Genetic variation of hydraulic and wood anatomical traits in hybrid poplar and trembling aspen

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Summary

- Intensive forestry systems and breeding programs often include either native aspen or hybrid poplar clones, and performance and trait evaluations are mostly made within these two groups. Here, we assessed how traits with potential adaptive value varied within and across these two plant groups.
- Variation in nine hydraulic and wood anatomical traits as well as growth were measured in selected aspen and hybrid poplar genotypes grown at a boreal planting site in Alberta, Canada. Variability in these traits was statistically evaluated based on a blocked experimental design.
- We found that genotypes of trembling aspen were more resistant to cavitation, exhibited more negative water potentials, and were more water-use-efficient than hybrid poplars. Under the boreal field test conditions, which included major regional droughts, height growth was negatively correlated with branch vessel diameter (D_v) in both aspen and hybrid poplars and differences in D_v were highly conserved in aspen trees from different provenances.
- Differences between the hybrid poplars and aspen provenances suggest that these two groups employ different water-use strategies. The data also suggest that vessel diameter may be a key trait in evaluating growth performance in a boreal environment.

Introduction

Trembling aspen (*Populus tremuloides* Michx.) and other poplars (e.g. *Populus balsamifera* L.; *Populus deltoides* Bartr. ex Marsh.; *Populus trichocarpa* Torr. & A. Gray) play an important role in North American ecosystems, particularly in the boreal forest and the aspen parklands of the prairie provinces (Alberta, Saskatchewan, Manitoba) in western Canada (Richardson *et al.*, 2007). Poplars (*Populus* ssp.) are among the fastest growing temperate trees and are considered to be vegetational pioneers (Eckenwalder, 1996; Bradshaw *et al.*, 2000). Poplars also represent an attractive and valuable forest resource as they grow quickly and are easy to propagate from both seed and vegetative propagation (Peterson & Peterson, 1992; Cooke & Rood, 2007). For instance, tree breeders in western Canada carry out intensive selection and breeding programs for poplars, searching for trees that produce high-quality wood for pulp and for oriented strand board production, but are also able to withstand the dry cold climate of the Canadian prairies. Tree improvement programs often include either native aspen or nonnative hybrid poplar clones in their breeding programs, and performance and trait evaluations are mostly made within these two groups, as reflected by a large number of studies conducted on either aspen or hybrid poplars. However, a comprehensive comparison between these two groups is still lacking (Lieffers *et al.*, 2001), even though it may become very valuable information for species selection in the context of climate change.

When selecting suitable genotypes for a particular location, the 'local is best' concept is normally applied, where nearby seed sources are selected for reforestation. Using locally adapted planting material reflects physiological adaptations of numerous tree generations to the local climate and site conditions. However, an accelerated trend in global warming (Houghton, 2005) may require a human-based relocation of certain genotypes from their southern distribution limits up to places where natural

migration through seed dispersal would not be sufficient, given the magnitude of current and predicted climate change (Aitken *et al.*, 2008). In addition, hybrids among North American and Eurasian species of poplar are widely used for their superior growth characteristics. In both cases, physiological and field testing are required before large-scale deployment of this often nonlocal or novel plant material. These tests are typically common garden experiments that can differentiate environmental and genetic differences among genotypes in a shared environment (Gornall & Guy, 2007).

In central Alberta, it may be particularly beneficial to facilitate the introduction of aspen genotypes from more southern latitudes, as climate warming and decreases in precipitation for this region over the last 25 yr have been very pronounced. The province of Alberta, for instance, has experienced warming of *c.* 0.7°C and a reduction of mean annual precipitation of 20% over this period (Mbogga *et al.*, 2009). In 2002, a severe regional drought led to massive aspen dieback and mortality in the aspen parklands of southern Alberta (Hogg *et al.*, 2008). Historically, droughts have always been part of the climate in the Canadian prairies (Roberts *et al.*, 2006; Bonsal & Regier, 2007). However, more frequent and more severe droughts have been recorded in the recent past (including another exceptional drought in 2009), and this poses a serious threat for local vegetation.

Since most poplar species are known to be sensitive to water deprivation (Blake *et al.*, 1996; Shock *et al.*, 2002), the question of how aspen and hybrid poplars will respond to drier conditions is becoming an important issue. Although poplar species are among the most susceptible trees to drought, considerable genotypic variability exists in water-use efficiency, growth performance, hydraulic traits, and tolerance to moderate water deficits, particularly in hybrid poplar clones (Morrison *et al.*, 2000; Monclus *et al.*, 2006; DesRochers *et al.*, 2007; Silim *et al.*, 2009; Fichot *et al.*, 2010). Even greater differences are likely to exist between hybrid poplars and aspen as a group, but a comprehensive comparison of hydraulic traits between these two groups has, to our knowledge, not been conducted.

