

Original Article

Leaf size serves as a proxy for xylem vulnerability to cavitation in plantation trees

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ABSTRACT

Hybrid poplars are an important renewable forest resource known for their high productivity. At the same time, they are highly vulnerable to water stress. Identifying traits that can serve as indicators for growth performance remains an important task, particularly under field conditions. Understanding which trait combinations translate to improved productivity is key in order to satisfy the demand for poplar wood in an uncertain future climate. In this study, we compared hydraulic and leaf traits among five hybrid poplar clones at 10 plantations in central Alberta. We also assessed the variation of these traits between 2- to 3-year-old branches from the lower to mid-crown and current-year long shoots from the mid to upper crown. Our results showed that (1) hybrid poplars differed in key hydraulic parameters between branch type, (2) variation of hydraulic traits among clones was relatively large for some clones and less for others, and (3) strong relationships between measured hydraulic traits, such as vessel diameter, cavitation resistance, xylem-specific and leaf-specific conductivity and leaf area, were observed. Our results suggest that leaf size could serve as an additional screening tool when selecting for drought-tolerant genotypes in forest management and tree improvement programmes.

Key-words: boreal forest; cavitation; drought stress; forest management; leaf area; plant hydraulics; plasticity; poplar plantations; water transport.

INTRODUCTION

Populus has emerged as a model tree species (Cooke & Rood 2007; Jansson & Douglas 2007). In the last decade, much has been learned about the transcriptional regulation of xylem formation (Zhong & Ye 2013), the transcriptome-level drought response (Raj *et al.* 2011) and various physiological traits of *Populus* genotypes (Fichot *et al.* 2015; Hacke 2015). If their large water requirements are met, poplars may show fast

growth rates. In addition, most *Populus* species and their hybrids are easy to propagate, and desired characteristics in genotypes can be maintained by clonal propagation (Stettler *et al.* 1996). In the Canadian prairies, millions of clonal poplars have been planted over the past century (Talbot *et al.* 2011). In Alberta, hybrid poplar plantations have been established on marginal farmland, with limited silvicultural intervention beyond the control of competing vegetation.

Many hybrid poplar clones have been developed in tree improvement programmes (Eckenwalder 2001; Riemenschneider *et al.* 2001). Large differences exist across clones in terms of morphology (e.g. leaf size and degree of apical dominance), water-use strategy, hybrid vigor and stress tolerance. Characterizing the genetic variability of these traits in both the field and the greenhouse is an ongoing effort, and much remains to be learned.

Due to the observed and projected changes in both temperature and precipitation in parts of western Canada (Mbogga *et al.* 2009), several recent studies investigated traits related to drought tolerance of hybrid poplar clones (e.g. Arango-Velez *et al.* 2011; Arshad *et al.* 2011). In a comparative study of hybrid poplars and trembling aspen, (Schreiber *et al.* 2011) found that hybrid poplars were more vulnerable to cavitation and less water-use efficient when compared with aspen. Although trembling aspen (*Populus tremuloides*, Michx.) is considered to be more drought tolerant than most *Populus* species and hybrids, massive drought-induced mortality of aspen has been observed along the southern edge of the Canadian boreal forest (Michaelian *et al.* 2011). It therefore seems reasonable to expect that future growth and productivity of other *Populus* species and their hybrids may also be constrained by water availability in adjacent regions. However, at the same time, it is also important to note that adaptive capacity and resilience within populations could also play an important role in mitigating the presently observed effects of drought on forest health in the future (Williams *et al.* 2008).

The primary objective of this study was to characterize hydraulic and wood anatomical traits related to drought resistance. We studied five hybrid poplar clones that are currently used in commercial plantations in Alberta.

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Measurements were conducted at the branch level. We were particularly interested in the relationships between the vulnerability of xylem to drought-induced cavitation, xylem transport efficiency, vessel diameter and leaf size. This latter trait can easily be measured, and the genotypes used in this study showed striking differences in their average leaf size (Fig. 1). Little is known about the relationship between leaf size and the hydraulic properties of stems. In some cases, positive correlations between leaf size and vulnerability to xylem cavitation have been reported (Markesteijn *et al.* 2011; Kröber *et al.* 2014). Drought-tolerant species with cavitation-resistant stem xylem tended to have small leaves. This agrees with the outcome of nitrogen (N) fertilization experiments with hybrid poplar plants. In these experiments, high N plants had much larger leaves than plants receiving lower levels of N. High N plants also had more vulnerable xylem, increased transport efficiency and wider vessels than control plants (Hacke *et al.* 2010; Plavcová *et al.* 2013).

We also included vessel diameters (D_V) in our analysis. This was expected to be a key trait, because previous studies on poplar have found that D_V correlated with cavitation resistance (Hacke *et al.* 2010; Plavcova & Hacke 2012; Hajek *et al.* 2014), the amount of native xylem embolism (Schreiber *et al.* 2013) and growth (Schreiber *et al.* 2011). The relationship between D_V and growth is interesting as it will likely depend on the environment. In the absence of severe drought and frost, wider vessels may facilitate faster growth, at least in the short term. In contrast, at boreal planting sites, sustainable growth appears to be associated with narrow vessels (Schreiber *et al.* 2011; Schreiber *et al.* 2015), presumably because narrow vessels are more resistant to freezing-induced embolism (Davis *et al.* 1999).

