Effect of Water Deficit on Gas Exchange, Osmotic Solutes, Leaf Abscission, and Growth of Four Birch Genotypes (*Betula* L.) Under a Controlled Environment

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Abstract. Water was withheld from 2-year-old seedlings or rooted cuttings of four birch genotypes (*Betula alleghaniensis* Britton, *B. davurica* Pall., *B. nigra* L. ‘Cully’, and *B. papyrifera* Marsh.) until the combined weight of the container and plant decreased below 40% of its original value to induce plant predawn water potential between -1.5 MPa and -2.1 MPa, after which plants were supplied with a requisite amount of water to reach 40% of its original value for 5 weeks under controlled conditions to investigate changes in gas exchange, osmotic solutes, leaf abscission, and growth compared with well-watered (WW) plants. Observations indicated that three of the four genotypes (except *B. papyrifera*) expressed three stages of photosynthetic response during water deficit: 1) a stress stage, 2) an acclimation stage, and 3) an adapted (or tolerance) stage. The stages were characterized by decreasing, increasing, and stabilized $Pn_{ws/ww}$ (net photosynthesis presented as a ratio of water-deficit stressed (WS) plants to WW plants), respectively. A strong relationship between Pn and g_s observed in the WS plants of the four genotypes, suggested inhibition of Pn by stomatal closure. After exposure to water deficit for 5 weeks, $Pn_{ws/ww}$ recovered to 70% of the initial value for *B. alleghaniensis* and *B. nigra* ‘Cully’ and 98% for *B. davurica* and *B. papyrifera*. WS plants had higher foliar concentrations of chlorophyll a and b (nmol/g) and potassium (%) than the WW plants. Increased levels of polyols (mg/g) were detected only in the WS plants of *B. alleghaniensis*. Increased levels of carbohydrates or organic acid under water deficit were not detected. A significant increase in leaf abscission in the WS plants of *B. papyrifera* compared with the other genotypes could be a morphological adaptation to water deficit conditions and facilitate recovery of $Pn_{ws/ww}$ during the acclimation stage.

Betula L., especially white-barked birch genotypes, are popular ornamental plants in the northern United States. In their natural origins, they often inhabit cool and moist regions, including bogs, stream banks, lake-shores, cool and damp woods, and moist slopes in cool coves (Atkinson, 1992; Farrar, 1995). Water deficits may pose an environmental stress to birch trees in landscapes and

nursery production. Urban surfaces and compacted soils diminish precipitation infiltration into tree root zones, and turf and other vegetation compete with trees for available water (Zwack and Graves, 1998). Under condition of high temperatures, trees increase transpiration and water uptake from soil to reduce leaf temperature, which could cause symptoms of water-deficit stress such as wilting as the soil dries down without precipitation or supplemental irrigation (Kramer and Boyer, 1995).

Water-deficit stresses trigger a myriad of physiological and morphological responses. Water-deficit stress generally reduces stomatal conductance (g_s), net photosynthesis (Pn), leaf water potential, and growth rate (Cregg, 1994; Matthews and Boyer, 1984; Pääkkönen et al., 1998a). Plants adapt to water-deficit stress by increasing water uptake and reduc-

ing water loss (Fort et al., 1998). The development of a more extensive root system was found to increase water uptake in some plants (Malinowski and Belesky, 2000). Plants can also reduce water loss through changes in leaf morphology and total leaf area (Connor et al., 2005; Graves, 1994; Nash and Graves, 1993; Pääkkönen et al., 1998a).

Osmotic solutes such as carbohydrates, organic acids, and mineral nutrients accumulate in plants during water-deficit stress and help to maintain turgor and metabolic activities (Arndt et al., 2000; Hare et al., 1998). In *Ziziphus mauritiana* Lam., changes in sugar metabolism correlated with significant increases in concentrations of hexose sugars, cyclitol, and proline during water-deficit stress, suggesting that altered solute partitioning may be an important factor in water-deficit stress tolerance (Clifford et al., 1998). Arndt et al. (2001) reported that the majority of osmoregulation was attributed to increases in hexose sugars, sucrose, malate, and potassium for *Z. rotundifolia* Lam. exposed to water stress. Besides carbohydrates, polyols might also play important roles in osmoregulation. Usually, specific polyols are characteristic of particular plants (Williamson et al., 2002).

However, active osmotic adjustment was also reported to develop only in situations in which water stress was imposed gradually for an extended period of time (Arndt et al., 2000). The relationship between water stress and changes in concentrations of different types of osmotic solutes remains unclear and could vary from species to species. Information is lacking on changes of polyols in birch.

Physiological responses of birch exposed to water deficit (Pääkkönen et al., 1998a, 1998b) and comparative water-deficit stress tolerance of birch genotypes (Graves et al., 2002; Ranney et al., 1991) have been investigated. Aspelmeier and Leuschner (2004) observed that none of the *B. pendula* clones exhibited nonstomatal limitation of photosynthesis and that the reduction of g_s was the first and most plastic response to water deficit in *B. pendula* clones, which allowed the maintenance of high predawn leaf water potentials during the water stress. However, very little information exists regarding the mechanisms of water-deficit stress tolerance of different birch genotypes. The goal of this research was to develop an understanding of the physiological responses to water deficit and the stress tolerance mechanisms of *B. alleghaniensis* Britton, *B. davurica* Pall., *B. nigra* L. ‘Cully’, and *B. papyrifera* Marsh. The decision to investigate these four birch genotypes was based on field evaluation of 20 birch genotypes grown under field conditions under two irrigation regimes in Fayetteville, AR (Gu et al., 2004). The four genotypes showed different growth responses under these conditions and, therefore, were chosen to further investigate what physiological and biochemical responses might explain these different growth responses.

