# Comparative drought resistance among six species of birch (*Betula*): influence of mild water stress on water relations and leaf gas exchange

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## Summary

Responses of plant water relations and leaf gas exchange to mild water stress were monitored and compared among six species of birch; monarch birch (*Betula maximowicziana* Reg.), river birch (*B. nigra* L.), paper birch (*B. papyrifera* Marsh.), European white birch (*B. pendula* Roth.), 'Whitespire' Japanese birch (*B. platyphylla* var. *japonica* Hara. 'Whitespire'), and gray birch (*B. populifolia* Marsh.). Before imposition of water stress, 'Whitespire' Japanese birch and river birch maintained the highest stomatal conductances and net rates of photosynthesis of the species examined. After irrigation was withheld, stomatal conductance and rate of net photosynthesis gradually declined for most species. After 28 days without irrigation, 'Whitespire' Japanese birch maintained significantly higher stomatal conductance and rate of net photosynthesis than did the other species despite having one of the lowest midday water potentials. There was no evidence of osmotic adjustment by any of the species in response to the imposed drought. However, there was substantial variation in the water potential at the turgor loss point among the species, from a high of −1.34 MPa for river birch to a low of −1.78 MPa for 'Whitespire' Japanese birch. Stomatal conductance and net photosynthesis under mild water stress (average predawn leaf water potential of −0.61 MPa) were negatively correlated with leaf osmotic potential at full turgor and leaf water potential at the turgor loss point. Thus, the greater net photosynthesis of 'Whitespire' Japanese birch under water stress compared with the other species appears to have resulted from a superior capacity to maintain turgor at low leaf water potentials, which in turn provided for greater stomatal conductance and $CO_2$ uptake. These results indicate that 'Whitespire' Japanese birch is better adapted to dry sites than the other species.

## Introduction

The genus *Betula* has many species with traits of ornamental value. Some of the ornamental species are native to cool, moist climates and perform poorly on dry sites, e.g., European white birch (*B. pendula* Roth.), paper birch (*B. papyrifera* Marsh.), and river birch (*B. nigra* L.) (Weaver 1978, Dirr 1983). Among the approximately 40 species of birch, however, there may be some that are well adapted to drier environments.

The capacity to maintain turgor pressure as plant water potential decreases can contribute to drought resistance. Turgor is a prerequisite for cell expansion (Lockhart 1965) and stomatal opening (Ehret and Boyer 1979, Richter et al. 1981) and is often positively correlated with photosynthesis and plant growth under water stress (Johnson 1978, Ludlow 1987, Osonubi and Davies 1978). The capacity to maintain turgor

pressure at decreasing plant water potentials is primarily a function of bulk tissue osmotic potential and tissue elasticity (Tyree and Jarvis 1982). Tissues with lower osmotic potentials and greater elasticity (lower bulk modulus of elasticity) will maintain positive turgor to lower tissue water potentials.

The objective of this experiment was to evaluate and compare water relations and leaf gas exchange of six species of birch subjected to mild water stress.

## Materials and methods

### Plant material

Between March 20 and April 4, 1989, bare-root seedlings of monarch birch (*Betula maximowicziana* Reg.), (see Santamour and Meyer 1977, for species description), river birch (*B. nigra* L.), paper birch (*B. papyrifera* Marsh.), European white birch (*B. pendula* Roth.), 'Whitespire' Japanese birch (*B. platyphylla* var. *japonica* Hara. 'Whitespire'), and gray birch (*B. populifolia* Marsh.), ranging in height from 0.6 to 1.0 m, were planted in 11.4-liter, black, plastic containers filled with a mixture of milled pine bark (< 13 mm)/sand/sphagnum peat moss (14/5/5 v/v) amended with 4.2 kg m$^{-3}$ dolomitic limestone, 1.2 kg m$^{-3}$ superphosphate, 0.9 kg m$^{-3}$ KNO$_3$, 3.6 kg m$^{-3}$ Esmigran (Sierra Chemical Co., Milpitas, CA), 59 ml m$^{-3}$ Sequestrene Fe 330 (Ciba-Geigy Co., Greensboro, NC), and 40 ml m$^{-3}$ Solubor (sodium borate, U.S. Borax and Chemical Co., Los Angeles, CA). Plants were initially grown outdoors at the Mountain Horticultural Crops Research Station, Fletcher, NC and received daily irrigation. On June 6, 1989, all plants were pruned to a height of 0.6 m above the stem–root junction. On July 20, 1989, one tree of each species was transplanted to each of six plywood boxes (1.0 m × 2.2 m × 0.4 m) filled to a depth of 0.3 m with 0.66 m$^3$ of the same growing medium. The boxes were placed in a polyethylene-covered house. Plants were grown in natural lighting and were well irrigated for 57 days (until September 15, 1989) when they were similar in size and approximately 1.2 m in height. Thereafter, irrigation was withheld. The experimental design was a randomized complete block with six replications. Data were assessed statistically by the analysis of variance using the SAS software package (SAS Institute, Cary, NC).

