

Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree

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Summary

1. Xylem vessel diameters represent an important plant hydraulic trait to ensure sufficient water supply from the roots to the leaves. The ability to adjust the hydraulic pathway to environmental cues is key in order to satisfy transpirational demands and maximize growth and survival.

2. We evaluated the variability of vessel diameters in trembling aspen in a reciprocal transplant experiment. We tested six provenances from three ecological regions of North America planted at four test sites in western Canada. All test sites were established at the same time with the same provenances arranged in a randomized complete block design.

3. Vessel diameter showed a strong interaction of population and test site suggesting a high degree of phenotypic plasticity in this trait. Gaussian kernel density estimates support plastic as well as genetic contributions in vessel diameter control trending from bimodal distributions at the most northern test site towards unimodal distributions at the warmest and mildest test site.

4. Furthermore, we used test site-specific climate data in form of a 2-year, 5-year and 10-year average of 21 directly and derived climatic variables and found that average site-specific vessel diameters correlated strongly with summer moisture availability. A previously found negative relationship with vessel diameter and tree height in central Alberta was also found at two other boreal test sites but reversed at a wetter and milder sub-boreal test site.

5. In summary, vessel diameters were highly plastic in response to different environments and varied with summer moisture availability. The variability of vessel diameter and tree height correlations suggests that vessel diameter alone cannot serve as a reliable proxy for long-term growth performance beyond boreal environments. Instead, selecting aspen populations with a high degree of plasticity in this trait appears to be the safest option for assisted migration and seed transfer programmes under climate change.

Key-words: aspen, assisted migration, climate change, common garden, forest management, genotype–environment interaction, plant hydraulics, plasticity, *Populus tremuloides*, precipitation

Introduction

Plants have to ensure a functional water conducting system in order to maximize growth performance and survival. Drought can negatively impact plant hydraulic conductance leading to air-filled and dysfunctional xylem vessels through cavitation (Tyree & Zimmermann 2002). In order to keep cavitation and the resulting embolism at tolerable

levels, and at the same time satisfying transpirational demands, plants can adjust their hydraulic architecture. Changes in the leaf-to-xylem area ratio, root-to-leaf area ratio, stomatal control or vessel dimensions can modify a plant's water-use strategy under given environmental or experimental conditions (Hacke *et al.* 2000; Chaves *et al.* 2002; Fisher *et al.* 2007; Martínez-Vilalta *et al.* 2009; Choat *et al.* 2011; Schreiber *et al.* 2011; Plavcová & Hacke 2012).

Because they cannot move, plants are particularly well adapted to respond to environmental change through a

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process known as phenotypic plasticity. Phenotypic plasticity is the ability of the genotype to respond to different environments by producing distinct phenotypes within its life span (Sultan 2000; Pigliucci 2005; Nicotra *et al.* 2010). Depending on the trait, long- and short-term plastic responses can contribute to plant development and the ability to cope with diverse environments (Sultan *et al.* 1998; Alpert & Simms 2002; Hetherington & Woodward 2003; Sultan 2003). Besides phenotypic plasticity, adaptation and migration are two other mechanisms through which plants can respond to environmental change; however, these adjustments require multiple generations of natural selection, seed dispersal and successful seed establishment (Aitken *et al.* 2008).

Identifying environmental factors that are responsible for phenotypic change in functional traits represents an important field of study given current and predicted climate change (Fonti *et al.* 2009; Matesanz, Gianoli & Valladares 2010; Nicotra *et al.* 2010; Plavcová & Hacke 2012; Franks, Weber & Aitken 2013). Particularly, traits related to water transport may be of interest due to increased drought-induced forest mortality across the globe (Bréda *et al.* 2006; Allen *et al.* 2010; Choat *et al.* 2012; Worrall *et al.* 2013). With respect to such hydraulic traits, many studies have looked at interspecific differences in different environments or along environmental gradients (Maherali, Pockman & Jackson 2004; Hacke *et al.* 2006; Jacobsen *et al.* 2007; Jansen, Choat & Pletsers 2009). However, commonly observed correlations of hydraulic traits in interspecific comparisons may not necessarily be found in comparisons with populations of a single species, especially under field conditions (Martinez-Vilalta *et al.* 2009; Schreiber *et al.* 2011).