Xylem traits, along with root and soil properties, can play an important role in limiting canopy water supply (Sperry *et al.*, 2002; McDowell *et al.*, 2008). Xylem properties may be especially important in riparian cottonwoods (Rood *et al.*, 2000) and hybrid poplars, which are known to be highly vulnerable to cavitation (Fichot *et al.*, 2010). As a result of cavitation and subsequent embolism, hydraulic conductivity in the xylem (K_h) declines as the xylem pressure becomes more negative. This dependence of K_h on xylem pressure is often referred to as a vulnerability curve (Sperry *et al.*, 2002). Comparisons of more or less distantly related taxa have shown that, at the interspecific level, cavitation resistance is often correlated with the water potential range that plants experience in their natural habitat (Hacke *et al.*, 2000; Pockman & Sperry, 2000). Interspecific comparisons have also linked differences in cavitation resistance with trends in xylem structure and transport efficiency (Maherali *et al.*, 2004; Hacke *et al.*, 2006; Jacobsen *et al.*, 2007; Jansen *et al.*, 2009). However, such correlations may not be found when comparing closely related genotypes (Cochard *et al.*, 2007) or populations of a single species (Martinez-Vilalta *et al.*, 2009). For instance, a tradeoff between xylem safety and xylem transport efficiency was absent across eight hybrid poplar genotypes (Fichot *et al.*, 2010), although it was found in a survey of 29 angiosperm species of diverse growth form and family affinity (Hacke *et al.*, 2006).

In the present study, we measured genetic differences in hydraulic and wood anatomical traits of six aspen genotypes and seven hybrid poplar clones growing at a boreal planting site in Alberta, Canada. Aspen genotypes represented three provenances (Alberta, British Columbia, and Minnesota; Table 1). We assessed how traits varied within and across these two plant groups. We asked whether relationships between hydraulic traits seen in broad interspecific surveys would also be resolvable at a finer phylogenetic scale, that is, across the studied genotypes of the genus *Populus*. We also evaluated the potential of linking differences in xylem traits with growth performance. Growth was measured as height and diameter at breast height (DBH), integrated over 16 and 11 yr in hybrid poplar and aspen trial data, respectively.

Table 1 Geographic origin of aspen seed sources and height and diameter at breast height (DBH) measured after 11 growing seasons in the field in a provenance field trial in central Alberta, Canada

Region	Provenance #	Latitude	Longitude	Elevation (m)	Height11 (m)	DBH11 (cm)
British Columbia	9	58°12′N	123°20′W	1177	5.6 (0.2)	7.0 (0.5)
British Columbia	10	58°36′N	122°20′W	335	6.0 (0.5)	8.0 (0.5)
Alberta	25	55°36′N	113°25′W	762	8.8 (0.3)	9.5 (0.6)
Alberta	26	54°56′N	112°44′W	545	7.7 (0.3)	8.8 (0.5)
Minnesota	39	47°12′N	93°48′W	405	11.3 (0.2)	13.5 (0.6)
Minnesota	41	47°30′N	93°36′W	433	11.0 (0.2)	13.9 (0.5)

Standard error of the mean is given in brackets.
DBH11, aspen diameter at breast height after 11 growing seasons; height11, aspen height after 11 growing seasons.

A long-term goal is to identify easily accessible traits that can serve as predictors of growth performance under field conditions in this boreal environment. Finally, we assessed which of the measured traits in aspen were conserved by geographic source (provenance) and which varied independently. The plantations were designed as long-term field experiments and represent a good opportunity to investigate the previously outlined issues in a common garden setting.

Materials and Methods

Plant material

The hybrid poplar and aspen plant material used in this study came from field trials located at the Alberta-Pacific Forest Industries Inc. (Al-Pac) pulp mill site near Boyle (54°49′N, 113°31′W), Alberta, Canada. The clonal hybrid poplar trial was established in 1993, whereas the aspen trial is part of a common garden experiment with open pollinated single tree seed sources from Minnesota, Alberta, and British Columbia, planted in 1998. Both trials were planted in a randomized complete block design with five (hybrid poplar trial) and six (aspen provenance trial) replications per clone or seed source in five-tree row plots. The aspen trial is also surrounded by two rows of border trees to minimize error caused by environmental effects. For this study we sampled eight trees (unless noted otherwise) from each clone and provenance. The same trees were used for all analyses, including growth measurements. The common garden trials contain a large amount of plant material, and we selected a representative sample of genotypes with contrasting performance for this study (Tables 1, 2). Growth performance was evaluated by tree height and DBH, measured 16 and 11 yr after trials were established for the hybrid poplars and aspen, respectively. Since height and DBH were closely correlated, correlations seen with height could also be seen for DBH and vice versa. In addition to high, average, and poorly performing hybrid poplars, we added the Walker clone as a reference because it is well

tested and widely used in shelterbelts and plantations in western Canada (Morrison et al., 2000; Silim et al., 2009) (Table 2).

A total of 104 samples were collected over a period of 7 wk in June and July. The sampling was carried out once a week and the material was processed within the next 4 d. In order to minimize time effects, hybrid poplar and aspen provenances were sampled so that differences caused by different sampling times were superimposed on spatial blocks of the experimental design. This undesired potential source of error could therefore be accounted for in the analysis as a block effect. In order to minimize destructive sampling, and for practical reasons, all hydraulic and wood anatomical measurements (Table 3) were conducted on branch segments. Samples were from 2- to 3-yr-old branches, which were taken from sun-exposed areas within the canopy using a telescope pruner. The material was packed in plastic bags with moist tissues and stored at 4°C in a walk-in refrigerator. The leaves from each branch segment and all remaining leaves distal to the segment were collected and stored in separate bags to determine leaf area and carbon isotope composition.