### Plant water relations

Leaf water potential ($\Psi$) was determined with a pressure chamber (Plant Moisture Status Console, Soil Moisture Equipment Corp., Santa Barbara, CA). Components of $\Psi$ were estimated by pressure-volume methodology (Tyree and Hammel 1972). Leaf samples were collected for pressure-volume measurement during four separate 3-day periods: (1) before withholding irrigation (September 12–14, 1989), (2) between 5–7 days without irrigation (September 19–21, 1989), (3) between 12–14 days without irrigation (September 26–28, 1989), and (4) between 25–27 days without irrigation (October 10–12, 1989). Samples were collected and measured on a block by block basis whereby all experimental units in a given block were sampled on a given day providing for three replicates for each time period. Fully exposed

sun-leaves (fifth most recently fully expanded leaf) were collected before dawn. With the petioles submerged in distilled water, they were enclosed in a polyethylene bag and allowed to rehydrate for 2 hours before measurement. Leaf water potential and corresponding leaf weights were then measured periodically on each sample over a range of $\Psi$ from 0 to $-4.0$ MPa (typically at intervals from 0.3 to 0.4 MPa). Between measurements, samples were allowed to transpire freely outside the pressure chamber (Hinckley et al. 1980, Ritchie and Roden 1985). A mixture of 98% $N_2$ and 2% $O_2$ was used to pressurize the chamber. Chamber pressure was changed at a rate not exceeding 0.02 MPa s$^{-1}$ to avoid tissue injury. Pressure-volume data were analyzed using a segmented, non-linear regression model (model "PVD," Schulte and Hinckley 1985; SAS, NLIN procedure, SAS Institute, Cary, NC). Curves typically consisted of eight data points with four in the region below the point of turgor loss. The $R^2$ values for individual regression analyses were greater than 0.98 in all cases.

The $\Psi$ at the turgor loss point ($\Psi_{tlp}$) was calculated as:

$$\Psi_{tlp} = \frac{\Psi_{\pi,sat}}{1 - \dfrac{1 - RWC_{tlp}}{SWF}} ,$$

where $\Psi_{\pi,sat}$ is the bulk tissue osmotic potential at full saturation, $RWC_{tlp}$ is the relative water content at the turgor loss point, and SWF is the symplastic water fraction at full saturation. Bulk modulus of elasticity was defined as the change in turgor pressure per change in the relative symplastic water content (Jones and Turner 1980). Differentiation and calculation at full turgor, i.e., where the relative water content $= 1$ and turgor pressure $= -\Psi_{\pi,sat}$, gives the maximum bulk modulus of elasticity ($\varepsilon_{max}$):

$$\varepsilon_{max} = \frac{\Psi_{\pi,sat} \times b}{RSWC_{tlp} - 1} ,$$

where $b$ is a constant estimated by the regression analysis (model "PVD") and $RSWC_{tlp}$ is the relative symplastic water content at the turgor loss point.

*Gas exchange measurements*

Net photosynthesis ($P_n$), stomatal conductance to water vapor ($g_s$), and photosynthetically active radiation (PAR, 400–700 nm) were measured between 1030 and 1400 h EDT with a portable gas exchange system (Li-Cor model LI-6200, Lincoln, NE). Supplemental light was provided with a halogen lamp to ensure a minimum irradiance of 1200 μmol m$^{-2}$ s$^{-1}$ of photosynthetically active radiation. Gas exchange readings were recorded as $CO_2$ was depleted from 330 to 318 ppm for each leaf. Following measurement of gas exchange rates, leaves were immediately excised and $\Psi$ was measured. Samples were measured and collected on a block by block basis.

## Results

### Gas exchange measurements

Before the initiation of water stress, there was a nearly twofold variation in $g_s$ and rates of $P_n$ among the six species (Figure 1). Of the species examined, river birch and 'Whitespire' Japanese birch had the highest rates of $P_n$, whereas paper and monarch birch had the lowest. Rankings of $g_s$ were typically similar to those of $P_n$.

