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Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization, and shading

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Abstract

Variation in xylem structure and function has been extensively studied across different species with a wide taxonomic, geographical, and ecological coverage. In contrast, our understanding of how xylem of a single species can adjust to different growing condition remains limited. Here phenotypic and developmental plasticity in xylem traits of hybrid poplar (*Populus trichocarpa* × *deltoides*) was studied. Clonally propagated saplings were grown under experimental drought, nitrogen fertilization, and shade for >30 d. Xylem hydraulic and anatomical traits were subsequently examined in stem segments taken from two different vertical positions along the plant's main axis. The experimental treatments affected growth and development and induced changes in xylem phenotype. Across all treatments, the amount of leaf area supported by stem segments (A_L) scaled linearly with stem native hydraulic conductivity (K_{native}), suggesting that the area of assimilating leaves is constrained by the xylem transport capacity. In turn, K_{native} was mainly driven by the size of xylem cross-sectional area (A_X). Moreover, the structural and functional properties of xylem varied significantly. Vulnerability to cavitation, measured as the xylem pressure inducing 50% loss of conductivity (P50), ranged from -1.71 MPa to -0.15 MPa in saplings subjected to drought and nitrogen fertilization, respectively. Across all treatments and stem segment positions, P50 was tightly correlated with wood density. In contrast, no relationship between P50 and xylem-specific conductivity (K_S) was observed. The results of this study enhance our knowledge of plant hydraulic acclimation and provide insights into common trade-offs that exist in xylem structure and function.

Key words: Cavitation, hydraulic conductivity, phenotypic plasticity, vessels, wood density, xylem embolism.

Introduction

Light, nutrients, and water are primary resources required by plants for their growth and reproduction (Larcher, 2003). Over the course of evolution, plant species have acquired a suite of traits allowing them to utilize these resources and persist under environmental conditions characteristic for their habitat. This process is known as adaptation (Lambers *et al.*, 1998). However, the availability of the resources can be rather variable during a plant's lifetime. For instance, periods of sufficient soil moisture supply can be interrupted by drought (Hogg *et al.*, 2008). Irradiance can rapidly increase when the surrounding vegetation is removed or

decrease when a plant becomes shaded by faster-growing neighbours (Lieffers *et al.*, 1999). The availability of inorganic nutrients such as nitrogen or phosphorus can become altered as a result of competition, flood pulse inundation, or increased runoff from fertilized fields (Rennenberg *et al.*, 2010). Plants are, to a certain extent, able to adjust to such changes because plant functional traits exhibit phenotypic plasticity. This continuous adjustment of physiological and structural properties during a plant's lifetime in order to optimize life processes under new environmental constraints is known as acclimation (Lambers *et al.*, 1998).

Long-distance water transport in plants is a physiological process of paramount importance which is intimately linked with the acquisition and use of all three resources mentioned above. Both adaptation and acclimation can be seen in xylem structure and function. Xylem structure and function have been studied in a wide range of different species (e.g. Carlquist, 1988, 2001; Wheeler *et al.*, 2007), and vast adaptive variation has been demonstrated (e.g. Maherali *et al.*, 2004; Sperry *et al.*, 2006; Chave *et al.*, 2009; McCulloh *et al.*, 2010). Among the most important hydraulic traits are the xylem-specific conductivity (K_S) and the vulnerability to drought-induced cavitation. While the former is a measure of transport efficiency, the latter characterizes xylem safety. Both traits exhibit large interspecific variation. K_S differs ~100-fold across diffuse porous angiosperms, reflecting differences in the size and number of vessels (Sperry *et al.*, 2006; McCulloh *et al.*, 2010). Vulnerability to cavitation measured as the pressure at 50% loss of hydraulic conductivity (P50) spans from values close to -0.2 MPa to values below -14 MPa (Maherali *et al.*, 2004; Sperry, 2011). Moreover, these two traits seem to be in a trade-off relationship, such that xylem of a given species cannot be both highly transport efficient and highly resistant to cavitation (Maherali *et al.*, 2004; Hacke *et al.*, 2006). Wood density with values ranging from 0.1 g cm⁻³ to 1.2 g cm⁻³ is another functionally important trait (Chave *et al.*, 2009). Wood density has been linked not only to wood mechanical strength (Niklas, 1992) but also to the resistance of xylem to cavitation (Hacke *et al.*, 2001a).

The structure and hydraulic function of xylem can also vary within a single species in response to growing conditions. Variation in ring widths, conduit diameters, or wood densities has often been used to reconstruct information about past environmental conditions and to infer the hydraulic function of xylem (for a review, see Fonti *et al.*, 2010). While xylem anatomy can provide a good proxy of xylem function in some cases, solid knowledge of how specific growing conditions influence xylem anatomy and how patterns in xylem structure link with xylem function is an essential prerequisite for such approaches. Changes in K_S and P50 in response to environmental conditions such as drought (Beikircher and Mayr, 2009; Fichot *et al.*, 2010), irradiance (Cochard *et al.*, 1999), salinity (Stiller, 2009), nutrient availability (Harvey and van den Driessche, 1997; Hacke *et al.*, 2010), and soil type (Hacke *et al.*, 2000; Mayr *et al.*, 2010) have been shown. However, a broader range of species and environmental conditions should be tested and links to genetic underpinnings should be elucidated (Lamy *et al.*, 2011; Wortemann *et al.*, 2011) in order to gain a better understanding of the acclimation potential of xylem.