With respect to crown architecture and branch morphology, poplars form indeterminate long shoots up to several meters in length particularly in the upper crown. Short shoots on the other hand are more frequent in the lower crown and only consist of a whorl of small leaves (Dickman *et al.* 2001). Leaves on current-year long shoots generally have larger individual leaf areas and higher photosynthetic rates compared with short

shoots (Nelson & Michael 1982; Dickman *et al.* 2001). In the present study, we measured and compared hydraulic traits with leaf size on 2- to 3-year-old branches from the lower to mid-crown (mainly composed of leaves from short shoots) and vigorously growing current-year long shoots from the middle to upper crown.

Given our study design, we were also able to investigate the effect of frost and drought on xylem vulnerability, a phenomenon known as cavitation fatigue (Hacke *et al.* 2001; Christensen-Dalsgaard & Tyree 2014). We did this by comparing cavitation resistance of 2- to 3-year branches and current-year long shoots. A recent study on two Japanese tree species reported that 1- or 2-year-old xylem was more vulnerable to cavitation than current-year xylem (Fukuda *et al.* 2015). We may therefore expect that 2- to 3-year-old xylem will be more vulnerable than current-year xylem that has not experienced drought or frost.

Lastly, we also calculated the integrals for xylem and leaf-specific vulnerability curves to assess a species' hydraulic capacity over the measured range of xylem pressures. These calculated *areas under the curves* (AUCs) are not meant to replace other well-established discrete points used to characterize a vulnerability curve, such as the pressure that causes 50% loss in hydraulic conductivity (P_{50}) or the maximum xylem-specific conductivity (K_S). However, AUCs allow us to determine the hydraulic capacity between any two points along the tested xylem pressures and therefore provide a simple and flexible approach comparing important curve characteristics between individuals and species. To make better predictions of plant drought responses on large scales, Sperry & Love (2015) used a similar approach, however, integrating soil-canopy conductance vulnerability curves, rather than xylem-specific conductivity curves, in order to derive transpiration supply and loss functions for modeling purposes.

In summary, the main objectives of this study were (1) to assess whether leaf size and hydraulic traits differ between 2- to 3-year-old branches and current-year long shoots, (2) to examine how much variation exists in key hydraulic traits among clones, and (3) to test whether correlations and trade-offs exist between these traits.

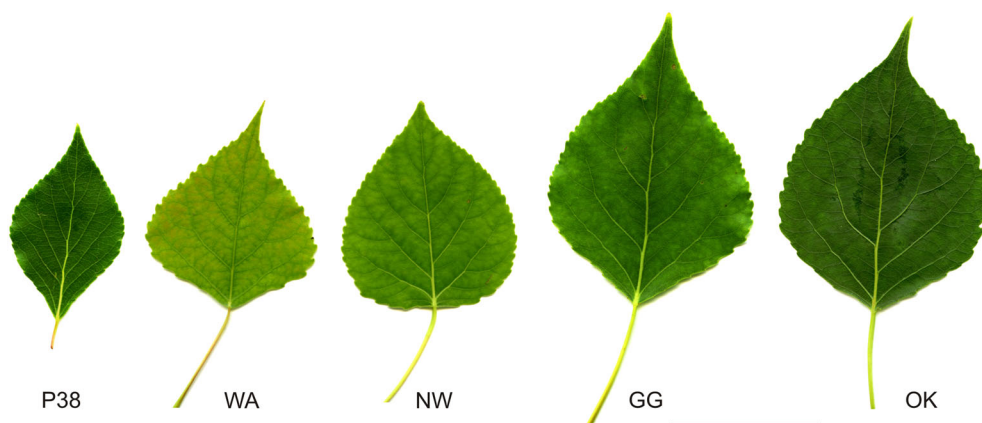


Figure 1. Leaves of the hybrid poplar clones P38P38 (P38), Walker (WA), Northwest (NW), Green Giant (GG) and Okanese (OK). The scale bar on the right is 5 cm. Leaves were collected from 2- to 3-year-old branches.

MATERIAL AND METHODS

Plant material

The plant material came from 10 operational hybrid poplar plantations established in the spring of 2007. The plantations are owned and maintained by Alberta-Pacific Forest Industries Inc. (Al-Pac). The studied clones were Green Giant, Okanese, Northwest, P38P38 and Walker. Each clone was replicated twice at spatially separated locations (Table 1). Trees were planted at a spacing of 3 × 3 m. Within each plantation, six similar-sized neighbouring trees were marked for further analysis, and data were pooled by clone ($n = 12$ trees per clone). Height and diameter at breast height (DBH, 1.3 m) measurements were taken in April 2012. One branch per tree was sampled at approximately 5 m height with a telescope pruner on 11 June 2013, resulting in a total of 60 2- to 3-year old branches. From each branch, one segment was prepared for hydraulic and anatomical analyses (see subsequent discussion). The hydraulic measurements were processed within five days of sampling. Two conductivity apparatuses were used in the laboratory, and samples were randomly distributed between the two systems, allowing for 12 stem segments to be measured per day. A second set of samples were collected on 20 August 2013. Six vigorously growing current-year long shoots were collected from the five clones. Each clone was sampled at only one plantation, resulting in a total of 30 individual branches ($n =$ six trees per clone). Samples were taken at approximately 6–7 m height. Hydraulic measurements were conducted within six days after sampling. For both sampling dates (June and August), branches > 1 m in length were cut and kept in moist plastic bags during transport back to the laboratory. Upon arrival at the University of Alberta, the material was stored in a walk-in refrigerator at 8 °C. Table 2 shows the measured parameters and the abbreviations used for this study.