After irrigation was withheld, $g_s$ and rates of $P_n$ gradually decreased in most species (Figure 1). Of the six species, river birch appeared to be the most sensitive to mild water stress, as indicated by early reductions in $g_s$ and $P_n$, which declined to low levels after 28 days without irrigation. 'Whitespire' Japanese birch appeared to be least sensitive to water stress, maintaining a higher $g_s$ and rate of $P_n$ throughout the stress period than the other species.

### Plant water relations

In all species, midday $\Psi$ on Day 1 (before stress) varied little from measurements taken after 28 days without irrigation, despite a drier growing medium as indicated by lower predawn $\Psi$ and similar levels of evaporative demand as indicated by the similar vapor pressure deficits (Figure 2). The gradual reduction in $g_s$, and hence transpiration, probably compensated for the decreasing water potential of the grow-
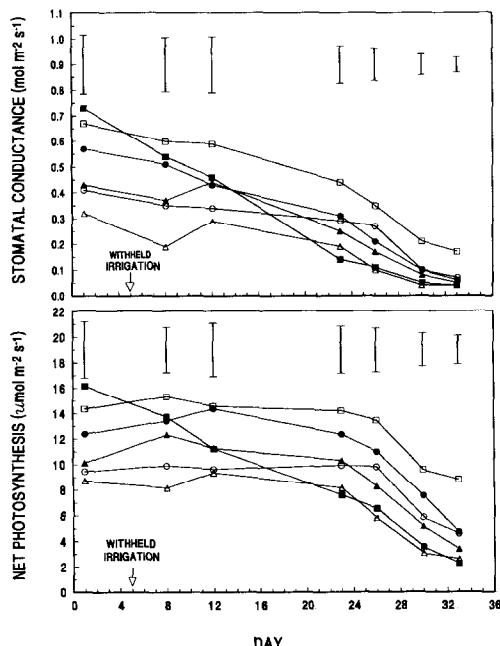


Figure 1. Stomatal conductance and net photosynthesis for 'Whitespire' Japanese birch (□), gray birch (●), paper birch (○), European white birch (▲), Monarch birch (△), and river birch (■) in response to water stress. Vertical bars represent $LSD_{0.05}$ values.
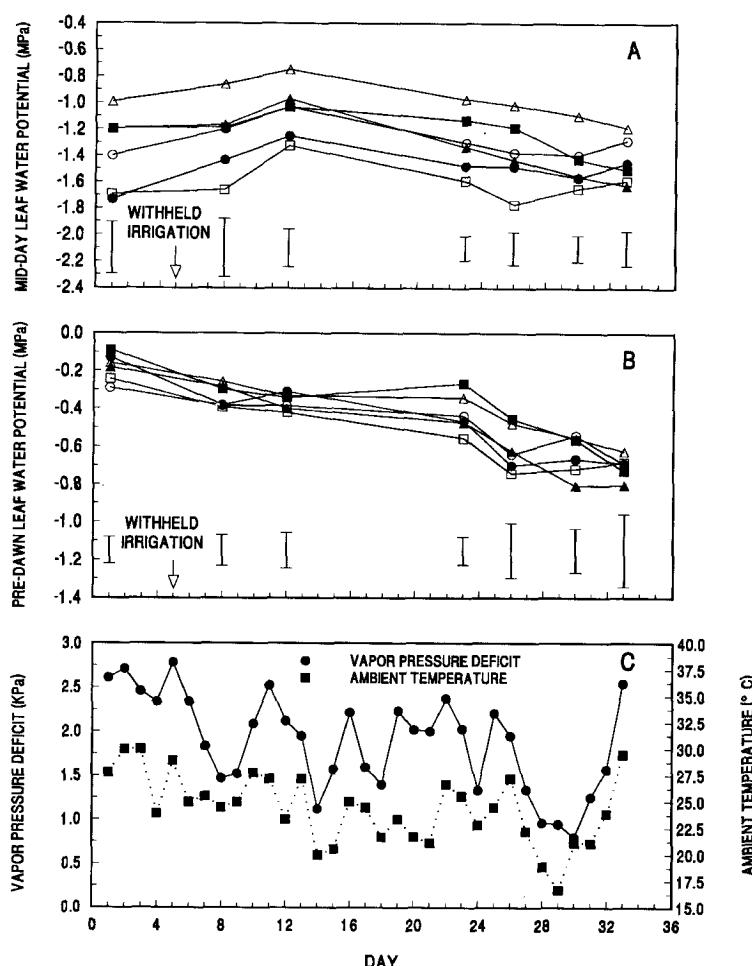
Figure 2. Midday leaf water potential (measured between 1030 and 1400 h), (A), and predawn leaf water potential, (B), for 'Whitespire' Japanese birch (□), gray birch (●), paper birch (○), European white birch (▲), Monarch birch (△), and river birch (■) in response to water stress, midday ambient vapor pressure deficit (C), and midday ambient temperature (C). Vertical bars in A and B represent LSD$_{0.05}$ values.