In this study, phenotypic plasticity of xylem traits was assessed using clonally propagated saplings of hybrid poplar (*Populus trichocarpa* × *Populus deltoides*, clone H11-11). Given its parentage, it was expected that highly vulnerable and highly conductive xylem (Tyree *et al.*, 1994; Sperry, 2011) would be found in this hybrid poplar. These xylem characteristics are well suited for the exploitative ecological strategy of cottonwoods in their natural environment. The riverine floodplains that are inhabited by riparian cottonwoods are a highly dynamic environment which is continuously modified by repeated disturbances (Braatne *et al.*, 1996). Therefore, it was expected that large phenotypic plasticity

would be found in the hybrid poplar in response to experimental drought, nitrogen fertilization, and shading.

Variation in the anatomical and hydraulic parameters of xylem in response to treatments was assessed in stem segments sampled from two vertical positions along a plant's main axis. Based on the above-mentioned literature, it was anticipated that increased vulnerability to cavitation in saplings subjected to fertilization and shade and increased resistance in response to drought would be found. Xylem-specific hydraulic conductivity was expected to increase in fertilized plants and decrease in drought-stressed and shaded plants. Furthermore, it was decided to test whether the hydraulic and anatomical traits were coupled in a similar way to that observed at the interspecific level. If so, it would be expected that correlations between vulnerability of xylem to cavitation and xylem transport efficiency, as well as between vulnerability and wood density, would be found.

Materials and methods

Plant material and experimental conditions

Saplings of hybrid poplar (*Populus trichocarpa* × *Populus deltoides*, clone H11-11) were grown under drought (DR), nitrogen fertilization (F), and shading (SH). Due to logistical concerns and a limited amount of space in the growth facility used, the experimental treatments were imposed one at a time in three independent, temporally separated experiments. For each experiment, saplings were produced from rooted cuttings and maintained in a growth chamber under the following standard growing conditions: 16/8 h day/night cycle, 24/18 °C day/night temperature, ambient irradiance 350 μmol m⁻² s⁻¹. Plants were kept well watered, and fertilized with a complete water-soluble fertilizer (20-20-20 N-P-K, Plant Products, Brampton, ON, Canada) at 1 g l⁻¹ dilution on a weekly basis. After a 7–9 week long period of sapling establishment, plants were randomly assigned to either a treatment (DR, F, or SH) or a control (DRC, FC, or SHC) group. Plants subjected to the DR treatment received 50–200 ml of water every other day. This limited irrigation resulted in repeated wilting but did not cause severe desiccation damage and extensive leaf die-off. In contrast, control plants (DRC) were kept well watered at all times, receiving 500–1000 ml of water daily. Plants subjected to the F treatment were supplied with 400 ml of 7.5 mM NH₄NO₃ in 0.5× Hocking's complete nutrient solution (Hocking, 1971) every other day, while control plants (FC) received 400 ml of 0.75 mM NH₄NO₃ in 0.5× Hocking's complete nutrient solution. These two fertilization protocols were previously shown to provide high (F) and adequate (FC) levels of nitrogen (N) for the growth of this hybrid poplar genotype (Pitre *et al.*, 2007a, b; Hacke *et al.*, 2010). In order to keep plants well watered, plants were irrigated with tap water on the days when the fertilizer solution was not applied. The general purpose N-P-K fertilizer was not applied in addition to F and FC treatments. The SH treatment was imposed by enclosing plants in shade boxes made of gardening fabric. The shade boxes reduced ambient irradiation by 80%, from 350 μmol m⁻² s⁻¹ in control plants (SHC) to 70 μmol m⁻² s⁻¹ in shaded plants (SH). Experimental treatments were imposed for ~5 weeks. The exact duration of the three individual experiments (i.e. the period between planting the rooted cuttings and plant harvesting) and the duration of the experimental treatments (i.e. the period between the onset of a treatment and plant harvesting) are indicated in Supplementary Table S1 available at JXB online.

Sampling strategy

After measuring a plant's final height and stem diameter at a height of 10 cm above the root collar (D_{stem}), plants were cut at their base, placed in a dark plastic bag with a moist paper towel, and immediately brought to the lab. Bags with the plant material were stored at 4 °C in a refrigerator

until they were used for hydraulic measurements (but no longer than for 4 d). For these measurements, stem segments 20–25 cm in length were sampled from two different positions along the plant's main stem. The first set of segments was sampled from a basal region of the stem at a fixed height of 5–30 cm above the root collar ('basal' segments). The second set of segments was taken from a position closer to the apex of a plant ('distal' segments). The height at which the distal segments were collected differed among the three experiments, reflecting the different growth rates of plants exposed to different experimental treatments. Distal segments were located at ~60% of the final plant height. Hence, they were sampled at a height of 40–60 cm above pots in DR, 60–85 cm in DRC, 95–120 cm in F, 85–110 cm in FC, and 65–90 cm in both SH and SHC plants. Distal segments underwent their entire growth and development under treatment conditions. Basal segments, in contrast, completed primary growth and initiated secondary growth under control conditions before experimental treatments began. Nevertheless, a significant portion of secondary xylem was formed after the onset of treatments.