Hydraulic measurements

Hydraulic conductivity was measured on 14.2-cm-long segments with diameters ranging between 5 and 9 mm. Branches were successively cut under water to avoid introducing embolisms (Venturas *et al.* 2015). The final segments used for conductivity measurements were located >30 cm from the basal end of the branch. Measurements were carried out using two conductivity apparatuses (Sperry *et al.* 1988). Hydraulic conductivity was calculated as the quotient of flow rate through the segment and pressure gradient. Each tubing apparatus consisted of an elevated water reservoir connected to an electronic balance (CP225D and CP224S, respectively; Sartorius, Göttingen, Germany) via Tygon tubing. The balances were interfaced with a computer, and hydraulic conductivity was logged every 10 s. Each segment was inserted into the tubing apparatus, and the native conductivity was measured. To remove native embolisms, all segments were flushed with filtered (0.2 µm) measuring solution (20 mM KCl + 1 mM CaCl₂) at 40 kPa for 15 min, and the maximum hydraulic conductivity (K_h) was determined. Each segment was then spun in a centrifuge (Sorvall RC5C Plus) for 10 min at increasing negative

Table 1. Clone name and parentage information of studied clones including geographic coordinates (latitude, longitude) for plantations, elevation, distance between plantations with the same clone, and mean height and DBH per clone at age six for plantation #1 and #2

Clone name (abbreviation)	Parentage ($P. = Populus$)	Plantation #1 (lat, long)	Plantation #2 (lat, long)	Elevation #1/#2 (m)	Distance (km)	Height #1/#2 (m)	DBH #1/#2 (mm)
Green Giant (GG)	$P. deltoides \times P. \times petrowskyana^*$	54°45'N, 113°06'W	54°46'N, 112°42'W	596/590	26	5.6 (0.09)/6.2 (0.25)	62 (2.20)/69 (3.96)
Okanese (OK)	$P. Walker \times P. \times petrowskyana^*$	54°53'N, 112°57'W	54°25'N, 113°28'W	600/666	62	7.0 (0.13)/4.6 (0.11)	71 (4.89)/48 (2.06)
Northwest (NW)	$P. deltoides \times P. balsamifera$	54°41'N, 112°44'W	54°33'N, 113°07'W	627/645	29	5.1 (0.22)/5.4 (0.22)	60 (4.03)/65 (5.71)
P38P38 (P38)	$P. balsamifera \times P. simonii$	53°42'N, 112°45'W	54°21'N, 112°51'W	703/700	73	6.4 (0.26)/5.7 (0.25)	71 (5.01)/61 (4.41)
Walker (WA)	$P. deltoides \times P. \times petrowskyana^*$	53°42'N, 112°45'W	53°47'N, 113°19'W	709/674	38	8.5 (0.03)/6.8 (0.13)	86 (1.94)/64 (2.33)

* $P. \times petrowskyana = P. laurifolia \times P. nigra$.

$P.$, *Populus*; DBH, diameter at breast height; lat, latitude; long, longitude.

Standard error of the mean is given in parentheses.

$n = 6$.

Table 2. A list of measured physiological parameters including abbreviations used in the text, the definition of the parameter and units

Abbreviation	Definition	Units
K_h	Hydraulic conductivity	$\text{kg s}^{-1} \text{m MPa}^{-1}$
PLC	Percent loss of hydraulic conductivity	%
D_V	Vessel diameter	μm
P_{50}	Pressure causing 50% loss of hydraulic conductivity	MPa
AUC_{K_S}	Xylem-specific conductivity (K_S) integrated over the measured pressure gradient or Area Under the Curve	$\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$
AUC_{K_L}	Leaf-specific conductivity (K_L) integrated over the measured pressure gradient, or Area Under the Curve	$10^{-4} \text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$
A_L , tot	Total leaf area per branch	m^2
A_L , ave	Average leaf area per branch	cm^2
C_L	Total leaf count per branch starting from measured segment	n/a
$A_L : A_S$	Leaf area to sapwood area ratio	$\text{m}^2 \text{cm}^{-2}$

pressure intervals of 0.5 MPa. After each interval, K_h was measured. This process was repeated until more than 90% loss of K_h occurred. The results were then graphically displayed as a vulnerability curve (Hacke *et al.* 2015). The xylem pressure at which 50% loss of hydraulic conductivity occurred (P_{50}) was determined for all segments by fitting a Weibull function to the data points. To compare K_h of the two conductivity apparatuses, some stem segments were measured in both systems. There was no systematic difference in conductivity between the two apparatuses, and values were in close agreement (data not shown).

Xylem-specific hydraulic conductivity (K_S) was calculated by dividing maximum K_h by the cross-sectional sapwood area (A_S) of the measured branch segment. A_S was captured using a stereomicroscope (MS5; Leica, Wetzlar, Germany) and measured with the image analysis software Image-Pro Plus 6.1 (Media Cybernetics, Silver Spring, MD, USA). K_S is a measure of the transport efficiency of the xylem (Tyree & Zimmermann 2002).

Leaf-specific hydraulic conductivity (K_L) was calculated by dividing maximum K_h by the total leaf area (A_L), that is, all leaves directly connected as well as upstream of the measured branch segments. Leaf area measurements were performed using a LI-3100 Area Meter (Li-Cor, Lincoln, NE, USA). K_L is a measure of the hydraulic sufficiency (or adequacy) of the segment to supply water to the leaves (Tyree & Zimmermann 2002).