ing medium, reducing the medium-to-leaf water potential gradient and resulting in relatively stable midday Ψ throughout the experiment.

Analysis of variance of pressure-volume data showed that there was no sampling time, or sampling time by species, interaction ($P > 0.10$) for Ψ$_{\pi,sat}$, Ψ$_{tlp}$, RWC$_{tlp}$, RSWC$_{tlp}$, or ε$_{max}$. Thus, data from the four sampling periods were combined. The absence of a sampling time effect indicates that neither osmotic adjustment nor any other change in tissue water relations affecting turgor maintenance occurred in response to the imposed water stress. However, the species showed substantial variation in their turgor maintenance capacities (Figure 3). 'Whitespire' Japanese birch had one of the greatest capacities for maintaining turgor as Ψ decreased, whereas river birch had one of the lowest. The bulk tissue osmotic potential at full
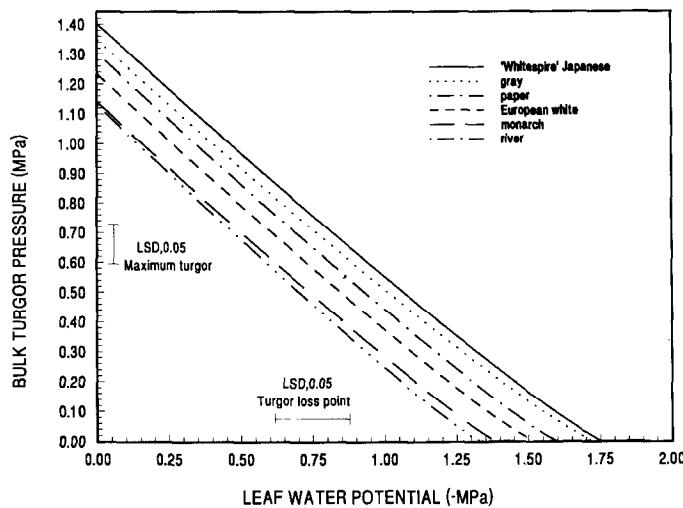
Figure 3. Bulk turgor pressure as a function of leaf water potential. Curves were generated by calculating tissue osmotic potential and turgor pressure (model "PVD", Schulte and Hinckley 1985) over a range of relative water contents using the parameter values given in Table 1 and by assuming that leaf water potential is equal to the sum of turgor and osmotic potential.

turgor of 'Whitespire' Japanese birch was significantly lower than those of gray, monarch, and river birches (Table 1). Rankings of $\Psi_{tlp}$ were similar to those of $\Psi_{\pi,sat}$. Because there was no significant difference in tissue elasticity, as indicated by $\varepsilon_{max}$, variations in $\Psi_{tlp}$ appear to have resulted primarily from variation in $\Psi_{\pi,sat}$. There was no significant difference in $RWC_{tlp}$ or $RSWC_{tlp}$.

The capacity of these species to maintain $g_s$ and $P_n$ under water stress was related to their capacity to maintain turgor. Under mild stress (e.g., Day 26, mean predawn $\Psi = -0.61$ MPa), both $P_n$ and $g_s$ were negatively correlated with $\Psi_{\pi,sat}$ (Figure 4) and $\Psi_{tlp}$ (Figure 5). As the growing medium became drier, $g_s$ continued to decrease and

Table 1. Osmotic potential at full hydration ($\Psi_{\pi,sat}$), water potential at the turgor loss point ($\Psi_{tlp}$), relative water content at the turgor loss point ($RWC_{tlp}$), relative symplastic water content at the turgor loss point ($RSWC_{tlp}$), and maximum bulk modulus of elasticity ($\varepsilon_{max}$) derived from pressure-volume measurements for six species of birch (*Betula*).