Xylem pressure

Xylem pressure was measured using equilibrated mature leaves attached between the fifth and eighth node counted from the top of a plant. One leaf per plant was measured, from five plants per treatment group. Leaves were sealed in a plastic bag covered with aluminium foil the night before harvesting to inhibit transpiration and to ensure equilibration of water potentials. Immediately after bagged leaves were excised, xylem pressure was measured using a Scholander-type pressure chamber (Model 1000; PMS Instrument Company, Albany, OR, USA). Xylem pressure measurements were conducted for F and DR experiments only.

Growth measurements

Final height and stem basal diameter (D_{stem}) of each sapling were assessed with measuring tape and calipers, respectively. Leaf area (A_L) was measured with an area meter (LI-3100, Li-Cor, Lincoln, NE, USA). A plant's total A_L and the A_L supported by basal stem segments were determined for each plant. The supported A_L was calculated as the sum of A_L distal to the segment and half of the A_L directly attached to the segment.

Hydraulic measurements

Segments used for hydraulic measurements were trimmed underwater to a final length of 14.2 cm. To measure native hydraulic conductivity (K_{native}), stem segments were fitted to a tubing apparatus, and the gravity-induced water flow rate through the segments was recorded with an electronic balance (CP225 D; Sartorius, Göttingen, Germany) interfaced with a computer. A pressure head of 4–5 kPa was used to induce the flow. Stem hydraulic conductivity was then calculated as the flow rate for a given pressure gradient. Subsequently, segments were flushed for 15–20 min at 50 kPa in order to remove native embolism. Conductivity measurements were repeated to determine hydraulic conductivity after flushing (K_{flush}). Stem segments were then attached in a custom-built centrifuge rotor and spun to progressively more negative pressures. Hydraulic conductivity was measured after each pressure increment until it dropped below 90% of the K_{flush} value. The percentage loss of hydraulic conductivity (PLC) relative to K_{flush} was plotted against the corresponding xylem pressure to generate vulnerability curves. Data points were fitted with a Weibull function and the xylem pressure corresponding to 50% loss of conductivity ($P50$) was determined for each segment.

After hydraulic measurements were completed, stem segments were sectioned near the middle of their length. The exposed cross-sectional surface was captured with a digital camera attached to a stereomicroscope (MS5; Leica Microsystems, Wetzlar, Germany) at $\times 10$ – $\times 16$ magnification. Xylem cross-sectional area (A_X) excluding pith and bark was measured with image analysis software (ImagePro Plus version 6.1, Media Cybernetics, Silver Spring, MD, USA). Xylem-specific hydraulic conductivity (K_S) was subsequently calculated as the maximal hydraulic conductivity (K_{max}) of a stem segment divided by the corresponding A_X . In the majority of stems, hydraulic conductivity increased

after flushing. However, in few instances, K_{flush} was slightly (i.e. <5%) lower than K_{native} , possibly due to a wounding response or clogging of the pit membranes. Thus, the K_{max} of a stem was determined as either K_{flush} or K_{native} , whichever value was higher.

Vessel diameter and wood density measurements

The same stem segments previously used for measuring hydraulic conductivity and cavitation resistance were used for measuring vessel diameter and wood density. Stem cross-sections (~40–60 μm thick) were prepared from the middle portion of stem segments using a sliding microtome (SM2400, Leica) or by hand with a fresh single-edge razor blade. Sections were stained with toluidine blue and observed with a light microscope (DM3000, Leica). Images were captured at $\times 100$ magnification using a digital camera (DFC420C, Leica). Vessel diameters were measured in complete radial sectors delimited by xylem rays spanning from the pith to the cambium. Between three and 10 different sectors were selected for each stem, providing a total of 300–500 vessel diameter measurements per stem. The average vessel diameter (D_v) per stem was subsequently calculated. Five to six stems were analysed for each treatment and position.

For measuring wood density (d_w), samples 2 cm in length were excised from stem segments and split longitudinally into two subsamples. The bark was peeled off and the pith was carefully removed. Wood specimens were then submerged in a beaker filled with water placed on an electronic balance (CP224 S, Sartorius). The displaced water weight was recorded and converted into fresh wood volume. Specimens were then oven-dried at 70 °C for 2 d. d_w was calculated as the ratio between a sample's dry weight and its fresh volume. The d_w of each stem segment was finally determined by averaging the values of the two subsamples. Five to eight stems were analysed for each treatment and position.