Leaf related measurements and growth

Leaf carbon isotope composition ($\delta^{13}C$) was used as an integrated measure for stomatal control and water-use efficiency (Farquhar et al., 1989). The analysis was conducted by the Stable Isotope Laboratory in the Department of Renewable Resources at the University of Alberta, Canada. The collected leaves were dried in an oven at 80°C for a minimum of 48 h and were ground with a ball grinder until a fine powder was yielded. Leaf water potentials (ψ-leaf) were measured at midday on a cloudless hot summer's day (21 August 2009; maximum daily temperature, 27°C) on a subset of three trees per hybrid poplar clone and aspen provenance. The measurements were carried out using a pressure chamber (Model 1000; PMS Instrument Company, Albany, OR, USA). Transpiring leaves were cut,

Table 2 Parentage information of clones selected for high, average and poor performance with respect to height and diameter at breast height (DBH) measured after 16 growing seasons in a clonal field trial in central Alberta, Canada

Performance group	Code/clone name	Parentage (P. = Populus)	Height16 (m)	DBH16 (cm)
High	P38P38	P. balsamifera × P. simonii	13.9 (0.5)	16.7 (1.3)
High	Brooks#1/Griffin	P. deltoides × P. × petrowskyana*	14.4 (0.4)	20.4 (1.7)
Average	4435	P. balsamifera × P. × euramericana	11.6 (0.7)	9.9 (1.6)
Average	TACN 1/Berlin	P. laurifolia × P. nigra	13.0 (0.1)	15.8 (1.0)
Poor	DTAC 22	P. deltoides × P. trichocarpa	7.1 (0.5)	5.4 (0.3)
Poor	DTAC 24	P. deltoides × P. trichocarpa	7.9 (0.6)	7.3 (0.9)
Reference	FNS 44-52/Walker	P. deltoides × P. × petrowskyana	14.7 (0.5)	15.4 (1.1)

DBH16, hybrid poplar diameter at breast height after 16 growing seasons; Height16, hybrid poplar height after 16 growing seasons.
*P. × petrowskyana: P. laurifolia × P. nigra.
The clone 'Walker' was included as a reference because it is widely known and used in Alberta and Saskatchewan for shelterbelt plantations.
Standard error of the mean is given in brackets.

Table 3 List of all physiological traits measured in this study with symbols and units

Symbol	Definition	Units
PLC_N	Percentage loss hydraulic conductivity/native embolism	%
P_{50}	Pressure causing 50% loss of hydraulic conductivity	MPa
P_e	Air entry pressure	MPa
ψ-leaf	Leaf water potential	MPa
D_V	Vessel diameter	µm
K_S	Xylem-specific hydraulic conductivity	kg m^{-1} MPa^{-1} s^{-1}
K_L	Leaf-specific hydraulic conductivity	10^{-4} kg m^{-1} MPa^{-1} s^{-1}
$A_L : A_S$	Leaf-to-sapwood area ratio	m^2 cm^{-2}
$\delta^{13}C$	Leaf carbon isotope composition	‰
d_W	Wood density	g cm^{-3}
Height16	Hybrid poplar height after 16 growing seasons	m
DBH16	Hybrid poplar diameter at breast height after 16 growing seasons	cm
Height11	Aspen height after 11 growing seasons	m
DBH11	Aspen diameter at breast height after 11 growing seasons	cm
MAT	Mean annual temperature	°C
MGP	Mean growing season precipitation (May–September)	mm
MAP	Mean annual precipitation	mm

bagged, and ψ-leaf was immediately measured in the field. Tree height and DBH were measured in the autumn when all leaves were shed. Height was measured with a laser hypsometer and DBH was measured using a digital caliper.

Hydraulic measurements

Branches were harvested in the field in lengths of at least 1 m and brought to the laboratory in plastic bags. Segments were cut from the center of these branches under water to avoid blocking additional vessels with air and to avoid including vessels that were embolized during harvesting. Hydraulic conductivity (K_h) was measured on 14.2-cm-long branch segments using a tubing apparatus (Sperry *et al.*, 1988) and a methodology described in detail in Hacke & Jansen (2009). Silicone injections (Hacke *et al.*, 2006) on branches of four of the hybrid poplar clones showed that < 1% of vessels were open in the 14.2-cm-long segments. Hydraulic conductivity was calculated as the quotient of flow rate through the segment and pressure gradient. The tubing apparatus consisted of an elevated water reservoir connected to an electronic balance (CP225D; Sartorius, Göttingen, Germany) via Tygon tubing. The balance was interfaced with a computer using Collect 6 software (Labtronics, Guelph, ON, Canada) and logged K_h every 10 s. Each branch segment was inserted in the tubing system and its native conductivity was measured. Subsequently, segments were flushed to remove native embolism and to obtain the maximum conductivity for a given segment. All segments were spun in a centrifuge to increasingly negative xylem pressure, and K_h was re-measured on the conductivity apparatus after spinning (Li *et al.*, 2008). The percentage loss in conductivity from the original value was plotted against the negative pressure, and curves were fitted with a Weibull function. The xylem pressure corresponding to 50% loss of K_h (P_{50}) was calculated for each

segment based on the Weibull fit. Values of P_{50} were then averaged for each genotype.