Anatomical measurements

All anatomical measurements were conducted on the same segments that were used for hydraulic measurements. Segments were cut in their centre, and cross sections of 30–35 μm were taken with a sliding microtome (Leica SM2400) and analysed using a Leica DM3000 microscope at $\times 100$ magnification. Images of each cross section were captured with a Leica DFC420C camera and analysed using image analysis software (Image-Pro Plus 6.1). Individual vessels were measured manually in three sectors per cross section. Mean hydraulic vessel diameters (D_V) were calculated based on the Hagen–Poiseuille equation. The vessel diameter that corresponds to the average lumen conductivity was calculated as $D_V = ((\sum d^4)/n)^{1/4}$, where

n is the number of vessels measured and d is the individual vessel lumen diameter (Tyree *et al.* 1994; Wheeler *et al.* 2005).

Data analysis

All data analyses and graphics were carried out using the R programming environment 3.1.1 (R Core Team, 2014) plus the additional R packages *plyr* (Wickham 2011), *ggplot2* (Wickham 2009), *car* (Fox & Weisberg 2011), *candisc* (Friendly & Fox 2013) and *Bolstad2* (Curran 2013). The data were pooled by clone because the experimental setup did not allow for proper testing of a genotype by environment effect among clones because each clone was planted at a unique location.

Normality and homogeneity of variances were assessed using the R functions *shapiro.test()* and *levenesTest()*, and data transformations were performed when necessary to conduct generalized canonical discriminant analysis (CDA) and analysis of variance (ANOVA). The objective of discriminant analysis is to maximize group separation using linear functions of the measured variables (Rencher 2002). CDA was calculated using the *candisc()* function from the R package *candisc*. Balanced one-way ANOVAs were carried out for all physiological parameters between all clones using the *avov()* function. For significant ANOVAs, multiple mean comparisons were performed using the *TukeyHSD()* function with a family-wise confidence level set at 0.95 (Supporting Information Tables S1 and S2).

We also performed numerical integration to quantify total xylem-specific and leaf-specific hydraulic conductivity over the measured pressure gradient by calculating the area under the curve (*AUC*) for each individual segment in the 2- to 3-year old branches ($n = 60$) and current-year shoots ($n = 30$):

$$AUC_{K_S} = \int_{P_X=0}^{P_X(K_S=0)} -K_S(P_X)dP_X ;$$

$$AUC_{K_L} = \int_{P_X=0}^{P_X(K_L=0)} -K_L(P_X)dP_X$$

Numerical integration, that is, the numerical approximation of definite integrals, was performed using the *sintegral()* function from the R package *Bolstad2*. To provide a multispecies comparison of the *AUC* parameter, we also integrated xylem-specific hydraulic conductivity for two additional species, that is, *Amelanchier alnifolia* (Wheeler *et al.* 2005) and *Populus tremuloides* (Schreiber *et al.* 2011).

RESULTS

Variation in key hydraulic traits between 2- to 3-year-old branches and current-year long shoots

All clones had relatively vulnerable xylem. Current-year long shoots appeared to be slightly more cavitation resistant and also showed a larger spread of P_{50} values compared with 2- to 3-year old branches (Fig. 2). However, the mean P_{50} for each branch type was not significantly different (Table 3). In the 2- to 3-year-old branches, a clear grouping between the Green Giant and Okanese clones (more vulnerable) and the Walker, P38P38 and Northwest clones (less vulnerable) was observed (Fig. 2(a) and Supporting Information Fig. S1). A similar grouping was also apparent in current-year long shoots, however, with more variability and a switch of the Walker clone into the more vulnerable group consisting of the Green Giant and Okanese clones (Fig. 2(b) and Supporting Information Fig. S2).

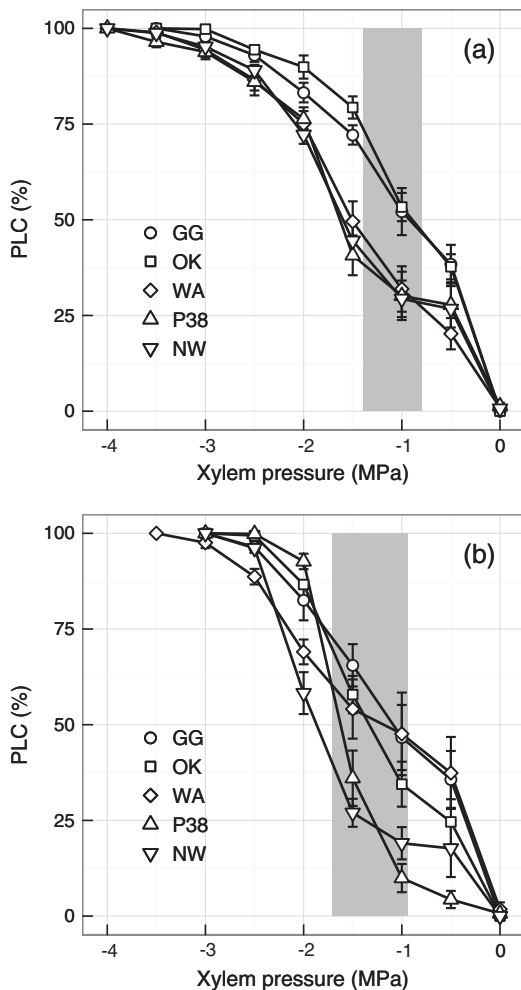


Figure 2. Percent loss hydraulic conductivity (PLC) for 2- to 3-year-old branches (a) and current-year long shoots (b). Error bars represent the standard error of the mean with $n = 12$ and $n = 6$ for (a) and (b), respectively. The grey rectangles show the range of calculated P_{50} values and serve as a visual aid to compare cavitation resistance in both groups. GG, Green Giant; OK, Okanese; WA, Walker; P38, P38P38; NW, Northwest.