Statistical analyses

Since the three experimental treatments were imposed as three independent and temporally separated experiments, the effects of treatments were statistically evaluated by comparing treated plants with their controls within a single experiment. In order to evaluate the effect of treatments on plant growth parameters, independent two-sample *t*-tests were performed. Analysis of variance (ANOVA) was carried out in order to dissect the effect of treatment and stem segment position on xylem hydraulic and structural parameters (A_X , D_v , d_w , K_S , and $P50$). The following linear model was used to fit the measured data:

$$\text{var} \sim \text{treat} + \text{pos} + (\text{treat} \times \text{pos})$$

where *var* represents the tested variable, *treat* and *pos* are the fixed effect factors 'treatment' and 'position', and *treat* × *pos* is the interaction term. Planned comparisons between means of treated versus control plants within the same stem segment position (i.e. either basal or distal) and between means of basal and distal stem segments within either treated or control plant groups were carried out using the least significance difference procedure (Sokal and Rohlf, 1995). To elucidate potential relationships between selected growth, hydraulic, and anatomical parameters, we tested for significant linear correlations between the group means across all three experiments. The results of all statistical analyses were deemed significant at $P \leq 0.05$. Probability (*P*) values for the planned comparisons were adjusted to 0.0125 using the Bonferroni correction procedure for multiple comparisons (Sokal and Rohlf, 1995). The statistical software package R 2.10.1 (R Development Core Team 2009, Auckland, New Zealand) was used to perform the analyses. Throughout this manuscript, group means are cited with their standard deviations.

Results

Plant growth

The growth characteristics of hybrid poplar saplings differed between treated and control plants as well as between

experiments (Supplementary Table S2 at JXB online). The average final height ranged from 77.1 ± 2.1 cm to 171.3 ± 6.1 cm in DR and F plants, respectively. Similarly, the D_{stem} was smallest in DR (6.3 ± 0.2 mm) and largest in F (9.5 ± 0.4 mm). Furthermore, total A_L exhibited differences ranging from 0.27 ± 0.02 m² to 0.99 ± 0.07 m². As for the effect of treatments, DR plants exhibited reduced growth in height and girth and had smaller A_L compared with controls. The opposite trend in growth was observed in F relative to FC plants. The height growth increment was higher in SH, while their radial growth was reduced in comparison with controls.

Leaf area, xylem area, and stem hydraulic conductivity

The supported A_L scaled linearly with A_X across all plant groups ($R^2=0.933$, $P=0.002$) (Fig. 1A). A_X was tightly correlated with a stem's K_{max} ($R^2=0.976$, $P<0.001$). The level of native embolism was low in most basal stem segments; therefore, their K_{max} corresponded well with their K_{native} . As a result, the supported A_L was positively correlated with both K_{native} ($R^2=0.921$, $P=0.002$) (Fig. 1B) and K_{max} ($R^2=0.921$, $P=0.002$).

Variation in stem morphology and xylem anatomy

The structural and hydraulic parameters of xylem varied between treated and control plants and between the two stem segment positions (Table 1). In all xylem parameters tested, the ANOVA results showed a significant effect of at least one of the factors (*treat*, *pos*) or of their interaction (*treat* × *pos*) (Supplementary Table S3 at JXB online). The results of planned comparisons testing for a significant difference between group means are presented in Table 2.

Variation in A_X in response to treatments reflected changes in plant radial growth. In basal segments, A_X decreased in DR and SH plants and increased in F plants in comparison with their controls (Table 1). Similar patterns in xylem formation were observed in distal segments although there was no significant difference in A_X between SH and SHC plants (Table 2). The A_X of distal segments was 45–60% smaller compared with basal segments even though the external stem diameters of distal and basal segments were often similar. The difference in A_X was mainly caused by a larger pith area in distal segments compared with their basal counterparts (Fig. 2). Furthermore, the vascular cylinder in distal segments had an irregular (rather than more or less cylindrical) shape, and the patterns associated with primary growth were still apparent in stem cross-sections. For instance, clusters of primary xylem could be readily distinguished adjacent to the vertices of the pentagonal pith (arrowheads in Fig. 2B). Despite these signs of juvenility, the transition from primary to secondary growth was clearly completed and a substantial amount of secondary xylem was produced along the entire length of distal segments.

Differences in xylem development and differentiation were paralleled by variation in xylem anatomy. In basal segments, mean vessel diameters (D_v) ranged between 35.2 ± 1.1 μm and 43.4 ± 0.8 μm in SHC and DRC plants, respectively (Table 1). In basal segments, D_v decreased in DR while it increased in F plants when compared with their controls. D_v was not significantly

different between SH and SHC plants (Table 2). In distal segments, D_v showed even more variation in response to treatments. The smallest (30.1 ± 1.2 μm) and the largest D_v (48.6 ± 1.2 μm) were measured in DR and F plants, respectively (Table 1). The relative changes in D_v in response to treatments were consistent with the trends observed in basal segments. With the exception of DR plants, distal segments exhibited wider vessels than their basal counterparts.

Large differences were also observed in wood density (d_w), with values ranging from 0.244 ± 0.016 g cm⁻³ in the distal segments of F plants to 0.404 ± 0.010 g cm⁻³ in the basal segments of SHC plants (Table 1). Basal segments typically showed higher d_w than the corresponding distal segments. In basal segments, d_w was significantly lower in F and SH plants in comparison with their respective controls, while it did not differ between DR and DRC plants (Table 1). In distal segments, d_w was lower in F and SH segments relative to their controls, following the same pattern as in basal segments. In contrast, the distal segments in DR exhibited denser wood than DRC plants (Table 1).