The threshold xylem pressure at which loss of conductivity begins to increase rapidly was determined according to the method of Domec & Gartner (2001). This air entry pressure (P_e) is less frequently reported than the P_{50}, but it is a useful parameter when linking vulnerability curves with stomatal control of xylem pressure (Sparks & Black, 1999; Meinzer *et al.*, 2009). In the present study, P_e was compared with ψ-leaf. The difference between these two parameters was used to assess the degree of safety against the onset of cavitation.

Specific conductivity (K_S) was measured by dividing the maximum K_h of a stem segment by its cross-sectional sapwood area. The sapwood area was measured with a stereomicroscope (MS5; Leica, Wetzlar, Germany). Specific conductivity is a measure of the transport efficiency of the xylem. Leaf specific conductivity (K_L) was calculated by dividing the maximum K_h of a stem segment by the leaf area distal to the base of the segment; that is, leaves attached to the segment were included in the measurements. K_L is a measure of the hydraulic sufficiency of the segment to supply water to leaves (Tyree & Zimmermann, 2002). Leaf area was measured with a LI-3100 area meter (Li-Cor, Lincoln, NE, USA).

Xylem anatomy

All xylem anatomical measurements were carried out on the same branch segments used for measuring hydraulic conductivity and cavitation resistance. Vessel diameters were measured on cross-sections of 30–35 µm thickness. Sections were prepared with a microtome (Leica SM2400) and analyzed with a Leica DM3000 microscope at ×200 magnification. Images of each cross-section were captured

with a Leica DFC420C camera and analyzed using image analysis software (Image-Pro Plus 6.1; Media Cybernetics, Silver Spring, MD, USA). Vessel diameters were measured in three radial sectors representing the two outermost growth rings. 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 $D_v = ((\Sigma \, d^4)/n)^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter. Wood density was measured following the methods of Hacke *et al.* (2000) and Pratt *et al.* (2007). Segments were cut into 3-cm pieces and split in half. Bark and pith were removed. Xylem density was measured by water displacement on an analytical balance (CP224S; Sartorius). Samples were dried in an oven at 70°C for at least 48 h and density was measured as dry mass (g)/fresh volume (cm³).

Statistical analyses

Aspen and hybrid poplar plantations were nearby separate trials established at different times. Since they were not part of the same randomized experimental design, we did not apply a formal statistical evaluation of differences between aspen and hybrid poplars. Instead, we present box plots to illustrate the differences between these two groups (Fig. 1).

For statistical analyses of intragroup differences between physiological and wood anatomical traits, we calculated means of row plots summarized at the clone and provenance levels, taking advantage of the blocked experimental design (Tables 4, Supporting Information, Tables S1, S2). Analysis of variance was carried out with PROC MIXED of the SAS statistical software package (SAS Institute, 2008),

where block and genotype within groups were specified as random factors.

Results

Physiological differences between hybrid poplars and aspen provenances

Many of the measured hydraulic and wood anatomical traits differed between the hybrid poplars and aspen provenances (Fig. 1). In particular, traits such as P_{50}, ψ-leaf, leaf-to-sapwood area ratio ($A_L : A_S$), K_L, and $\delta^{13}C$ differed considerably. Compared with aspen, hybrid poplars were more vulnerable to cavitation and correspondingly exhibited higher (less negative) leaf water potentials (Fig. 1). Branches of hybrid poplars tended to show higher K_L values than aspen branches. This was mainly a result of lower $A_L : A_S$ ratios of hybrid poplars, as xylem-specific conductivities were similar in both plant groups. Native embolism varied between 36.7 and 58.7% and did not differ between plant groups. Wood densities were similar, but showed greater variation within hybrid poplars than within aspen provenances.

Xylem cavitation resistance, leaf water potentials and safety margins

Vulnerability curves for hybrid poplars and aspen provenances were similar in shape, but aspen curves were shifted toward more negative xylem pressure, that is, greater resistance to cavitation (Fig. 2). Most hybrid poplars and all aspen provenances exhibited relatively steep sigmoidal curves with a well-defined cavitation threshold. The P_{50} varied from −1.51 to −1.97 MPa in hybrid poplars, and
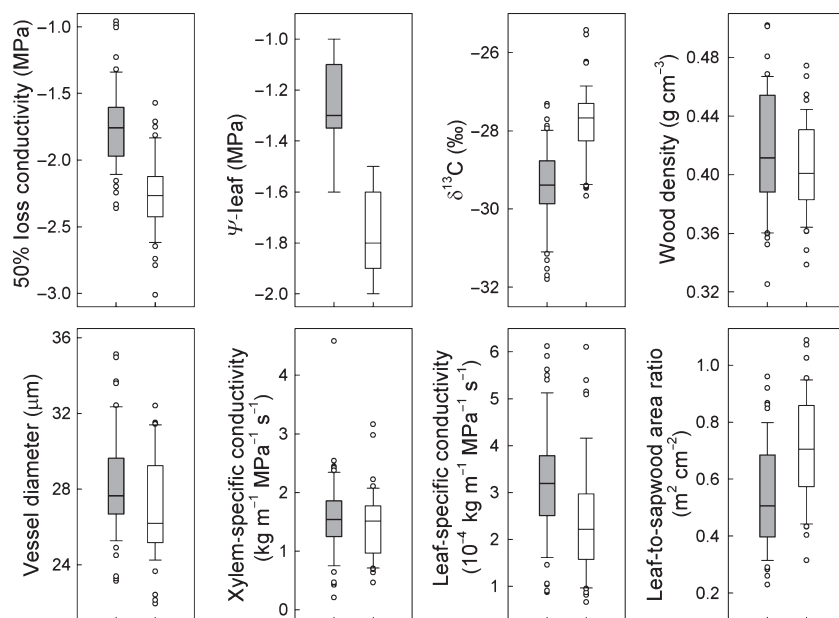