Studies at the intraspecific level can help to illuminate species-specific correlations among hydraulic traits and provide information on how variable these traits are under different environmental conditions. However, as Fonti & Jansen (2012) pointed out, there is a lack of studies at the intraspecific level as well as studies that monitor long-term modifications of xylem over the full life span of trees. Intraspecific studies, ideally replicated across multiple environments, could be of value to better understand variation in key hydraulic traits within a single species. These types of reciprocal transplant experiments, also known as provenance tests or common garden experiments, can disentangle genotype and environmental effects and therefore represent a powerful tool for testing hypotheses related to climatic adaptation in trees (Mátyás 1996). However, with respect to hydraulic traits, these controlled long-term studies are rare (but see Schreiber *et al.* 2011; Lopez *et al.* 2013). Results of such provenance tests are not only interesting from a scientific perspective, but can also be very informative for forest management applications (e.g. Ying & Yanchuk 2006). For instance, knowing how key hydraulic traits vary across environments could facilitate decision-making for reforestation efforts in order to select the most suitable planting stock for a given target location.

Currently, little is known about the variation of vessel diameter within species or genotypes across different environments. In general, it can be assumed that smaller vessels are advantageous for avoiding freezing-induced embolism and minimizing the impact of drought-induced cavitation (Langan, Ewers & Davis 1997; Davis, Sperry & Hacke 1999; Hacke & Sperry 2001; Pittermann & Sperry 2003, 2006; Fisher *et al.* 2007; Stuart *et al.* 2007; Choat *et al.* 2011; Schreiber *et al.* 2013b; Medeiros & Pockman 2014). While the underlying mechanisms are different, in both cases dysfunctional xylem conduits are the result, which ultimately impairs water transport to the canopy. Bubble formation during freezing is mainly a consequence of gas segregation during the freezing process and the speed of ice formation (Sevanto, Holbrook & Ball 2012) and is strongly correlated with vessel diameter, presumably because conduit diameter scales with the size of the bubbles formed (Davis, Sperry & Hacke 1999; Pittermann & Sperry 2003). Drought-induced cavitation on the other hand occurs by 'air seeding' in which air is pulled from adjacent embolized conduits through pores in the pit membrane (e.g. Sperry & Tyree 1988).

Studies looking at vessel diameter distributions in annual growth rings revealed that plants grown in different environments produce varying size classes of xylem conduits. A recent study by Medeiros & Pockman (2014) found that *Larrea* species growing at high latitudes show a higher frequency of smaller vessels than plants growing at low latitudes, suggesting increased protection against freeze-thaw embolism. Similarly, Fisher *et al.* (2007) showed that vessel diameter in the Hawaiian trees species *Metrosideros polymorpha* were smaller in a subalpine habitat that experiences freezing than in a lowland rain forest habitat. On the other hand, Sperry *et al.* (1994) found that trees in Alaska had less freezing-induced embolism than their Utah counterparts, presumably because of fewer freeze-thaw cycles in Alaska. Besides temperature and number of freeze-thaw cycles, some studies also linked moisture availability in the field with conduit size (e.g. Villar-Salvador *et al.* 1997; Fonti & Garcia-Gonzalez 2008). Arend & Fromm (2007) showed that vessel cross-sectional area was positively related to water availability during earlywood formation, but not during latewood production. In Norway spruce, Montwé, Spiecker & Hamann (2014) showed decreased tracheid lumen diameter and increased cell wall thickness as a result of a 7-year experimental drought. After the drought treatment ended lumen diameters and cell wall thickness quickly returned to pre-drought measurements suggesting a high degree of plasticity in these traits. However, plastic responses of the same genotypes across multiple environments have rarely been studied.

Building on previous work at a single planting environment (Schreiber *et al.* 2011), the objectives for this study were to (i) disentangle the genetic and environmental influence on vessel diameter variations across multiple environments as part of a reciprocal transplant experiment with trembling aspen in western Canada; (ii) compare vessel

diameter distributions among populations and environments using kernel density estimation; (iii) identify climatic parameters that may explain differences in vessel diameters across environments; (iv) evaluate whether vessel diameter can be used as a proxy for growth potential in boreal and sub-boreal regions of western Canada.