Fig. 1. Relationship between supported leaf area (A_L) and a stem's capacity to transport water measured as (A) xylem cross-sectional area (A_X) and (B) native hydraulic conductivity (K_{native}) in hybrid poplar saplings grown under drought (DR), nitrogen fertilization (F), shade (SH), and control conditions (DRC, FC, and SHC). Means $\pm$ SD are shown.

Table 1. Structural and hydraulic parameters of xylem of basal (upper case letters) and distal (lower case letters) stem segments in hybrid poplar saplings grown under drought (DR, dr), nitrogen fertilization (F, f), shade (SH, sh), and control conditions (DRC, drc; FC, fc; SHC, shc).

	A_x (mm ²)	D_v (μm)	d_w (g cm ⁻³)	K_s (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	P50 (MPa)
DR	15.9 (0.7)	38.0 (1.8)	0.332 (0.021)	5.6 (0.6)	-1.18 (0.16)
DRC	31.6 (2.1)	43.4 (0.8)	0.349 (0.008)	7.9 (0.7)	-1.38 (0.04)
dr	6.3 (0.5)	30.1 (1.2)	0.334 (0.027)	3.1 (0.6)	-1.71 (0.02)
drc	12.8 (2.3)	45.9 (0.9)	0.288 (0.009)	3.8 (0.5)	-0.96 (0.20)
F ^a	51.3 (5.0)	42.6 (1.6)	0.311 (0.014)	8.3 (1.0)	-1.14 (0.16)
FC ^a	40.3 (3.8)	38.9 (0.4)	0.372 (0.021)	7.2 (0.6)	-1.42 (0.07)
f	28.1 (3.5)	48.6 (1.2)	0.244 (0.016)	6.5 (1.9)	-0.15 (0.08)
fc	18.0 (2.9)	45.3 (1.0)	0.295 (0.008)	7.9 (0.7)	-0.79 (0.19)
SH ^b	21.3 (1.4)	35.2 (1.1)	0.318 (0.005)	4.8 (0.3)	-1.14 (0.11)
SHC ^b	27.2 (2.0)	36.7 (0.8)	0.404 (0.010)	6.6 (0.1)	-1.53 (0.06)
sh	11.4 (2.0)	41.4 (1.2)	0.276 (0.010)	5.1 (0.7)	-0.63 (0.06)
shc	11.6 (1.8)	40.6 (1.5)	0.314 (0.013)	2.7 (0.3)	-1.03 (0.24)

Values are means with standard deviations given in parentheses ($n=5-8$)

A_x , xylem cross-sectional area; D_v , mean vessel diameter; d_w , wood density; K_s , xylem-specific hydraulic conductivity; P50, the pressure at 50% loss of hydraulic conductivity.

^a Data from Plavcová *et al.* (2012)

^b Data from Plavcová *et al.* (2011).

Variation in the hydraulic parameters of xylem

Changes in xylem structure were paralleled by differences in xylem hydraulic parameters (Tables 1, 2). Xylem-specific hydraulic conductivity (K_s) varied ~3-fold, from 2.7 ± 0.3 kg m⁻¹ s⁻¹ MPa⁻¹ to 8.3 ± 1.0 kg m⁻¹ s⁻¹ MPa⁻¹ in DR and F plants, respectively (Table 1). In basal segments, K_s was lower in DR and SH plants in comparison with their controls. K_s tended to be higher in F than in FC plants; however, the difference was not statistically significant (Table 2). In distal segments, K_s was higher in SH and did not differ in DR and F plants with respect to their respective controls.

Cavitation resistance varied profoundly in response to experimental treatments and segment location (Tables 1, 2). The P50 of basal segments differed <0.5 MPa across all three treatments, ranging from -1.14 MPa in both SH and F plants to -1.53 MPa in SHC plants. While the vulnerability of basal stem segments did not change significantly in response to the DR treatment, stems of SH and F plants were more vulnerable than their controls. In distal segments, P50 values exhibited a large variation of 1.5 MPa between the most vulnerable (F plants, -0.15 ± 0.08 MPa) and the most resistant plants (DR plants, -1.71 ± 0.20 MPa). With the exception of the DR treatment, distal segments were more vulnerable than their basal counterparts. In fact, distal segments

Table 2. Probability (P) values of least significance difference (LSD) tests comparing means of treated versus control plants within the same stem segment position (i.e. either basal or distal) and between means of basal and distal stem segments within either treated or control plant groups.

Comparison	A_x (mm ²)	D_v (μm)	d_w (g cm ⁻³)	K_s (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	P50 (MPa)
DR-DRC	<0.001	<0.001	NS	<0.001	NS
dr-drc	<0.001	<0.001	<0.001	NS	<0.001
DR-dr	<0.001	<0.001	NS	<0.001	<0.001
DRC-drc	<0.001	0.022	<0.001	<0.001	<0.001
F-FC	<0.001	<0.001	<0.001	NS	<0.001
f-fc	<0.001	<0.001	<0.001	NS	<0.001
F-f	<0.001	<0.001	<0.001	0.0045	<0.001
FC-fc	<0.001	<0.001	<0.001	NS	<0.001
SH-SHC	<0.001	NS	<0.001	<0.001	<0.001
sh-shc	NS	NS	<0.001	<0.001	<0.001
SH-sh	<0.001	<0.001	<0.001	NS	<0.001
SHC-shc	<0.001	<0.001	<0.001	<0.001	<0.001

DR, dr, drought-stressed plants; F, f, plants fertilized with high levels of nitrogen; SH, sh, shaded plants; DRC, drc, FC, fc, SHC, shc, control plants.