In this study, we tested the hypothesis that osmotic solutes, especially polyols, facilitate photosynthesis acclimation to short-term

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Fig. 1. Predawn leaf water potential of well-watered (diamond symbols) and water-stressed (square symbols) plants for four birch genotypes on 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 d(s) after treatment (DAT) in Expt. 1 (closed symbols) and on 0, 1, 2, 3, 4, 5, 8, 9, 10, 11, 17, 19, 22, 24, 25, 31, and 35 DAT in Expt. 2 (open symbols). Values represent mean $\pm$ SE (Tukey's adjusted test; $P \leq 0.05$; $n = 6$).

Fig. 2. Response of net photosynthesis (P_n) to water deficit in four birch genotypes on 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 d after treatment (DAT) in Expt. 1 (closed symbols) and on 0, 1, 2, 3, 4, 5, 8, 9, 10, 11, 17, 19, 22, 24, 25, 31, and 35 DAT in Expt. 2 (open symbols). Values are presented as a ratio of water-stressed plants to well-watered plants ($P_{nws/ww}$). Data were subjected to PROC MODEL to produce trend lines; $n = 6$.

water-deficit stress in four birch genotypes under greenhouse conditions.

Materials and Methods

Two-year-old bare root seedlings or rooted cuttings from different nursery sources were planted into 4-L pots with Sun Gro SB500 (Sun Gro Horticulture, Bellevue, WA) on 20 Feb. 2004 and grown in an outdoor lathhouse until moved into a greenhouse (University of Arkansas–Fayetteville) on 15 Apr. 2004. The day/nighttime greenhouse temperature was set as 30 °C/25 °C. Six 1000-W metal halide high-intensity discharge lights were set to supplement if the ambient light intensity fell below 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Ten grams Osmocote 14N–6P–11K (The Scotts Company, Marysville, OH) were applied at planting. Plants were trained to a single shoot to provide a simple vegetative model plant system with one single terminal meristem, which allowed easy overall

growth measurement to evaluate physiological response to water-deficit stress.

Water-deficit stress treatment was similar to “target water potential” described by Cregg (2004). In a preliminary experiment, plants were watered to container capacity and allowed to dry down until no additional weight loss (the combined weight of the container and plant) was observed. The combined weight of the container and plant as well as the predawn leaf water potential (Ψ_{predawn}) were measured daily to determine the relationship between the percentage of the original combined weight and Ψ_{predawn} . In this study, 40% of the original combined weight was chosen to induce Ψ_{predawn} between -1.5 MPa and -2.1 MPa in water-stressed plants (Fig. 1).

Plants were watered daily to container capacity until experiments were started by withholding water from plants subjected to water-deficit stress (WS) treatment on 2 May 2004. The combined weight of the container

and plant was measured daily at ≈ 1900 HR (CDST). Water was withheld from WS plants until the combined weight decreased below 40% of its original value. A requisite volume of water was applied everyday to each container to reach 40% of its original value. The well-watered (WW) plants were watered daily to container capacity. The gas exchange and water potential measurements were taken at 1- to 3-d intervals after treatment (DAT) for 5 weeks as described subsequently. The experiment was ended after the fifth week, because the tagged leaf for photosynthesis measurement on *B. papyrifera* had abscised. The experiment was repeated on 4 June 2004 using a different set of trees with measurements taken at 1- to 6-d intervals.

The Ψ_{predawn} was measured as described by Oosterhuis and Wulleschleger (1987) at 700 HR (CDST) with PST 55–15 thermocouple psychrometers connected to a Wescor HR-33T microvoltmeter (Wescor, Logan, UT). Leaf discs were punched and enclosed in psychrometer chambers, which were immersed in a 25 °C water bath for 4 h before measurements.

The fifth unfolded leaf from the shoot apex was tagged on each tree at the beginning of the experiment. Leaf gas exchange was measured from 900 HR to 1300 HR (CDST) with a closed-chamber infrared gas analyzer CIRAS-1 (PP Systems, Haverhill, MA) with the Parkinson's leaf cuvette. All gas exchange measurements were taken on a 2.5-cm² section on the center of half of the tagged leaf to minimize variability attributable to leaf age and position. The microprocessor

Table 1. Variables of net photosynthesis ($P_{n_{\text{WS/WW}}}$) stages in response to water deficit in four birch genotypes.

	Variables of net photosynthesis ($P_{n_{\text{WS/WW}}}$) stages			
	<i>B. alleghaniensis</i>	<i>B. davurica</i>	<i>B. nigra</i> Cully	<i>B. papyrifera</i>
Slope I	−0.033	−0.037	−0.082	−0.095
Slope II	0.48	0.08	0.12	0.02
Stress severity ^z (%)	27	41	10	37
Recovery level ^y (%)	70	98	70	98

^zStress severity is the value of $P_{n_{\text{WS/WW}}}$ at the end of stage I—stress stage, presented as percentage to the initial $P_{n_{\text{WS/WW}}}$ value.