Fig. 1 Box plots of hydraulic and wood anatomical properties contrasting poplar clones (gray) with aspen provenances (white). The central box in each box plot represents the 25th and 75th percentiles with the median (50th percentile). Whiskers indicate the 10th and 90th percentiles. Every outlier is shown as a circle.

Table 4 Group means of physiological parameters and growth traits for hybrid poplar and regional means for aspen provenances

Physiological parameter	Hybrid poplar				Aspen		
	High	Average	Poor	Walker	MN	AB	BC
Native embolism (%)	40.3 A (5.5)	40.5 A (5.4)	54.6 A (3.3)	36.7 (6.6) A	41.0 A (3.7)	58.7 A (4.9)	48.3 A (5.6)
50% loss conductivity (MPa)	−1.81 A (0.07)	−1.68 A (0.07)	−1.64 A (0.08)	−1.98 A (0.09)	−2.37 A (0.07)	−2.15 A (0.07)	−2.28 A (0.07)
ψ-leaf (MPa)	−1.18 AB (0.05)	−1.51 B (0.05)	−1.12 A (0.05)	−1.24 AB (0.07)	−1.68 A (0.05)	−1.90 A (0.05)	−1.72 A (0.06)
Vessel diameter (μm)	27.15 B (0.42)	28.50 AB (0.41)	30.66 A (0.72)	25.41 B (0.57)	24.82 B (0.41)	27.01 AB (0.50)	29.37 A (0.57)
Xylem specific conductivity	1.73 A (0.12)	1.37 A (0.16)	1.77 A (0.23)	1.32 A (0.10)	1.10 B (0.09)	1.66 A (0.15)	1.60 A (0.13)
Leaf specific conductivity ($\times 10^{-4}$)	3.79 A (0.30)	2.67 A (0.31)	3.09 A (0.27)	3.25 A (0.35)	1.72 A (0.18)	2.33 A (0.29)	3.16 B (0.34)
Leaf area : xylem area	0.52 A (0.05)	0.54 A (0.04)	0.61 A (0.05)	0.42 A (0.03)	0.74 A (0.04)	0.80 A (0.04)	0.59 A (0.05)
δ^{13}C (‰)	−29.6 A (0.4)	−29.8 A (0.1)	−28.7 A (0.2)	−30.0 A (0.3)	−27.5 A (0.3)	−27.8 A (0.2)	−28.0 A (0.3)
Wood density (g cm^{-3})	0.39 A (0.00)	0.40 A (0.01)	0.46 A (0.01)	0.42 A (0.01)	0.41 A (0.01)	0.40 A (0.01)	0.39 A (0.01)
16 and 11 yr height (m)	14.4 A (0.3)	12.8 A (0.5)	7.6 B (0.4)	14.5 A (0.5)	11.2 A (0.1)	8.2 B (0.3)	5.8 C (0.3)
16 and 11 yr DBH (cm)	18.8 B (1.2)	14.0 AB (1.5)	6.5 B (0.6)	15.3 AB (1.1)	13.7 A (0.4)	9.2 B (0.4)	7.5 B (0.4)

DBH, diameter at breast height; ψ-leaf, leaf water potential; MN, Minnesota; AB, Alberta; BC, British Columbia.
Significant differences among performance groups or regions are indicated by different letters (α = 0.05). Standard error of the mean is given in brackets. We did not test for significant differences between aspen and hybrid poplar because traits were confounded by test site and age of trees.
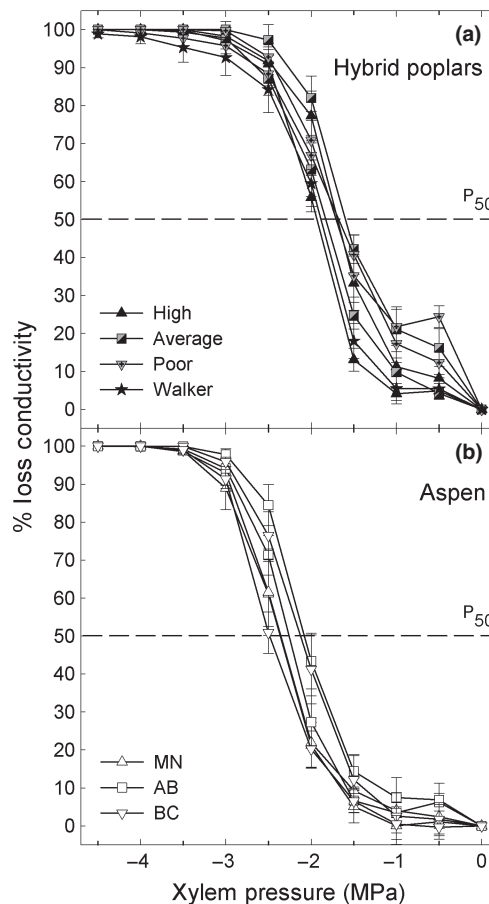