Upper case letters indicate basal segments, while lower case letters refer to distal segments.

A_x , xylem cross-sectional area; D_v , mean vessel diameter; d_w , wood density; K_s , xylem-specific hydraulic conductivity; P50, the pressure at 50% loss of hydraulic conductivity; NS, non-significant ($P > 0.0125$).

Fig. 2. Representative cross-sections of (A) basal and (B) distal stem segments from one of the control plants (FC). In the basal segment (A), secondary xylem (X) represents the majority of stem cross-sectional area, while the area of pith (p) is relatively small. In contrast, a large pentagonal pith is surrounded by a relatively narrow layer of secondary xylem in the distal segment (B). Signs of juvenility are apparent in the distal segment. Clusters of primary xylem and secondary xylem formed early after the transition to secondary growth are apparent in the distal segment cross-section (arrowheads). Scale bars=1 mm.

of DR plants had the most resistant xylem across all the plant groups and segment positions tested in this study (Table 1).

The differences in P50 between basal and distal segments were associated with a marked change in the shape of their vulnerability curves (Fig. 3). While all basal segments showed typical sigmoidal-shaped vulnerability curves, the shape of the curves of distal segments varied from sigmoidal through linear to r-shaped, depending on the treatment. The distal segments of F plants were extremely vulnerable. These segments exhibited 75% PLC at a modest xylem pressure of -0.25 MPa, resulting in an r-shaped vulnerability curve (Fig. 3C). In distal segments of

DR plants, in contrast, embolism did not exceed 20% at xylem pressures less negative than -1.25 MPa (Fig. 3A). Vulnerability curves generated by the centrifuge method were in good agreement with the native PLC values plotted against the native xylem pressures measured in the F and DR experiments (square symbols in Fig. 3). Xylem pressure was not measured for the SH experiment.

P50s of both basal and distal segments scaled tightly with d_w (basal $R^2 = 0.928$, $P = 0.002$; distal $R^2 = 0.925$, $P = 0.002$) (Fig. 4A). In contrast, P50 was not correlated with K_s (Fig. 4B). P50 was significantly correlated with D_v in distal segments (distal

Fig. 3. Vulnerability curves (circles) and native values of percentage loss of conductivity (PLC) plotted against the native xylem pressure (squares) for basal (filled symbols) and distal (open symbols) stem segments in saplings grown under (A) drought (DR) and (B) well-watered conditions (DRC), and under (C) high N (F) and (D) adequate N (FC) fertilization. Note the profoundly different shape of the vulnerability curves in distal segments (open circles), ranging from (A) sigmoidal through (B and D) linear to (C) r-shaped. Means $\pm$ SD are shown.

Fig. 4. Relationship between P50 and (A) wood density, (B) xylem-specific hydraulic conductivity (K_s), and (C) mean vessel diameter for basal (filled circles, upper case letters) and distal (open circles, lower case letters) stem segments in hybrid poplar saplings grown under drought (DR, dr), nitrogen fertilization (F, f), shade (SH, sh), and control conditions (DRC, drc; FC, fc; SHC, shc). Means $\pm$ SD are shown. Solid and dashed lines represent significant linear correlations for basal and distal segments, respectively.

$R^2 = 0.766$, $P = 0.023$); however, this correlation was mainly driven by the two extremely different distal segments in DR and F plants (Fig. 4C).

Discussion

Scaling of A_x and A_L and variation in K_s

Plant growth and development were greatly affected when saplings of hybrid poplar were grown under experimental drought (DR), shade (SH), or nitrogen fertilization (F) for >30 d (Supplementary Table S2 at JXB online). In addition, some variability in growth and hydraulic parameters existed between the three sets of control plants (DRC, SHC, and FC) that grew under similar but not identical conditions. Across all plant groups, the A_x was tightly correlated with the supported A_L (Fig. 1A). Since A_x represents the principal anatomical basis of a stem's capacity to transport water, the allometric relationship between A_x and A_L translates into tight scaling between A_L and a stem's hydraulic conductivity (K_{native}) (Fig. 1B). These relationships, reflecting a well-established allometric relationship between stem basal diameter and leaf area (e.g. Harrington and Fownes, 1993; McCulloh *et al.*, 2012), arguably help to maintain an adequate supply of water from roots to transpiring leaves (Shinozaki *et al.*, 1964).

A tight correlation between A_L and A_x would in principle not be required if a plant could radically change the hydraulic parameters of its xylem. For instance, if the xylem could become much more transport efficient, a smaller A_x per unit A_L would be sufficient to provide an adequate water supply. Despite an almost 2-fold variation in K_s values across the basal segments in the current data set, these differences in transport efficiency did not significantly alter the overall linear relationship between A_L and A_x across the experimental group averages.