^yRecovery level is the percentage of $P_{n_{\text{WS/WW}}}$ at stage III—adapted stage to the initial $P_{n_{\text{WS/WW}}}$ value.

Fig. 3. Response of g_s to water deficit in four birch genotypes on 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 d after treatment (DAT) in Expt. 1 (closed symbols), and on 0, 1, 2, 3, 4, 5, 8, 9, 10, 11, 17, 19, 22, 24, 25, 31, and 35 DAT in Expt. 2 (open symbols). Values are presented as a ratio of water-stressed plants to well-watered plants ($g_{s_{\text{WS/WW}}}$). Data were subjected to PROC MODEL to produce trend lines; $n = 6$.

Table 2. Variables of g_S ($g_{SWS/WW}$) stages in response to water deficit in four birch genotypes.

	Variables of stomatal ($g_{SWS/WW}$) conductance stages			
	<i>B. alleghaniensis</i>	<i>B. davurica</i>	<i>B. nigra</i> Cully	<i>B. papyrifera</i>
Slope I	-0.045	-0.053	-0.010	-0.230
Slope II	0.007	0.011	0.004	0.004
Stress severity ^z (%)	26	32	22	20
Recovery level ^y (%)	46	65	34	33

^zStress severity is the value of $g_{SWS/WW}$ at the end of first stage, presented as percentage to the initial $g_{SWS/WW}$ value.

^yRecovery level is the percentage of $g_{SWS/WW}$ at the end of second stage to the initial $g_{SWS/WW}$ value.

was set to maintain cuvette conditions at 360 ppm CO₂ concentration, 50% relative humidity, 25 °C temperature, and photosynthetically active radiation of 1300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Foliar chlorophyll (Chl) concentrations were determined with the N,N-dimethylformamide (DMF) method described by Porra et al. (1989) on the last day of each experiment. Leaf samples (100 mg fresh weight) were weighed and ground to a powder with liquid nitrogen before being incubated in DMF in the dark for 1 h under room temperature. The mixture was centrifuged for 15 min at 6000 g. Absorbance was measured at 663.8 nm and 646.8 nm using a Shimadzu 160A spectrophotometer (Shimadzu Corp., Kyoto, Japan). The Chl concentrations were calculated according to the following formulas given by Porra et al. (1989)

$$\text{Chl } a = 13.43 \times A_{663.8} - 3.47 \times A_{646.8};$$

$$\text{Chl } b = 22.9 \times A_{646.8} - 5.387 \times A_{663.8};$$

$$\text{Chl } a + b = 19.43 \times A_{646.8} + 8.05 \times A_{663.8}.$$

Data were presented on the basis of leaf fresh weight (nmol/g FW).

All leaves above the tagged leaf on each tree were harvested at the end of each experiment, lyophilized, ground to a fine powder, and kept at -80 °C before the analysis of carbohydrates (arabinose, rhamnose, xylose, fructose, galactose, glucose, mannose, maltose, trehalose, sucrose, and raffinose), organic acids (shikimic acid, malic acid, quinic acid, citric acid, salicylic acid, and succinic acid), polyols (arabitol, myoinositol, mannitol, and xylitol), and mineral

nutrients (P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B).

Concentrations of [%; mg/g Dry weight (DW)] carbohydrates, organic acids, and polyols were determined following the procedures of Chapman and Horvat (1989) using a Hewlett-Packard 6890A gas chromatograph (Wilmington, DE) with a capillary column [15 × 0.25 mm (i.d.) fused silica, DB-1, 0.25- μm film thickness (J&W, Alltech, Deerfield, IL)]. Approximately 0.3 g leaf powder was extracted in 75% ethanol overnight before filtered through a 1.0- μm filter (Corning Nucleopore Track-etch Nucleopore polycarbonate membrane; VWR, West Chester, PA). Carbohydrates, organic acids, and polyols were converted to their oximes by the addition of hydroxylamine containing phenyl β -D-glucoside as the internal standard and subsequently converted to their TMS derivatives by the addition of N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane. A small amount of anhydrous sodium sulfate was added to ensure the dryness of the derivatized samples before they were analyzed by the gas chromatograph. Injector and detector temperatures were set at 225 °C and 280 °C, respectively. The oven temperature of the gas chromatograph was held at 150 °C for 4 min, programmed to

Fig. 4. Relation between g_S and net photosynthesis (P_n) of water-stressed trees of four birch genotypes in Expt. 1. Values represent mean $\pm$ SE (Tukey's adjust test; $P \leq 0.05$; $n = 6$).

Fig. 5. Relation between g_s and net photosynthesis (Pn) of water-stressed trees of four birch genotypes in Expt. 2. Values represent mean $\pm$ SE (Tukey's adjust test; $P \leq 0.05$; $n = 6$).

increase to 192 °C at 4 °C/min and hold at 192 °C for 30 s, and then programmed to increase to 240 °C at 10 °C/min and hold at 240 °C for 7 min.