Table 3. Welch's two sample t -tests between 2- to 3-year-old branches and current-year hybrid poplar shoots across clones for cavitation resistance at 50% loss of hydraulic conductivity (P_{50}), and the integrated xylem-specific and leaf-specific hydraulic conductivity over the measured pressure gradient (AUC_{K_S} , AUC_{K_L})

Branch type	P_{50}	AUC_{K_S}	AUC_{K_L}
2- to 3-year old branches	-1.15^A (0.06)	4.01^A (0.21)	6.38^A (0.40)
Current-year shoots	-1.29^A (0.09)	5.82^B (0.46)	11.82^B (0.98)

Different letters indicate significant differences ($\alpha = 0.05$). $n = 60$ (2- to 3-year-old branches) and 30 (current-year shoots).

Current-year long shoots had more efficient xylem in terms of xylem-specific and leaf-specific conductivity, K_S and K_L , respectively (Fig. 3).

We also calculated the area under the curve (AUC) for xylem-specific and leaf-specific hydraulic conductivity (K_S and K_L) as an alternative measure of water transport capacity over physiologically relevant xylem pressures. Figure 4 shows the xylem-specific hydraulic conductivity (K_S) of three species/genotypes. The hybrid poplar Okanese had the highest conductivity between 0 and -1 MPa. Aspen had the highest K_S at xylem pressures between -1 and -2.5 MPa, and *A. alnifolia* showed the highest conductivity at pressures more negative than -2.5 MPa. This ranking reflects the range of xylem pressures that these species/genotypes experience in the field. Because *A. alnifolia* xylem maintained hydraulic function over a wide range of xylem pressures, it exhibited the highest AUC_{K_S} . Within our data set, clones with more resistant xylem also tended to have higher AUC_{K_S} values than clones with more vulnerable xylem (Table 4; see also Figs S1 & S2 and Table S1 & S2).

Variation of key hydraulic traits among clones

All measured physiological traits showed significant variation among clones (Figs. S1 & S2, Table S1 & S2). A canonical discriminant analysis for 2- to 3-year old branches confirmed the observed groupings in Fig. 2(a) (Fig. 5(a)). The first two canonical dimensions (Can1 and Can2) explained 93% of the total variance in the data set. Green Giant and Okanese showed considerable overlap in their 95% confidence regions, while Walker, Northwest and P38P38 were more 'isolated' and spread out. The horizontal separation (Can1, 74.1% of total variance) was mainly driven by average A_L , D_V and P_{50} as well as AUC_{K_L} . A vertical separation between groups was much weaker and only visible for the Walker clone (Can2, 18.9% of total variance).

For current-year long shoots, the analysis showed considerable overlap of the 95% confidence regions among all clones with respect to the measured physiological traits (Fig. 5(b)). Only P38P38 did not overlap with any of the other clones. Current-year shoots of P38P38 showed the smallest D_V as well as the smallest total and average A_L of any clone (Figs S1 & S2, Table S1 & S2). This contributed to the horizontal

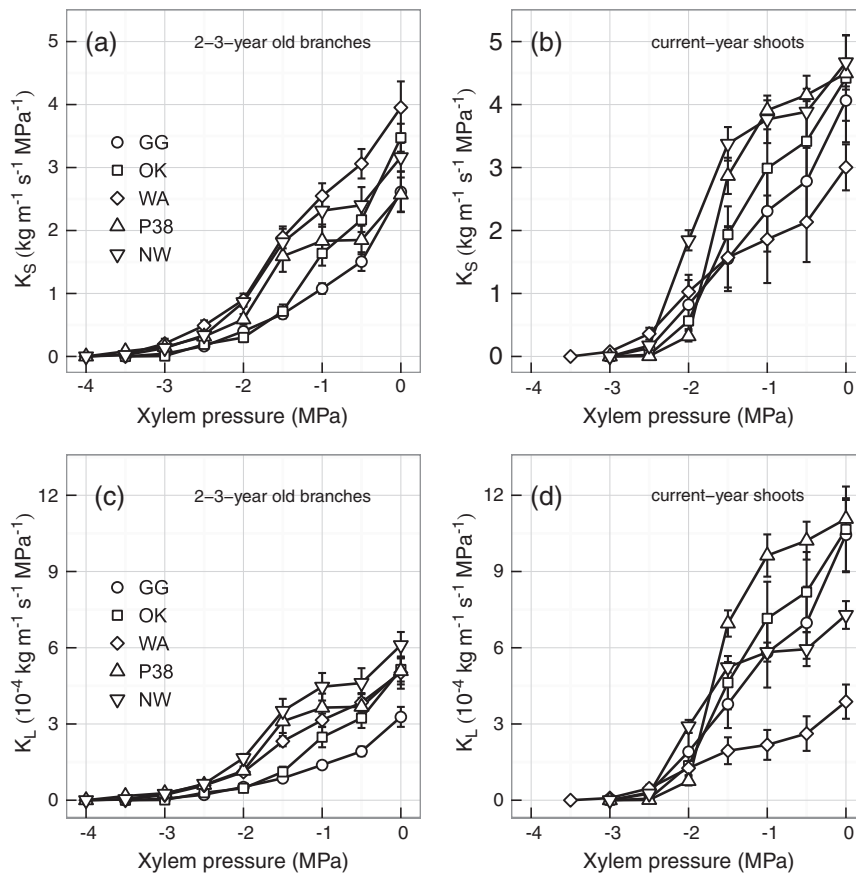


Figure 3. Xylem-specific hydraulic conductivity (K_S) and leaf-specific hydraulic conductivity (K_L) for 2- to 3-year-old branches (a, c) and current-year long shoots (b, d). Error bars represent the standard error of the mean with $n = 12$ and $n = 6$ for (a, c) and (b, d), respectively. GG, Green Giant; OK, Okanese; WA, Walker; P38, P38P38; NW, Northwest.