Fig. 2 Vulnerability curves of all hybrid poplar clones (a) and aspen provenances (b). The dashed lines indicate 50% loss of hydraulic conductivity. Closed symbols, hybrid poplar clones; open symbols, aspen provenances. Error bars represent the standard error of the mean. MN, Minnesota; AB, Alberta; BC, British Columbia.

from −2.05 to −2.44 MPa in aspen. Hence, there was no overlap in P_{50} between the two plant groups. The P_e varied between −0.72 and −1.44 MPa in hybrid poplars, compared with a range between −1.41 and −1.91 MPa in aspen. No clear relationship between cavitation resistance and growth performance was apparent in either plant group. No significant differences in P_{50} existed within hybrid poplars and aspen (Table 4). Variation in P_{50} was also not correlated with differences in D_v or d_w.

Leaf water potential varied from −1.07 to −1.47 MPa in hybrid poplars and from −1.57 to −1.93 MPa in aspen (Fig. 3). Safety margins can be implied by the difference between P_e and ψ-leaf. A genotype with a safety margin of zero would plot on the 1 : 1 line in Fig. 3(a). Higher and lower safety margins would plot below and above the diagonal, respectively. Although no correlation existed within hybrid poplars and aspen provenances, there was a significant ($P < 0.02$) correlation across all data points. The slope of the regression line did not differ from the 1 : 1 line, indicating that there was a general agreement between leaf water
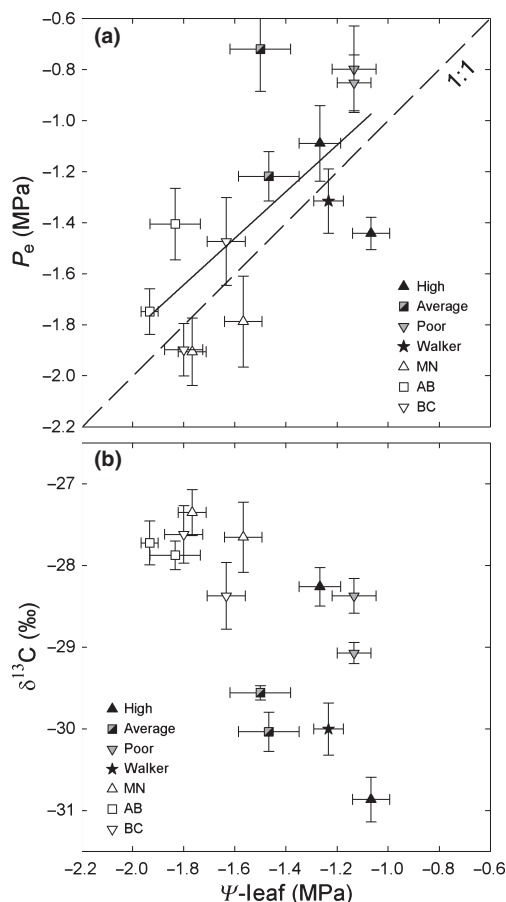
Fig. 3 Relationship between leaf water potential (ψ-leaf) and the air entry pressure (P_e) at which loss of hydraulic conductivity begins to increase rapidly (a) as well as leaf carbon isotope composition ($\delta^{13}C$; b). Closed symbols, hybrid poplar clones; open symbols, aspen provenances. The dashed line in (a) represents the 1 : 1 line separating the plot in lower and upper areas, indicating larger and smaller safety margins, respectively. Error bars represent the standard error of the mean. MN, Minnesota; AB, Alberta; BC, British Columbia.
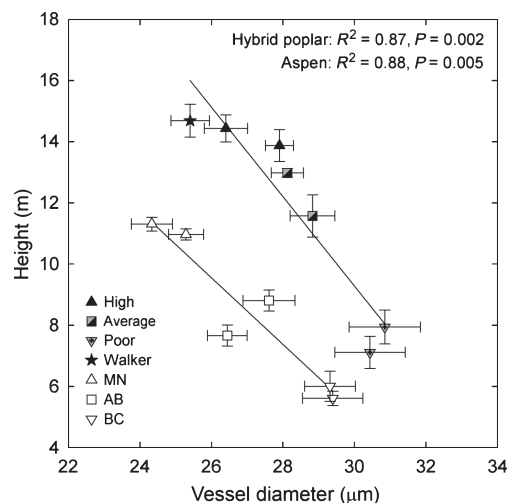

Fig. 4 Correlation between tree height and vessel diameter. Closed symbols represent hybrid poplar clones; open symbols represent aspen provenances. Error bars are representing the standard error of the mean. MN, Minnesota; AB, Alberta; BC, British Columbia.

potentials and cavitation threshold. Safety margins ranged from −0.78 to 0.38 MPa and did not differ between aspen and hybrid poplars (t-test, $P = 0.43$). It should be noted that in transpiring plants, ψ-leaf is more negative than the xylem pressure. Therefore, the actual safety margins will be larger than our estimates that were based on ψ-leaf values.