Mineral nutrient concentrations (percent for P, K, Ca, Mg; mg/g for Na, Fe, Mn, Zn, Cu, and B) were digested in nitric acid and hydrogen peroxide and analyzed using inductively coupled plasma-atomic emission spectrometry at the Altheimer Laboratory at the University of Arkansas.

At the end of the experiment, the fifth most recently expanded leaf (new leaf) and the tagged leaf (old leaf) were harvested. Leaf area was measured before leaves were dried in an oven at 60 °C for 8 h, and their dry weights were measured to calculate specific leaf weight, SLW (leaf dry weight/leaf area; mg/cm²).

Abscised leaves were collected daily for each tree. The number of retained leaves on each tree was counted at the end of each experiment. Leaf abscission was expressed as a percentage of total number of leaves. Initial and final height was measured on the first and last day of the experiments at 10 cm above the medium line and the relative change in shoot height was calculated as % = [(final height – initial height)/initial height] $\times$ 100.

The experiment design was a two-factor factorial (four genotypes $\times$ two irrigation

treatments) with six replications arranged in a completely randomized design. Analysis of variance was used to separate the effect of genotypes, treatment, and the interaction. Before being subjected to PROC MODEL analysis (SAS Institute, Cary, NC) to produce the trend lines of responses to water deficit, net photosynthesis, g_s , and water use efficiency (WUE; calculated as a ratio of net photosynthesis divided by evapotranspiration) from the repeated experiments are presented as the ratio of the WS plants to the WW plants ($Pn_{ws/ww}$, $g_{s_{ws/ww}}$, $WUE_{ws/ww}$) to minimize variability resulting from the environmental effects variance.

Results

Predawn leaf water potential. The $\psi_{predawn}$ of the four genotypes changed in a similar pattern after exposed to water deficit in both experiments (Fig. 1). The $\psi_{predawn}$ was ≈ -0.3 MPa before water-deficit treatment started. The WW plants maintained their $\psi_{predawn}$ above -0.5 MPa during experiments. In the WS *B. alleghaniensis*, $\psi_{predawn}$ decreased to -1.5 MPa by 18 DAT and 11 DAT in Expts. 1 and 2, respectively, and maintained above -2.0 MPa until the end of the experiments. The $\psi_{predawn}$ of the WS *B. davurica* and *B. nigra* 'Cully' decreased

on initiation of water deficit treatment and remained at ≈ -1.75 MPa. The $\psi_{predawn}$ of the WS *B. papyrifera* decreased to ≈ -2.0 MPa in Expts. 1 and 2.

Gas exchange. On the initiation of water deficit treatment, $Pn_{ws/ww}$ decreased in the birch genotypes tested (Fig. 2). The decrease was followed by an increase in $Pn_{ws/ww}$. During the experimental period, $Pn_{ws/ww}$ stabilized for three of the four genotypes (except *B. papyrifera*).

Based on $Pn_{ws/ww}$, three stages of response to water-deficit stress were observed that described the water-deficit responses of three genotypes investigated (except *B. papyrifera*). Those response stages can be described as follows: stage I—stress stage, characterized by decreasing $Pn_{ws/ww}$ or a trend line with negative slope I (slope I < 0); stage II—acclimation stage, characterized by increasing $Pn_{ws/ww}$ or a trend line with positive slope II (slope II > 0); and stage III—adapted or tolerance stage characterized by a constant $Pn_{ws/ww}$ or a horizontal trend line (slope III = 0). The absolute values of slope I described the rate at which the birch expressed stress effects resulting from water deficit. The values of $Pn_{ws/ww}$ at the end of the stress stage indicated the stress severity. The values of slope II and the durations of the acclimation stage described plant acclimation

Fig. 6. Response of water use efficiency (WUE) to water deficit in four birch genotypes on 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 d after treatment (DAT) in Expt. 1 (closed symbols) and on 0, 1, 2, 3, 4, 5, 8, 9, 10, 11, 17, 19, 22, 24, 25, 31, and 35 DAT in Expt. 2 (open symbols). Values are presented as a ratio of water-stressed plants to well-watered plants ($WUE_{ws/ww}$). Data were subjected to PROC MODEL to produce trend lines; $n = 6$.

to water-deficit conditions. During the adapted stage, plants had adapted to the lower moisture level and established new levels of gas exchange. The value of $Pn_{ws/ww}$ at the adapted stage indicated the level of recovery from water stress. Unlike the other three genotypes, only two trend lines (two stages) fit $Pn_{ws/ww}$ for *B. papyrifera*. It is possible that an adapted stage would have been observed for *B. papyrifera* if the experiment had continued.

Slope I for the stress stage ranged from -0.033 for *B. alleghaniensis* to -0.095 for *B. papyrifera* (Table 1). At the end of the stress stage, $Pn_{ws/ww}$ decreased to 10% of the initial $Pn_{ws/ww}$ value for *B. nigra* 'Cully' and 41% for *B. davurica*. Slopes II for the acclimation stage ranged from 0.48 for *B. alleghaniensis* to 0.02 for *B. papyrifera*. At the end of the 5-wk water deficit, $Pn_{ws/ww}$ recovered to 70% of the initial $Pn_{ws/ww}$ value for *B. alleghaniensis* and *B. nigra* 'Cully' and 98% for *B. davurica* and *B. papyrifera*.