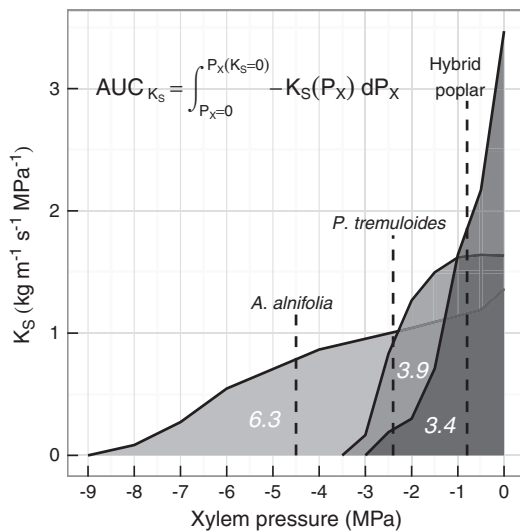


Figure 4. Illustration of the integrated xylem-specific conductivity (AUC_{K_S}) as a measure for the water transport capacity over the physiologically relevant range of xylem pressures. The area under the curve is given for *Amelanchier alnifolia* (Wheeler *et al.* 2005), *Populus tremuloides* (Schreiber *et al.* 2011) and the vulnerable hybrid poplar Okanese from the present study. Values for P_{50} are indicated by vertical bars, that is, -4.5 , -2.4 and -0.8 MPa, respectively.

differentiation of P38P38 in the discriminant analysis (Can1, 65.9% of total variance). The vertical separation between groups was mostly driven by C_L and $A_L : A_S$ (Can2, 26.5% of total variance).

Correlations and trade-offs between hydraulic traits

Average leaf area (A_L) was 3–4 times larger in current-year long shoots when compared with 2- to 3-year old branches (Supporting Information Fig. S3; see also Figs S1 & S2). At the same time, total A_L was slightly lower due to the high number of individual leaves (C_L) on 2- to 3-year old branches (Figs. S1 & S2), which also corresponded with a trend for lower leaf to xylem area ratios ($A_L : A_S$) in current-year shoots relative to 2- to 3-year-old branches. Hydraulic parameters such as vessel diameter (D_V), P_{50} , AUC_{K_S} and AUC_{K_L} were significantly correlated with A_L in both 2- to 3-year-old branches and current-year long shoots (Table 4). Average A_L emerged as a key trait, correlating strongly with both D_V and P_{50} in 2- to 3-year-old branches (Fig. 6).

DISCUSSION

The objectives of this study were (1) to assess whether hydraulic traits differ between 2- to 3-year-old branches and

Table 4. Correlation matrix among measured traits for 2- to 3-year-old branches (upper right half, $n = 60$) and current-year hybrid poplar shoots (lower left half, $n = 30$)

	D_V	P_{50}	AUC_{K_S}	AUC_{K_L}	$A_{L, tot}$	$A_{L, ave}$	C_L	$A_L:A_S$
D_V		0.42 (0.00)	-0.06 (0.62)	-0.18 (0.17)	0.40 (0.00)	0.35 (0.01)	-0.04 (0.75)	0.23 (0.08)
P_{50}	0.52 (0.00)		-0.52 (0.00)	-0.56 (0.00)	0.26 (0.05)	0.55 (0.00)	-0.28 (0.03)	0.22 (0.09)
AUC_{K_S}	-0.28 (0.14)	-0.84 (0.00)		0.81 (0.00)	-0.05 (0.68)	-0.39 (0.00)	0.34 (0.01)	0.00 (0.98)
AUC_{K_L}	0.02 (0.93)	-0.57 (0.00)	0.72 (0.00)		-0.44 (0.00)	-0.54 (0.00)	0.12 (0.37)	-0.54 (0.00)
$A_{L, total}$	0.64 (0.00)	0.58 (0.00)	-0.42 (0.02)	-0.48 (0.01)		0.42 (0.00)	0.43 (0.00)	0.79 (0.00)
$A_{L, average}$	0.69 (0.00)	0.51 (0.00)	-0.29 (0.12)	-0.37 (0.04)	0.85 (0.00)		-0.55 (0.00)	0.48 (0.00)
C_L	-0.15 (0.43)	0.10 (0.59)	-0.18 (0.34)	-0.14 (0.47)	0.21 (0.25)	-0.31 (0.10)		0.23 (0.08)
$A_L:A_S$	-0.43 (0.02)	-0.17 (0.36)	0.20 (0.30)	-0.48 (0.01)	0.14 (0.48)	0.05 (0.79)	0.15 (0.42)	

Values represent the Pearson correlation coefficient including P -values in parentheses rounded to two decimal places. Significant correlations are indicated in bold ($\alpha = 0.05$). Please refer to Table 2 for the full name of the measured traits.