The changes in K_s observed in response to individual experimental treatments (Tables 1, 2) were largely consistent with the initial hypotheses. In basal segments, K_s values were lower in resource-limited DR and SH plants. In contrast, the highest value of K_s was found in basal segments of F plants which received luxurious levels of nitrogen and were kept well irrigated all the time. These findings are in agreement with changes in K_s observed in other studies in response to low water availability (Beikircher and Mayr, 2009), shade (Raimondo *et al.*, 2009), and high nutrient supply (Hacke *et al.*, 2010).

Variation in cavitation resistance

In agreement with our initial hypothesis, xylem vulnerability to drought-induced cavitation changed in response to experimental treatments (Tables 1, 2). More vulnerable xylem in comparison with controls was found in both basal and distal segments of SH and F plants. These findings are in line with results obtained in other species in response to similar environmental cues (Cochard *et al.*, 1999; Harvey and van den Driessche, 1999; Barigah *et al.*, 2006). P50 was not statistically different between DR and DRC plants in basal segments; however, cavitation resistance increased in distal segments of DR saplings. As growth was significantly reduced in DR plants, the amount of xylem produced under treatment conditions was small in basal segments, and hence did not affect the overall vulnerability of the bulk xylem tissue. However, the increased resistance in distal segments of DR plants indicates that their xylem adjusted to sustain lower xylem pressures and prevent excessive cavitation. An increase

in cavitation resistance in response to low water availability has been previously documented in several species, including poplar, exposed to various levels of drought severity (Beikircher and Mayr, 2009; Awad *et al.*, 2010; Fichot *et al.*, 2010), while only a limited change has been found in three willow clones (Wikberg and Ögren, 2007).

Across the three experiments conducted in the current study, the average difference in P50 between treated and control plants was 0.3 MPa and 0.6 MPa for basal and distal segments, respectively. Even a relatively subtle change in P50 may have important implications for plant hydraulic performance because the loss of conductivity due to embolism increases very steeply around the P50 value. The smaller variation in P50 observed in basal in comparison with distal segments is not surprising given the experimental design. The basal segments completed their primary growth and started their secondary growth under the same conditions prior to the commencement of treatment. In distal segments, in contrast, the entire growth and development took place under treatment conditions.

With the exception of DR plants, distal segments were more vulnerable than their basal counterparts. The difference between basal and distal segments across the treatment groups was on average 0.6 MPa, with the largest difference of almost 1 MPa found in F plants. Age-related differences in xylem vulnerability have been previously studied using branches of field-grown trees. While some studies found that younger branches and roots were more vulnerable to cavitation (Sperry and Ikeda, 1997; Choat *et al.*, 2005), other studies do not support this trend (Sperry and Saliendra, 1994; Hacke and Sauter, 1996). Based on the vulnerability segmentation hypothesis (Zimmermann, 1983; Tyree and Ewers, 1991), it has been proposed that more distal organs such as leaf petioles and terminal branches should be more vulnerable than trunk xylem in mature trees. Distal plant parts are arguably expendable; their sacrifice can help to maintain favourable plant water balance by reducing the total transpiring surface (Rood *et al.*, 2000). This strategy might be particularly vital in poplar which is well known for its ability to regenerate by root suckering and re-sprouting from auxiliary buds (Galvez and Tyree, 2009; Lu *et al.*, 2010). Nevertheless, it is questionable whether such reasoning is relevant to the relatively young saplings measured in this current study.

The increased vulnerability of the distal segments could potentially be linked to their juvenility. In distal segments, primary xylem represented a substantial proportion of the bulk xylem (Fig. 2). Primary xylem has been shown to be more vulnerable than secondary xylem in 1-year-old branches of sugar maple (Choat *et al.*, 2005). However, little evidence was found for increased vulnerability of primary xylem in hybrid poplar in the current study. When native and artificially induced embolism was visualized by perfusing distal stem segments with safranin dye, more embolized vessels were detected in secondary xylem while the clusters of primary xylem vessels were largely functional (Supplementary Fig. S1 at JXB online). Thus, the increased proportion of primary xylem in distal segments cannot explain their increased vulnerability.

The distal segments of F plants appeared particularly vulnerable, as indicated by their r-shaped vulnerability curve (Fig. 3C). While some regarded r-shaped vulnerability curves as

measurement artefacts (Choat *et al.*, 2010; Cochard *et al.*, 2010), others provided evidence that these curves can be valid and that they are associated with the presence of extremely vulnerable vessels that embolize at near-atmospheric pressures (Christman *et al.*, 2012; Jacobsen and Pratt, 2012; Sperry *et al.*, 2012). r-shaped curves have been typically measured in large-vesselled plants such as oaks and grapevine (Taneda and Sperry, 2008; Jacobsen and Pratt, 2012); however, similar curves have been observed in poplar under distinct conditions of cavitation fatigue (Hacke *et al.*, 2001b) and xylem senescence (Sperry *et al.*, 1991). In the current study, native PLC measured in distal segments of F plants plotted against the values of native xylem pressure corresponded well with the vulnerability curves (Fig. 3C), providing support that these curves are valid in the present study. According to the air-seeding hypothesis, the population of particularly vulnerable vessels is characterized by extremely leaky pits. Thus, the pit membranes in distal segments of F plants might have been inherently more porous or more susceptible to pore enlargement during pit membrane deflection. Alternatively, it is possible that the low mechanical reinforcement of vessels, as evidenced by low wood density, resulted in irreversible damage to some of the pit membranes, rendering them extremely permeable to air.