In this study, *B. papyrifera* had a short stress stage (7 d) but a long acclimation stage

(29 d). In contrast, *B. alleghaniensis* had a long stress stage (18 d) but a short acclimation stage (1 d) to reach its tolerance stage (Fig. 2).

The $g_{Sws/ww}$ (Fig. 3) indicated a different pattern of response to water deficit compared with $Pn_{ws/ww}$. Only two stages were observed for $g_{Sws/ww}$ for each genotype. In the first stage, $g_{Sws/ww}$ decreased with slopes ranging from -0.045 for *B. alleghaniensis* to -0.230 for *B. papyrifera* (Table 2). At the end of the first stage, $g_{Sws/ww}$ reached the lowest values, which ranged from 20% of the initial $g_{Sws/ww}$ value for *B. papyrifera* to 32% for *B. davurica*. The recovery of $g_{Sws/ww}$ preceded the recovery of $Pn_{ws/ww}$ (acclimation stage) in all four genotypes (Figs. 2 and 3). In the second stage, $g_{Sws/ww}$ increased with slopes ranging from 0.004 for *B. nigra* 'Cully' and *B. papyrifera* to 0.011 for *B. davurica*. At the end of the second stage, $g_{Sws/ww}$ of *B. davurica* recovered to 65% of the initial value, whereas *B. papyrifera* only recovered to 33% of the initial value. Based on the models, $g_{Sws/ww}$ reached the lowest value

13 DAT for *B. alleghaniensis*, 10 DAT for *B. davurica* and *B. nigra* 'Cully', and 4 DAT for *B. papyrifera*.

The four genotypes displayed strong linear relationships between Pn and g_s under water-deficit conditions in both Expts. 1 (Fig. 4) and 2 (Fig. 5). In both experiments, Pn of *B. alleghaniensis* decreased faster with the decrease in g_s compared with the other genotypes.

Based on the model, $WUE_{ws/ww}$ also displayed two stages in response to water-deficit stress in the four genotypes (Fig. 6). In the first stage, $WUE_{ws/ww}$ increased on initiation of water-deficit treatment with slopes ranging from 0.019 for *B. alleghaniensis* to 0.045 for *B. nigra* 'Cully' (Table 3). The second stage of $WUE_{ws/ww}$ initiated ≈ 20 DAT in three of the four genotypes investigated except *B. papyrifera*. The second stage started on 35 DAT for *B. papyrifera*. Based on the models, $WUE_{ws/ww}$ reestablished at final levels as represented by the second stage ranging from 145% of the initial value for *B. alleghaniensis* to 200% of the initial value for *B. nigra* 'Cully'.

Chlorophyll concentrations. No interaction was observed between genotype and treatment for the concentration of Chl a or Chl b. The WS plants had significantly higher concentrations of Chl a and Chl b than WW plants for all four genotypes on an nmol/g (FW) basis (Table 4).

Table 3. Variables of water use efficiency ($WUE_{ws/ww}$) stages in response to water deficit in four birch genotypes.

	Variables of water use efficiency ($WUE_{ws/ww}$) stages			
	<i>B. alleghaniensis</i>	<i>B. davurica</i>	<i>B. nigra</i> Cully	<i>B. papyrifera</i>
Slope I	0.019	0.036	0.045	0.030
Acclimation level (%)	145	161	200	186

Table 4. Foliar chlorophyll (Chl) concentrations of well-watered and water-stressed plants of four birch genotypes (*B. alleghaniensis*, *B. davurica*, *B. nigra* Cully, and *B. papyrifera*).^z

Treatment	Chlorophyll concentrations (nmol/g)	
	Chl a	Chl b
Well-watered	316 ± 23	111 ± 8
Water-stressed	454 ± 21	163 ± 8

^zMeasurements were taken after 5 weeks of water-deficit treatment. Each value represents mean ± SE (Tukey's adjusted test; $P \leq 0.05$; $n = 6$).

Table 5. Foliar concentration of osmotic solutes (carbohydrates, polyols, and organic acids) of well-watered (WW) and water-stressed (WS) plants of four birch genotypes.^z