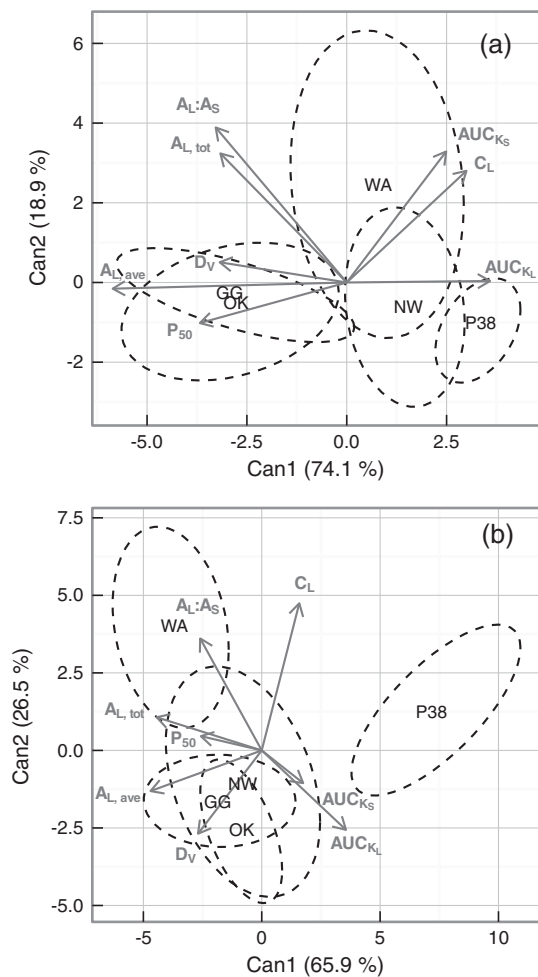


Figure 5. Canonical linear discriminant scores Can1 and Can2 for eight physiological parameters measured in 2- to 3-year-old branches (a) and current-year hybrid poplar shoots (b). Values in percent indicate the amount of total variance explained. Canonical scores for individual trees were removed from the figure to facilitate readability. Instead, dashed ellipses are shown representing the 95% confidence region for the group means (GG, Green Giant; OK, Okanese; WA, Walker; P38, P38P38; NW, Northwest). The vectors represent the correlations between the original variates and the canonical scores component loadings. The strength of the correlation is indicated by the vector length, and the direction indicates which clones have high values for the original variables. See Table 2 for the full name of the measured traits.

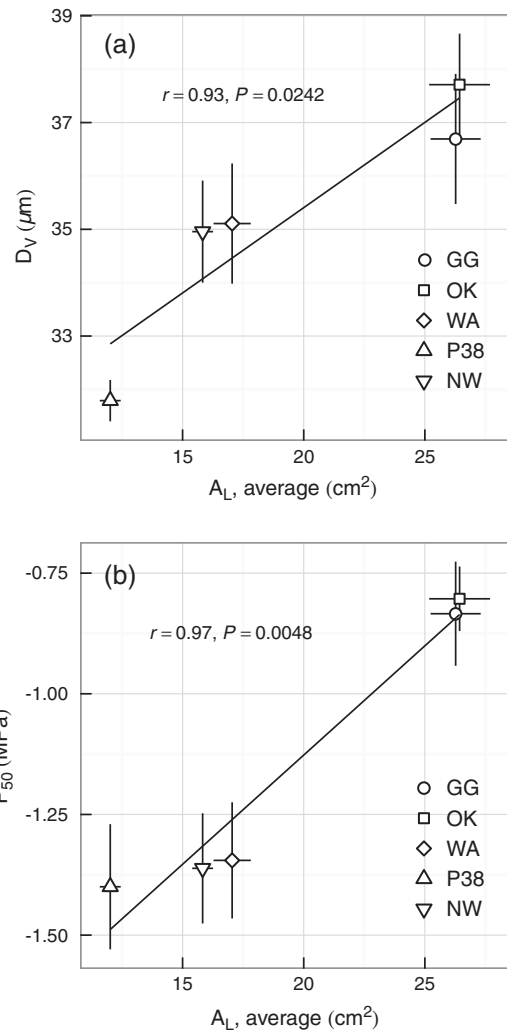


Figure 6. Correlation of average leaf area (A_L) of 2- to 3-year old branches with xylem vessel diameter (D_V) (a) and the pressure at which 50% hydraulic conductivity is lost (P_{50}) (b). Error bars represent the standard error of the mean with $n = 12$. GG, Green Giant; OK, Okanese; WA, Walker; P38, P38P38; NW, Northwest.

current-year long shoots, (2) to examine how much variation exists in key hydraulic traits among clones, and (3) to test whether correlations and trade-offs exist between these traits.

Our results showed that (1) hybrid poplars differed in key hydraulic parameters between branch type, that is, 2- to 3-year-old branches (lower to mid-crown) and current-year long shoots (mid to upper crown) (Figs. 2 & 3, Figs. S1 & S2), (2) variation of hydraulic traits among clones was relatively large for some clones and less for others (Fig. 5), and (3) from all measured hydraulic traits, D_V , P_{50} , the integrated xylem-specific and leaf-specific hydraulic conductivity (AUC_{K_S} and AUC_{K_L}) and A_L showed strong relationships to each other (Fig. 6, Table 4).

Variation in key hydraulic traits between 2- to 3-year-old branches and current-year long shoots

Our data suggest that vigorously growing current-year long shoots were hydraulically favoured compared with 2- to 3-year-old branches (Fig. 3). Current-year shoots maximize elongation to compete for light (Dickman *et al.* 2001). These shoots showed much higher leaf-specific conductivities than 2- to 3-year old branches, which implies that current-year long shoots experienced smaller pressure gradients to maintain a given transpiration rate (Tyree & Zimmermann 2002). The fact that fast-growing long shoots were hydraulically favoured is also in agreement with studies that showed higher photosynthetic capacities of leaves on current-year long shoots compared with leaves originating on short shoots (Isebrands & Nelson 1982; Nelson & Michael 1982). Leigh *et al.* (2011) studied long-shoot and short-shoot leaves in *Ginkgo biloba* and discussed the impact of hydraulic constraints during growth on leaf structure and physiology. Their work highlights the potentially close coordination of branch hydraulics and leaf development.