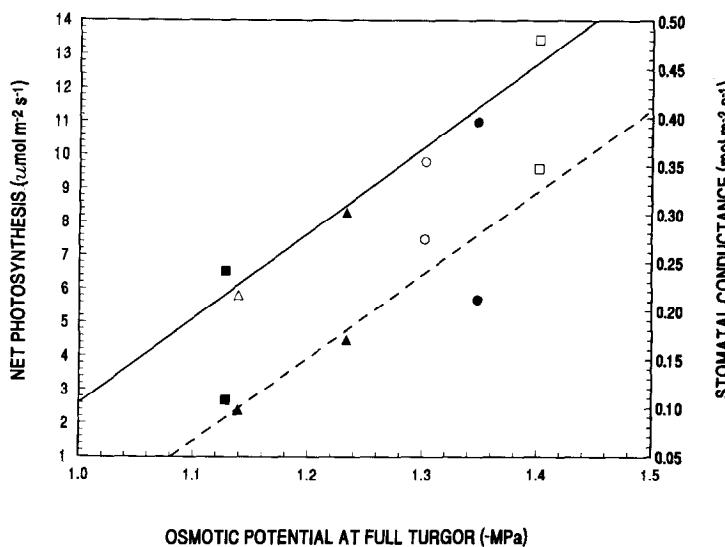| Species | $\Psi_{\pi,sat}$ (MPa) | $\Psi_{tlp}$(MPa) | $RWC_{tlp}$ | $RSWC_{tlp}$ | $\varepsilon_{max}$(MPa) |
|---|---|---|---|---|---|
| River | −1.13 | −1.34 | 0.90 | 0.85 | 11.96 |
| Monarch | −1.14 | −1.41 | 0.87 | 0.82 | 10.34 |
| European white | −1.23 | −1.55 | 0.91 | 0.80 | 10.53 |
| Paper | −1.30 | −1.66 | 0.88 | 0.80 | 10.99 |
| Gray | −1.35 | −1.76 | 0.89 | 0.77 | 8.79 |
| 'Whitespire' Japanese | −1.40 | −1.78 | 0.89 | 0.79 | 10.00 |
| $LSD_{0.05}$, $n = 12$. | 0.14 | 0.25 | 0.04 | 0.09 | 4.07 |

Figure 4. Net photosynthesis (——) and stomatal conductance (– – –) (measured on Day 26, which was 21 days after irrigation was first withheld) as a function of tissue osmotic potential at full turgor for 'Whitespire' Japanese birch (□), gray birch (●), paper birch (○), European white birch (▲), Monarch birch (△), and river birch (■). Net photosynthesis = $-22.76 + 25.35\,X$ ($R^2 = 0.97$). Stomatal conductance $= -0.87 + 0.86\,X$ ($R^2 = 0.96$).
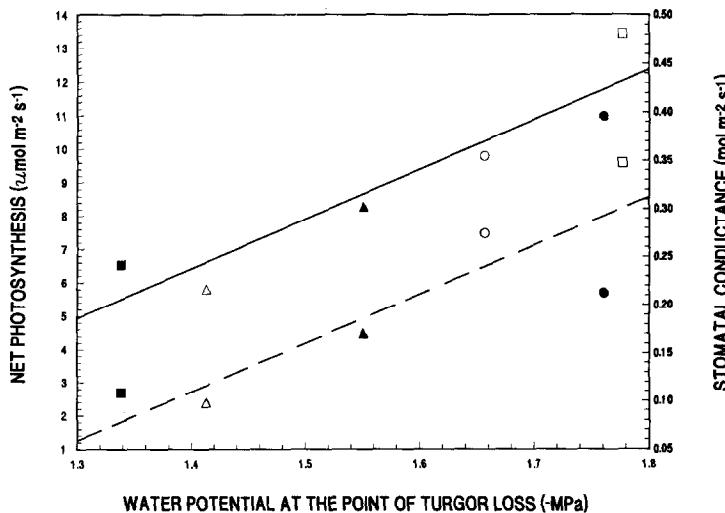


Figure 5. Net photosynthesis (——) and stomatal conductance (– – –) (measured on Day 26, which was 21 days after irrigation was first withheld) as a function of the water potential at the point of turgor loss for 'Whitespire' Japanese birch (□), gray birch (●), paper birch (○), European white birch (▲), Monarch birch (△), and river birch (■). Net photosynthesis = $-14.39 + 14.87\,X$ ($R^2 = 0.89$). Stomatal conductance $= -0.60 + 0.52\,X$ ($R^2 = 0.90$).

differences among species diminished.