Osmotic solutes	Foliar concn of osmotic solutes (mg/g DW)							
	<i>B. alleghaniensis</i>		<i>B. davurica</i>		<i>B. nigra</i> Cully		<i>B. papyrifera</i>	
	WW	WS	WW	WS	WW	WS	WW	WS
Arabinose	2 ab ^y	2 ab	4 c	3 bc	2 abc	1 a	3 bc	2 abc
Rhamnose	1 ns ^x	0 ns	3 ns	5 ns	1 ns	0 ns	0 ns	3 ns
Xylose	5 ns	1 ns	1 ns	2 ns	0 ns	0 ns	0 ns	1 ns
Pentose^w	8 ab	4 ab	8 ab	10 b	3 ab	1 a	4 ab	5 ab
Fructose	104 b	92 ab	310 c	281 c	63 ab	42 a	176 bc	158 b
Galactose	16 b	12 ab	19 b	33 c	5 ab	2 a	8 ab	6 ab
Glucose	79 ab	76 ab	236 c	205 c	55 ab	32 a	144 bc	93 ab
Mannose	3 a	4 ab	5 abc	7 c	3 a	2 a	7 bc	4 a
Hexose^v	202 ab	184 ab	569 c	526 c	126 ab	78 a	334 bc	261 ab
Maltose	2 ns	1 ns	1 ns	1 ns	1 ns	1 ns	1 ns	1 ns
Trehalose	1 ns	1 ns	1 ns	1 ns	1 ns	0 ns	0 ns	1 ns
Sucrose	56 ab	39 ab	66 ab	36 ab	23 a	17 ab	93 b	54 ab
Disaccharide^u	60 ab	42 ab	68 ab	38 ab	25 a	17 a	94 b	55 ab
Raffinose	36 ns	25 ns	39 ns	38 ns	18 ns	8 ns	18 ns	16 ns
Total carbohydrates^t	305 ab	254 ab	683 c	612 c	173 ab	104 a	449 bc	337 ab
Arabitol	5 a	19 b	10 ab	6 a	6 a	1 a	10 ab	8 ab
Myoinositol	6 a	7 ab	3 a	3 bc	3 a	3 cd	4 a	5 d
Mannitol	0 abc	0 abc	0 a	0 abc	0 abc	1 c	0 ab	0 bc
Xylitol	6 ab	12 b	2 a	6 ab	12 b	1 a	1 a	4 ab
Total polyols^s	18 a	38 b	15 a	16 a	20 a	6 a	15 a	18 a
Shikimic acid	1 bc	1 ab	1 ab	1 b	1 ab	0 a	1 bc	2 c
Malic acid	1 ns	1 ns	1 ns	1 ns	1 ns	0 ns	1 ns	1 ns
Quinic acid	37 cd	11 a	14 ab	14 ab	20 abc	11 a	41 d	29 bcd
Citric acid	0 ns	1 ns	0 ns	0 ns	0 ns	0 ns	0 ns	0 ns
Salicylic acid	1 ab	2 c	1 abc	1 ab	0 a	1 ab	1 ab	1 bc
Succinic acid	3 a	4 a	9 b	6 ab	2 a	2 a	3 a	4 a
Total organic acids^f	43 cd	19 ab	26 a–d	23 ab	24 abc	14 a	46 c	36 bcd

^zMeasurements were taken after 5 weeks of water-deficit treatment.

^yMeans within a row followed by different letters are significantly different at 5% level by Tukey's adjusted test; $n = 6$.

^xMeans within a row followed by "ns" are not significantly different at the 5% level by Tukey's adjusted test; $n = 6$.

^wPentose is calculated by adding detectable arabinose, rhamnose, and xylose together.

^vHexose is calculated by adding detectable fructose, galactose, glucose, and mannose together.

^uDisaccharide is calculated by adding detectable maltose, trhalose, and sucrose together.

^tTotal carbohydrates is calculated by adding pentose, hexose, disaccharide, and raffinose together.

^sTotal polyols is calculated by adding detectable arabitol, myoinositol, mannitol, and xylitol together.

^fTotal organic acids is calculated by adding detectable shikimic acid, malic acid, quinic acid, citric acid, salicylic acid, and succinic acid together.

Carbohydrates, polyols, organic acids, and mineral nutrients. The sum of fructose, glucose, and sucrose comprised from 78% to 92% (mg/g; DW/DW) of total detectable carbohydrates content in the leaves of the four birch genotypes investigated (Table 5). Other carbohydrates detected were arabinose, rhamnose, xylose, galactose, mannose, maltose, trehalose, and raffinose. Total detectable carbohydrate concentrations ranged from 104 mg/g to 683 mg/g (1% to 7%). Water-deficit treatment did not affect the foliar concentration of detectable carbohydrates.

Polyols (arabitol, myoinositol, mannitol, and xylitol) and organic acids (shikimic acid, malic acid, quinic acid, citric acid, salicylic acid, and succinic acid) were also detected in

birch leaves at relatively smaller amounts, ranging from 6 mg·g⁻¹ to 38 mg·g⁻¹ and from 14 mg·g⁻¹ to 46 mg·g⁻¹, respectively (Table 5). Of the four genotypes, only *B. alleghaniensis* had a significantly higher concentration of total polyols in the WS plants relative to the WW plants, and only *B. alleghaniensis* had a significantly lower foliar concentration of total organic acids in the WS plants compared with the WW plants.

Water-deficit treatment decreased P concentration (%) in leaves of all genotypes except *B. papyrifera* (Table 6), for which P was higher in WS plants. The concentration of B was decreased by water-deficit treatment in the birch leaves. Both K and S accumulated in response to water deficit. Water deficit increased Na concentration signifi-

cantly in *B. alleghaniensis* leaves. Interaction of birch genotypes and foliar concentration of mineral nutrients was detected in Ca, Mg, Na, Fe, Mn, Zn, and Cu.

Leaf abscission and growth. Water-deficit treatment did not affect SLW of new or old leaves in any genotype investigated in the study (data not presented). A significant interaction was observed in the percentage of leaf abscission between genotype and treatment (data not presented). Leaf abscission on the WW plants of all genotypes was less than 10% (Table 7). The WS plants of *B. papyrifera* had a significantly greater percentage of leaf abscission than the other three genotypes.

The effect of water deficit on the relative change in shoot height varied significantly among the genotypes (Table 8). Water-deficit treatment reduced the shoot height of *B. alleghaniensis* compared with the others.