Lower leaf water potentials in aspen trees corresponded with less negative $\delta^{13}C$ values than in hybrid poplars (Fig. 3b), suggesting aspen trees were more water-use-efficient. Variation in $\delta^{13}C$ was larger in hybrid poplars than in aspen provenances, and was not related to performance within groups (Table S1) or provenances (Table S2).

Height growth and links with other parameters

Of all parameters measured, only D_v showed strong correlations with height (and DBH) in both aspen and hybrid

poplars (Fig. 4). Surprisingly, greater height growth corresponded with narrower vessel diameters. Tree height varied between 5.6 and 11.3 m in the aspen provenances and between 7.1 and 14.7 m in the hybrid poplars. In other words, the best performers in each group were about twice as high as the slowest-growing genotypes. The absolute height values cannot be compared between the aspen and hybrid poplars since they were confounded by the micro-environment at the test site and by the age of the trees. Nevertheless, the fastest- and slowest-growing aspen genotypes had comparable growth rates to the fastest- and slowest-growing hybrid poplar clones with an approximate adjustment for age. Within the aspen as much as 87.4 % of the variance in height (and 82.4 % of the variance in DBH) could be explained by region (Table S2).

Like height, vessel diameters exhibited large variation within each plant group. Within hybrid poplars 50.4% of the variance in D_v could be explained by performance groups (Table S1), and the means between performance groups showed significantly smaller vessel diameters of Walker vs poor, and high vs poor performers (Table 4). Similarly, within the aspen, 55.5 % of the variance in D_v could be explained by region (Table S2), and the means showed significantly smaller vessel diameters for Minnesota vs British Columbia sources (Table 4).

Discussion

Differences in cavitation resistance between plant groups

Our results show that hybrid poplars and aspen differed greatly in some key hydraulic parameters, including cavitation

resistance and leaf water potentials. Hybrid poplars were more vulnerable to cavitation than aspen, and, correspondingly, maintained less negative leaf water potentials. The fact that most data points fell on or near the 1 : 1 line of the P_e vs ψ-leaf relationship (Fig. 3a) indicates that predicted safety margins from hydraulic failure were similar in both plant groups. The data shown in Fig. 3(a) also suggests that leaf water potentials were constrained by the cavitation threshold. This was an expected finding given the fact that all vulnerability curves showed a steep slope after the onset of cavitation (Fig. 2; see also Fichot et al., 2010).

The fact that hybrid poplars were found to be highly vulnerable to cavitation agrees with previous work on Populus species (Blake et al., 1996; Hacke & Sauter, 1996; Pockman & Sperry, 2000; Rood et al., 2000). Many of the hybrid poplars analyzed in this study were derived from cottonwoods (sensu Rood et al., 2003, 2007). Riparian cottonwoods are dependent on shallow groundwater, which is often linked to stream water. Given that there is access to such relatively stable water sources, phreatophytic cottonwoods can persist even in semi-arid regions (Rood et al., 2003). Trembling aspen, by contrast, has ecophysiological adaptations to nonriparian zones and is widespread on upland sites (Lieffers et al., 2001; Rood et al., 2007). Differences in cavitation resistance between the two plant groups agree with these ecological characteristics.

Correlations between cavitation resistance and other traits, aside from ψ-leaf, were weak or absent, as observed previously in a study on eight hybrid poplar genotypes (Fichot et al., 2010). Our failure to identify such correlations may have been a result, at least in part, of the fact that variation in P_{50} remained relatively small. Moreover, if cavitation resistance in poplar is determined by differences in pit membrane ultrastructure (Jansen et al., 2009), then variation in P_{50} will not necessarily be linked with traits such as D_v and d_w. If a direct causal link between cavitation resistance and other vessel traits does not exist, it may be possible to breed poplar genotypes that show improved transport safety while maintaining high transport efficiency.

$\delta^{13}C$ and leaf water potentials

Our results show that hybrid poplar and aspen also differed distinctly in their $\delta^{13}C$ and ψ-leaf values (Fig. 3), suggesting that aspen regulated its stomata more conservatively in order to avoid xylem cavitation and excessive water loss. Previous work has shown that stomata in aspen operate in a way that maintains ψ-leaf above a critical threshold value between −2 and −2.5 MPa (Hogg & Hurdle, 1997; Hogg et al., 2000). Considering that aspen clones can be quite large, tree water use is likely to exert a strong feedback on the future availability of soil moisture in the area occupied by the clone. This may have led to more selection pressure for increased water-use efficiency in the aspen (T. Hogg, pers. comm.). We

conclude that aspen appears to be more water-use-efficient than hybrid poplars at a boreal planting site.

Growth performance and vessel diameters

Height was negatively correlated with d_w in hybrid poplars ($r = −0.82$, $P < 0.02$; data not shown). In aspen, variation in d_w was much smaller than in hybrid poplars, and there was no clear relationship with height or DBH. Again, it should be noted that d_w was measured in branch segments. Stronger correlations between height and d_w may have been found if d_w had been measured in the trunk.

