desiccates it holds relatively less water than it did while it was wetter. This would not be expected if the plant was utilizing osmotic adjustment as a strategy to resist drought, which would likely give the opposite result.

3.4. Field screening

There was significant variation in tree survival in the second and third growing season at the Overton field site (Fig. 8). The Chi-square test for survival in both seasons for open-pollinated family was highly significant ($P \leq 0.0001$). In both years, the western genotypes generally had higher survival percentages than genotypes from more mesic, eastern sources (Fig. 8). This pattern is especially striking in the third season (2006) cumulative survival where none of the eastern families had above 25% cumulative survival (Fig 8b).

There was significant variation in the height growth index (Fig. 9) and the trunk diameter growth index for 2005 among families ($P \leq 0.0001$) (Fig. 10). In 2006, only the variation in trunk diameter growth indices was significant ($P \leq 0.0001$). A similar pattern to that observed in the survival percentages was evident. The western families grew faster in height (Fig. 9) and trunk diameter during 2005 and in trunk diameter during 2006, when compared to eastern genotypes (Fig. 10).

This pattern is similar to that observed in the greenhouse-based drought screenings. These results support the conclusions of the greenhouse-based studies that western populations of *Taxodium distichum* are generally more drought tolerant than eastern populations. Field performance under xeric conditions improved as populations were sampled from east to west in the U.S. and then south into Mexico, following a general environmental gradient of decreasing precipitation.

References