Some found that current-year xylem was more cavitation resistant than older xylem (Fukuda *et al.* 2015), presumably due to cavitation fatigue, which may be triggered by frost and/or drought stress (Hacke *et al.* 2001; Christensen-Dalsgaard & Tyree 2014). When comparing P_{50} values and PLC curves of the 2- to 3-year-old and current-year xylem, we did not find clear evidence for cavitation fatigue, that is, P_{50} was not significantly different between branch type (Fig. 2, Table 3). However, trees may simply have not experienced xylem pressures that would induce significant cavitation fatigue. Midday leaf water potentials on a sunny day in July 2013 were around -1.0 MPa at most of our sites (data not shown), and apparently, this was not negative enough to induce a significant shift in xylem vulnerability. Furthermore, water potentials of transpiring leaves tend to be considerably more negative than the stem to which they are attached because of the drop in potential across the hydraulic resistance of the leaf. This would make the stem water potential less negative than -1 MPa, reducing the risk of stem cavitation even more. Generally, all our studied trees were well-established, that is, they probably had an extensive root system that provided sufficient access to soil water resulting in limited water stress even during periods of high transpirational demand. It is possible, however, that drier conditions and soil water depletion would cause more negative water potentials, which in turn may induce cavitation fatigue.

Regarding frost-induced cavitation fatigue, we do not know why frost events did not result in more vulnerable 2- to 3-year-old xylem. The lack of frost-induced cavitation fatigue agrees with previous work on *Populus balsamifera* and several coniferous species collected in central Alberta. In these species, vulnerability curves were measured at different times of the year (spring, summer, fall and winter), and no significant shifts in cavitation resistance were found (L. Plavcova and A. Schoonmaker, unpublished manuscript).

Variation in key hydraulic traits among clones

Depending on the branch type, we found different clonal groupings with respect to the measured traits (Fig. 5). Green Giant and Okanese clustered together in both branch types, perhaps in part due to their similar parentage background (Table 1). By contrast, Walker, while sharing the same parent species as Green Giant and Okanese, was fairly variable in its hydraulic traits, sometimes even grouping opposite to Green Giant and Okanese (Fig. 5(a)). The lack of clonal differentiation among current-year long shoots (Fig. 5(b)) may indicate that all studied clones allocated their 'best xylem' to vigorously growing current-year shoots to ensure height growth and competition for light.

Correlations and trade-offs among hydraulic traits

Some of the strongest correlations we observed in this study were related to leaf size. This trait was correlated with P_{50} , D_V , AUC_{K_S} and AUC_{K_L} (Fig. 6, Table 4). But why were larger leaves and total leaf area correlated with wider vessels and more vulnerable xylem? We may hypothesize that fast and sustained leaf expansion in spring is facilitated by the presence of wide earlywood vessels. At the same time, wide vessels are often more vulnerable to air seeding than narrower conduits (e.g. Hacke *et al.* 2006). Although this trend is not always observed across species (Tyree *et al.* 1994), we suggest that the observed correlation between xylem vulnerability and leaf size may be a consequence of a more fundamental relationship between vessel diameter and leaf size, particularly in vigorously growing current-year long shoots (Table 4). Alternatively, the correlations shown in Fig. 6 may be a consequence of differences in water potential at the time when leaves and earlywood vessels were formed. Differences in water potential may result from different rooting patterns (depth, amount of fine roots, root biomass). Both of these hypotheses could be tested.

Our data are consistent with studies conducted on tropical trees, in which similar relationships with cavitation resistance and leaf size were found (Markestijn *et al.* 2011; Kröber *et al.* 2014).

On a cautionary note, we should point out that our measurements were focused at the branch level. Branches only represent one part of the soil-plant-atmosphere continuum. In order to gain a more complete understanding, measurements at the root and leaf level are also needed. While additional work on hydraulic and morphological traits in leaves is currently underway for these clones (Brocius and Hacke, unpubl.

manuscript), measurements at the root level would also be required to better understand the water-use strategy of individual clones. The observed rapid loss of hydraulic conductivity with decreasing xylem pressure in hybrid poplar clones (Fig. 4) suggests that these trees rely on an extensive root system with permanent access to stable sources of soil water.

In conclusion, we found a relationship between leaf size on the one hand and vessel diameter and vulnerability to cavitation on the other hand. Leaf size/area may therefore represent an additional and practical trait to add to existing key hydraulic traits when assessing productivity in forest management and tree improvement programmes. However, studies comparing leaf size and vulnerability to cavitation in stems are scarce. More work is required to evaluate the causal relationships (if any) between these parameters. Such work may inform us if and under what circumstances leaf size can serve as a proxy for xylem vulnerability to cavitation. Lastly, we hope that the integrated measure of xylem-specific and leaf-specific conductivity (AUC_{K_s}, AUC_{K_l}) presented in this study may also be reported in future studies to test its suitability for the hydraulic assessment of trees.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site.

Figure S1. Boxplots of physiological traits for 2-3 year-old hybrid poplar branches.

Figure S2. Boxplots of physiological traits for current-year hybrid poplar shoots.

Figure S3. Leaves of the clone Green Giant.

Table S1. Clone means of eight measured or calculated physiological traits for 2-3-year-old hybrid poplar branches (n= 12).

Table S2. Clone means of eight measured or calculated physiological traits for current-year hybrid poplar shoots (n= 6).