Discussion and Conclusions

Similar levels of water stress were induced in the four genotypes investigated as indicated by ψ_{predawn} (Fig. 1). Water status of *B. papyrifera* responded faster than the other three genotypes because water was controlled, which could be associated with the plant's physiological and anatomical differences (Gu et al., 2003), because plants of uniform size were selected at the beginning of treatment. *Betula papyrifera* had larger leaf area than the other three genotypes (data not presented), which could result in increased daily water loss from leaves and, therefore, faster change in water status when the water stress was imposed.

Previous studies have shown reductions of gas exchange in birch genotypes exposed to water stress (Graves et al., 2002; Pääkkönen et al., 1998a; Ranney et al., 1991). Decreasing gas exchange in response to water deficit was observed in all genotypes in the stress stage, and $g_{\text{SWS/WW}}$ decreased more sharply compared with $P_{\text{NWS/WW}}$ as indicated by the first segment of the trend lines in Figures 2 and 3. These results confirmed that over time, the g_{S} responded faster than net photosynthesis in plants exposed to water stress (Pankovic et al., 1999), and plants might be responding to water stress in a passive way (Picon et al., 1997). A strong relationship was observed between P_{N} and g_{S} in the WS plants of the four genotypes in both experiments (Figs. 5 and 6). These results suggested inhibition of P_{N} by stomatal closure in plants exposed to water-deficit conditions, which was consistent with previous studies in *B. pendula* (Aspelmeier and Leuschner, 2004) and other plant species (Medrano et al., 2002; Pankovic et al., 1999).

The gas exchange measurements in this study were made in the morning on a single leaf rather than the whole plant. Single-leaf measurement may not be the best indication of whole-plant gas exchange when significant leaf abscission occurs.

During the acclimation stage of the four genotypes, $P_{\text{NWS/WW}}$ increased after $g_{\text{SWS/WW}}$

Table 6. Foliar concentration of mineral nutrients of well-watered (WW) and water-stressed (WS) plants of four birch genotypes (*B. alleghaniensis*, *B. davurica*, *B. nigra* Cully, and *B. papyrifera*).^z

Mineral Nutrient	Foliar concn of mineral nutrients							
	<i>B. alleghaniensis</i>		<i>B. davurica</i>		<i>B. nigra</i> Cully		<i>B. papyrifera</i>	
	WW	WS	WW	WS	WW	WS	WW	WS
P (%)	0.50 ± 0.03 ^y	0.35 ± 0.03	0.85 ± 0.03	0.67 ± 0.03	0.55 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.47 ± 0.03
K (%)	1.21 ± 0.04	1.52 ± 0.04	1.44 ± 0.04	1.69 ± 0.04	1.17 ± 0.05	1.28 ± 0.05	1.21 ± 0.04	1.45 ± 0.04
Ca (%)	1.05 ± 0.04	1.06 ± 0.04	1.12 ± 0.04	1.16 ± 0.04	0.95 ± 0.5	0.85 ± 0.5	0.95 ± 0.04	0.76 ± 0.04
Mg (%)	0.46 ± 0.01	0.47 ± 0.01	0.46 ± 0.01	0.49 ± 0.01	0.51 ± 0.02	0.46 ± 0.02	0.41 ± 0.01	0.39 ± 0.01
S (%)	0.19 ± 0.01	0.23 ± 0.01	0.13 ± 0.01	0.23 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0.12 ± 0.01	0.24 ± 0.01
Na (mg/g)	239 ± 22	388 ± 22	404 ± 22	372 ± 22	208 ± 24	153 ± 24	221 ± 22	257 ± 22
Fe (mg/g)	183 ± 14	120 ± 14	77 ± 14	97 ± 14	78 ± 16	135 ± 16	100 ± 14	115 ± 14
Mn (mg/g)	99 ± 10	91 ± 10	144 ± 6	168 ± 6	72 ± 0	51 ± 1	230 ± 12	187 ± 12
Zn (mg/g)	204 ± 19	249 ± 19	261 ± 19	219 ± 19	295 ± 21	190 ± 21	269 ± 19	212 ± 19
Cu (mg/g)	6 ± 0	7 ± 0	6 ± 0	6 ± 0	8 ± 0	7 ± 0	8 ± 0	8 ± 0
B (mg/g)	48 ± 2	25 ± 2	50 ± 2	37 ± 2	60 ± 2	33 ± 2	48 ± 2	35 ± 2

^zMeasurements were taken after 5 weeks of water-deficit treatment.

^yEach value represents mean ± SE (Tukey's adjusted test; $P \leq 0.05$; $n = 6$).

Table 7. Effect of water deficit on percentage of leaf abscission of four birch genotypes (*B. alleghaniensis*, *B. davurica*, *B. nigra* Cully, and *B. papyrifera*) after 5 weeks of treatment.

Treatment	Leaf abscission (%)			
	<i>B. alleghaniensis</i>	<i>B. davurica</i>	<i>B. nigra</i> Cully	<i>B. papyrifera</i>
Well-watered	9 ± 2 ^z	4 ± 2	3 ± 2	7 ± 2
Water-stressed	21 ± 2	25 ± 2	36 ± 2	50 ± 2

^zEach value represents mean ± SE (Tukey's adjusted test; $P \leq 0.05$; $n = 6$).