Relationship between wood density and cavitation resistance

The correlation between wood density (d_w) and P50 was remarkably strong across all basal and distal segments measured in this study (Fig. 4A). This finding agrees with previous studies that identified a trade-off between vulnerability and xylem construction cost at the interspecific level (Hacke *et al.*, 2001a; Jacobsen *et al.*, 2005; Pratt *et al.*, 2007). Results from intraspecific comparisons are less conclusive regarding this trade-off. No relationship between d_w and P50 was found in a recent study comparing eight different genotypes of *Populus deltoides* × *Populus nigra* grown under two levels of irrigation (Fichot *et al.*, 2010). However, a significant correlation between P50 and another parameter related to xylem mechanical strength, the double vessel wall thickness, was found in the same study. Similarly, Awad *et al.* (2010) found that increased vulnerability scaled with decreasing cell wall thickness and vessel thickness to span ratio in plants of a single *Populus tremula* × *Populus alba* clone grown under three contrasting water regimes, while no significant correlation was found between P50 and d_w . In the current study, saplings were maintained in a controlled environment with their stems secured to supporting stakes. In plants supported by stakes, wood density may strongly reflect demands arising from cohesion-driven water transport, while the role of xylem in providing structural support to the plant body is likely to be less important than in plants growing in a natural environment. This might have contributed to the tight correlation observed between P50 and d_w in this study.

Safety versus efficiency trade-off

The mean values of K_s and P50 across all basal segments were $6.7 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ and -1.3 MPa , respectively, which ranks hybrid poplar among diffuse-porous species that are relatively

transport efficient but vulnerable to cavitation. Thus, these data support the notion that xylem cannot be superior in both cavitation resistance and transport efficiency (Maherali *et al.*, 2004; Hacke *et al.*, 2006; Lens *et al.*, 2011). However, within the present data set, there was no significant correlation between P50 and K_s in either basal or distal stem segments (Fig. 4B). A correlation between D_v and P50, which can also be regarded as an indicator of a safety versus efficiency trade-off, was significant only in distal segments, and this trend was mainly driven by two extreme data points (Fig. 4C). Thus, these findings indicate that increased resistance to cavitation is not necessarily associated with reduced transport efficiency. This is good news for tree breeders because it suggests that there is some limited room for simultaneous improvement of both hydraulic efficiency and cavitation resistance. In the current data set, basal segments were closer to this optimum than the distal segments (Fig. 4B). Interestingly, basal segments under treatments in which either water or light resources were severely limited (i.e. DR and SH plants) appeared less hydraulically optimized compared with the basal segments of F and all three control plant groups.

Conclusions

The hybrid poplar genotype used in this study inherited a genetic blueprint from the riparian cottonwoods *P. trichocarpa* and *P. deltoides*, which defines the general characteristics of its anatomy, morphology, and physiology. This study has specifically focused on the anatomy and hydraulic function of xylem. The data show that the xylem of hybrid poplar is efficient and highly vulnerable to drought-induced cavitation, as expected given its parentage. While these general properties of xylem cannot be radically changed, the data demonstrate that the xylem structure and function are, to a certain extent, variable. Differences in xylem cross-sectional area, mean vessel diameter, wood density, xylem-specific hydraulic conductivity, and vulnerability to cavitation were detected not only in response to experimental treatments (drought, nitrogen fertilization, and shade) but also in stem segments sampled from different vertical positions along a plant's main axis (basal and distal segments). Such developmental and phenotypic plasticity in xylem traits can potentially be used to cope with different and changing environmental conditions. However, it is difficult to predict what xylem phenotypes would be produced under field conditions.

The results also provide insights into xylem structure–function trade-offs and can help to elucidate mechanistic underpinnings of some of these patterns. The close correlation between wood density and P50 observed in this study is intriguing and suggests that there might have been a true functional link between these two traits in this study. It is likely that stronger cell walls helped to stabilize the pit fields, thereby protecting pit membranes from mechanically induced damage. More research is necessary to show if this situation is unique to this highly vulnerable hybrid poplar clone grown with stems structurally supported by stakes or if it can be extrapolated to a broader array of species and growing conditions. In contrast, no correlation between P50 and K_s was found across the data set, indicating that the safety versus efficiency trade-off is not, within certain bounds, inevitable.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Cross-section of a distal stem segment perfused with safranin dye to visualize functional and embolized xylem conduits.

Table S1. Overview of the experimental design: the duration of experiments, and the duration of treatments, and a brief description of the treatment conditions for treated and control plants.

Table S2. Growth-related parameters of hybrid poplar saplings grown under drought (DR), nitrogen fertilization (F), shade (SH), and control conditions (DRC, FC, SHC).

Table S2. Analysis of variance results: variation in xylem structural and hydraulic parameters in response to treatments and between stem segment positions.

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