The only other parameter that scaled with height in both hybrid poplars and aspen was D_v. The fact that strong negative correlations between tree height and D_v existed in both plant groups was unexpected. Another interesting finding was that differences in both height and D_v were highly conserved in trees from different aspen provenances. Trees from the two Minnesota provenances showed very similar values of height growth and D_v, as did trees from the two Alberta and the two British Columbia provenances (Fig. 4). The negative correlations between height and D_v seen in these mature trees contrast with observations on hybrid poplar saplings growing in a controlled environment without being subjected to abiotic stress. In such saplings, faster growth was correlated with wider vessels (Hacke et al., 2010). Why was height at our boreal planting site associated with narrower vessels at the expense of potentially lower transport efficiency?

At our study site, long-distance water transport in the xylem is not only constrained by drought-induced cavitation, but also by freezing. Wider vessels are more vulnerable to freezing-induced embolism than narrow vessels (Davis et al., 1999; Stuart et al., 2007). Relatively small differences in D_v can lead to large differences in vulnerability. Although we did not measure native embolism during winter, it seems reasonable to assume that trees with narrow vessels exhibited lower amounts of embolism in the winter than trees with wider vessels. Unlike other species, such as birch, poplars do not reverse winter embolism by developing root pressure (Sperry et al., 1994). The amount of winter embolism and differences in D_v may be significant in the context of this study because, despite some variation, a functional linkage exists between the embolism in late winter and the timing of spring budbreak across ring- and diffuse-porous angiosperms and conifers (Wang et al., 1992; Tyree & Zimmermann, 2002). Lower amounts of embolism may allow for a relatively early budbreak in spring and an adequate water supply to the developing foliage in Minnesota trees.

Available records for this common garden trial from 2008 indicate that Minnesota provenances did in fact leaf out c. 1 wk earlier than sources from central Alberta (Li, 2010), an observation opposite to normal latitudinal trends in budbreak, where sources from cooler environments break bud relatively earlier to take advantage of a shorter available

growing season (Leinonen & Hanninen, 2002). This departure from normal trends was explained as an adaptation of Minnesota sources to take advantage of favorable early-season growing conditions in Minnesota (Li, 2010). Minnesota receives 1.5 times more precipitation throughout the year (700 mm vs 463 mm for central Alberta and 449 for northeastern British Columbia) (Table S3), and when temperatures reach growing-season conditions (5°C) in spring, precipitation is 2.5 times higher in Minnesota (50 mm $month^{-1}$) than is typically recorded in Alberta and northeastern British Columbia (very dry with only 20 mm $month^{-1}$) (Fig. S1).

Our hydraulic data provide additional information that might help us to understand how Minnesota sources are adapted to their local climatic conditions, and why they grow exceptionally well in central Alberta, exceeding locally adapted sources by 30–40% in height and diameter growth. For a given spring temperature, Minnesota sources start growing early and are therefore more likely to be exposed to freeze–thaw events in spring. The small vessel diameters that we observed in this study for Minnesota sources may provide effective protection against embolism caused by freeze–thaw events in spring.

In hybrid poplars, differences in xylem anatomy were the result of differential genetic backgrounds rather than natural selection. Nevertheless, narrower vessels appear advantageous for growth within the hybrid poplar group as well: Walker exhibited the greatest height growth and also had the narrowest vessel diameters, followed, with increasingly larger vessel diameters, by the high, average, and poorly performing groups. A complicating factor in the analysis of D_v in trees of different height is the well-known fact that vessel diameters in the trunk vary with tree height (Tyree & Zimmermann, 2002; McCulloh & Sperry, 2005; Petit et al., 2010). When D_v is measured at the same height in trees of different sizes, as was done in our study, D_v may be expected to be wider in larger trees than in smaller ones (Weitz et al., 2006). We observed the opposite, suggesting that the trend in D_v was not just the consequence of a size effect.

While these explanations are speculative, they provide a framework to guide future research aimed at linking xylem traits, winter embolism, plant growth and climatic characteristics. Such work could be useful to identify genotypes that are well adapted to drought conditions as well as freeze–thaw cycles, which could become more frequent in a warmer and more variable future climate.

In conclusion, large differences in hydraulic traits existed between hybrid poplar clones and aspen provenances. Hybrid poplars exhibited less negative water potentials and were more vulnerable to drought-induced cavitation than aspen genotypes. Within groups, traits such as wood density and $\delta^{13}C$ showed wide variation within hybrid poplars but not within the aspen provenances. By contrast, vessel diameter and height growth varied substantially in both plant groups, and much of this variation in aspen was related to geographic seed source. In both plant groups, height growth was negatively correlated with vessel diameters. Vulnerability to freezing-induced embolism is closely related to vessel diameter, and genetically determined differences in vessel diameter could play an important role in explaining differences in tree performance.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Characterization of local climate conditions for the planting site and for the three aspen provenances locations Minnesota, Alberta and British Columbia.

Table S1 Analysis of variance for physiological parameters and growth traits measured in hybrid poplar clones

Table S2 Analysis of variance for physiological parameters and growth traits measured in aspen provenances

Table S3 Mean annual temperature (MAT), mean growing season precipitation (MGP), and mean annual precipitation (MAP) for the planting site as well as the aspen provenance locations

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