Table 8. Effect of water deficit on the relative change in shoot height of four birch genotypes (*B. alleghaniensis*, *B. davurica*, *B. nigra* Cully, and *B. papyrifera*) after 5 weeks of treatment.

Treatment	Relative change in shoot ht (%)			
	<i>B. alleghaniensis</i>	<i>B. davurica</i>	<i>B. nigra</i> Cully	<i>B. papyrifera</i>
Well-watered	87 ± 4 ^z	17 ± 4	37 ± 5	12 ± 4
Water-stressed	40 ± 4	12 ± 5	39 ± 2	12 ± 4

^zEach value represents mean ± SE (Tukey's adjusted test; $P \leq 0.05$; $n = 6$).

initiated recovery from the lowest value (Figs. 2 and 3). Leaf abscission was observed from the WS plants of each genotype during the acclimation stage (data not presented). Leaf abscission was more related to water stress than other environmental stresses (Grice, 1998) and is observed in many plants (Arndt et al., 2001; Connor et al., 2005). Water stress increased leaf abscission in birch trees (Table 7). Plants in response to water-deficit stresses may develop smaller leaf area (Turner, 1986; Wullschleger and Oosterhuis, 1991) or leaf abscission to reduce water loss (Connor et al., 2005; Fort et al., 1998; Pääkkönen et al., 1998a). Significant increase in leaf abscission was reported in *B. pendula* exposed to water stress (Pääkkönen et al., 1998b). Leaf abscission might be a mechanism to remobilize and redistribute assimilates for coping with water stress (Arndt et al., 2001; Yin et al., 2005). The observed drought-induced stimulation of photosynthesis could be regarded as a compensation mechanism for leaf abscission (Pääkkönen et al., 1998a, 1998b). The increased leaf abscission of *B. papyrifera* exposed to water stress compared with the other genotypes (Table 7) could be a morphological adaptation to reduce water loss from leaves and redistribute resources to newer and upper leaves under water deficit conditions and thus facilitate the recovery of $Pn_{ws/ww}$ during the acclimation stage (Fig. 2). From a practical standpoint, a significantly

lower level of leaf abscission in *B. alleghaniensis* under water stress would make it visually more acceptable in a landscape compared with the other three genotypes.

The most abundant carbohydrates detected in leaves of the four genotypes were glucose, fructose, and sucrose, which was in accord with Mononen et al. (2004). The water-deficit-induced increase in total foliar carbohydrates, polyols, and organic acids has been reported as an indicator of osmotic adjustment during water-deficit stress in many woody and herbaceous species (Arndt et al., 2001; Bacchus et al., 2000; Malinowski and Belesky, 2000; Pankovic et al., 1999; Patonnier et al., 1999; Richardson et al., 1992). Osmotic adjustment could help maintain plant turgor and facilitate physiological activities (Hare et al., 1998; Malinowski and Belesky, 2000), which was not previously observed in birch genotypes in response to imposed water deficit (Ranney et al., 1991). Contrary to the previous reports on accumulation of carbohydrates during water-deficit stress in other plants, increased levels of carbohydrates or organic acids under water-deficit conditions were not detected in birch leaves (Table 5). However, an increased level of polyols was detected in *B. alleghaniensis*. Although the absolute amount was relatively low relative to carbohydrates, their abundant hydroxyl groups might help to prevent metabolic inactivation under low water potential conditions (Galinski and Truper, 1994).

Potassium was detected at higher concentrations in leaves of the WS plants for the four genotypes (Table 6), which was different from the previous research report on *B. pendula* (Pääkkönen et al., 1998b). Potassium has been found to accumulate in many plants under water-deficit conditions (Martinez et al., 2003). Potassium is believed to be involved in maintaining turgor of guard cells and facilitating stomatal aperture (Taiz and Zeiger, 1998). Recovery of $g_{Sws/ww}$ and thus recovery of $Pn_{ws/ww}$ in the second stage (Fig. 3) could be attributed to potassium accumulation in the WS plants.

The Chl a and Chl b concentrations were consistently higher in remaining leaves of the WS plants for four genotypes (Table 4). Similarly, increased foliar chlorophyll concentrations under water-deficit conditions were reported in *B. pendula* Roth. (Pääkkönen et al., 1998a, 1998b), *Fagus sylvatica* L. (Le Thiec et al., 1994), and *Gossypium hirsutum* L. (Pettigrew, 2004). In contrast, Jagtap et al. (1998) reported 20% to 30% chlorophyll loss in sorghum under water stress. Leaf chlorosis (yellowing) and senescence normally occur before leaves abscission in plants under water stress, which is associated with chlorophyll degradation and translocating nutrients to newer leaves (Arndt et al., 2001). The inconsistency in chlorophyll change could result from the difference among species or the severity and duration of the water-deficit conditions. Accumulating photosynthetic pigments in leaves under water-stressed conditions could help capture more light energy per unit leaf area than leaves of well-watered plants and thus improve photosynthetic capacity per unit leaf area in water-stressed plants.

This study has provided insight into the physiological and morphological response of four *Betula* genotypes to water deficits. These observations, combined with field observations, will assist in our understanding of adaptation of birch genotypes to environments where water stress may occur.

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