## Discussion

Soon after withholding irrigation, $g_s$ began to decline gradually in all species, despite relatively constant midday $\Psi$ values throughout the experiment. In the absence of a decrease in midday $\Psi$, a reduction in $g_s$ in response to water stress might indicate that root-produced phytohormones are involved in triggering stomatal closure in response to a drying rhizosphere (Blackman and Davies 1985, Davies et al. 1987, Henson et al. 1989). An alternative hypothesis is that stomata responded directly to leaf water deficits, resulting in decreased transpiration and increased $\Psi$ and turgor pressure. Such a tightly coupled feedback system would tend to keep $\Psi$ constant (slightly above $\Psi_{tlp}$) until resistance to water uptake became so great that a reduction in transpiration could no longer compensate for the decreased soil water potential. Osonubi and Davies (1978) found that European white birch (B. pendula = B. verrucosa) often had a midday $\Psi$ near the turgor loss point, even under well-watered conditions, and that $g_s$ decreased in response to mild soil water deficits. Moreover, our observation that birch plants maintained fairly uniform midday $\Psi$, slightly above (ca. 0 to 0.3 MPa) their $\Psi_{tlp}$, despite variation in vapor pressure deficit and predawn water potential, supports the hypothesis that stomata were responding directly to leaf water deficits.

Stomatal closure can serve to limit water loss and to postpone development of damaging water deficits. The birch species with low $\Psi_{\pi,sat}$ values were better able to maintain turgor with decreasing $\Psi$, and they maintained higher $g_s$ and rates of $P_n$ and developed lower midday $\Psi$ under mild water stress than species with high $\Psi_{\pi,sat}$. Development of lower midday $\Psi$ does not necessarily mean, however, that these plants were under greater internal strain (sensu Levitt 1980). Because of a greater osmolality, tissues with low $\Psi_{\pi,sat}$ will maintain a higher relative water content at a given $\Psi$ than tissues with high $\Psi_{\pi,sat}$ (Tyree 1976, Hinckley et al. 1981). In this study, plants with low $\Psi_{\pi,sat}$ maintained turgor at low $\Psi$, yet all species lost turgor at similar relative water contents (Table 1). Thus, plants with low $\Psi_{\pi,sat}$ and $\Psi_{tlp}$ may well be able to tolerate greater water stress (i.e., low $\Psi$) before physiological processes are affected by either low turgor pressures (Hsaio 1973) or low relative water contents (Sinclair and Ludlow 1985).

A plant's capacity to resist drought results from the integration of a variety of adaptive characteristics and mechanisms (Jones 1980, Turner 1979). The effectiveness of any combination of traits in conferring drought resistance will depend on the specific environment (Pallardy 1981, Ranney et al. 1990). Several authors (Tyree 1976, Tyree and Jarvis 1982, Jones and Zur 1984) have noted that low tissue osmotic potentials will do little to increase water uptake from dry soils because there is typically little water remaining in soils at water potentials below $-1.0$ MPa, particularly sandy soils. However, in trees and other perennial plants that develop substantial, widespreading root systems (Bunger and Thomson 1938) water deficits may develop very gradually during periods of drought or with seasonal depletion of soil

water, resulting in prolonged exposure to mild water stress (Hinckley et al. 1981). Moreover, because of the hydraulic resistance to water transport in the soil-plant-atmosphere continuum, midday $\Psi$ values are typically much lower than are soil water potentials. As a result, midday $\Psi$ may decline to values near $\Psi_{tlp}$ under mild water stress (Hinckley et al. 1981). In this study, reduced stomatal conductance was apparent in some species under very mild water stress (predawn $\Psi = -0.35$ MPa). However, plants with low $\Psi_{\pi,sat}$ maintained greater turgor, $g_s$, and rates of $P_n$ during this period. Thus, the most beneficial effect of low tissue osmotic potential may be not on the extraction of water at low soil water potentials, but on the maintenance of $g_s$ and $P_n$ during transient, midday water deficits that may occur early during drought. According to this view, low tissue osmotic potentials may be a useful indication of a species adaptation to chronic, mild water stress.

The differential response to water stress of the six birch species suggests the potential for selecting among birch species for adaptation to dry sites. In this study, 'Whitespire' Japanese birch was better able to maintain turgor pressure, stomatal conductance, and net photosynthesis under mild water stress than the other species tested and would be a preferred species for sites where mild water stress commonly occurs.

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