Material and methods

EXPERIMENTAL DESIGN

The plant material for this study was collected from a large-scale reciprocal transplant experiment in western Canada established by the industrial members of the Western Boreal Aspen Cooperative in 1998. A total of 43 provenances from six ecological regions were replicated at five test sites along a boreal climate gradient in western Canada. Each test site contains 43 provenances from six ecological regions arranged as randomized complete blocks with six blocks per provenance planted in five-tree row plots and surrounded by two rows of border trees. The spacing between individual trees within rows is 2 m and between rows 3 m (see Schreiber *et al.* (2013a) for a full description of the transplant experiment). The sampled test plantations for the present study are located in north-east British Columbia (BC), northern Alberta (nAB), central Alberta (cAB) and Alberta foothills (ABf) (Table 1). Here, we subsampled six provenances from three ecological regions for xylem vessel measurements, that is two provenances from British Columbia (BC1, BC2), two from the boreal plains of central Alberta (AB1, AB2) and two from the boreal shield ecoregion in Minnesota (MN1, MN2). This selection covered the full extent of the transplant experiment, enabling us to study genotype by environment interactions with provenances from as distant as 2300 km in a south-east to north-west direction and 11° in latitude. For each provenance, we sampled one branch from eight individual trees with similar diameters totalling 48 branches per test site. Sampling was carried out with a telescope pruner at a fixed height of approximately 5 m.

Due to logistic difficulties inherent in a large-scale experiment as this, sampling had to take place in different years. The central Alberta and Alberta foothills test sites were sampled during the summer of 2009, the northern Alberta test site during the summer of 2011, and the British Columbia test site in early spring of 2014 approximately 2 weeks prior bud break, which means the latest growth increment occurred in 2013. Height measurements for the entire common garden experiment were taken in 2006 after nine growing seasons in the field. The established provenance means were used as a measure of growth potential for this study.

ANATOMICAL MEASUREMENTS

Branches were typically between 3 and 4 years of age. No systematic variations in vessel diameter across growth rings were found (Appendix S1 in Supporting Information). Branch vessel diame-

ters were measured manually on cross sections of 30–35 µm thickness. Sections were prepared with a microtome (Leica SM2400) and analysed using a Leica DM3000 microscope at ×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; Media Cybernetics, Silver Spring, MD, USA). Vessel diameters were measured in three to four radial sectors (depending on section quality) representing the two outermost growth rings but excluding the most recent ring if branches were sampled in the middle of the growing season (Fig. 1). Each sector included approximately 100 individual vessels resulting in a total of 65 876 individual vessel measurements. Vessel diameters from the central Alberta test site have previously been published (Schreiber *et al.* 2011) but will be included in the present data set to enable a comprehensive analysis of vessel diameter variation in trembling aspen across western Canada. Mean hydraulic vessel diameters (VD) were calculated based on the Hagen–Poiseuille equation. The vessel diameter that corresponds to the average lumen conductivity was calculated as $VD = ((\sum d^4)/n)^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter (Tyree, Davis & Cochard 1994; Wheeler *et al.* 2005). Besides calculating the lumen diameters by provenance, we also calculated an average lumen diameter for each test site across all provenances representing a site-specific response of vessel diameter to the experienced environmental conditions of this ecological region.

CLIMATE DATA

We extracted climate data from gridded climate surfaces for western North America using the free for download software package ClimateWNA (Wang *et al.* 2012; Hamann *et al.* 2013). This program estimates a range of biologically relevant seasonal and annual climate variables for historic time periods from 1901 to 2012 using geographic coordinates and elevation for the desired locations. In this study, we calculated test site-specific climate data in form of a 2-year, 5-year and 10-year average of 21 directly and derived climatic variables to interpret the observed variation in vessel diameters across test sites. To account for differences in the timing of sampling, these averages start with the year in which the latest measured growth ring was formed. Furthermore, we used the 1998–2012 period in order to characterize the experienced climate at each test site since establishment (Appendix S2).

DATA ANALYSIS

All data exploration, analysis 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), *reshape2* (Wickham 2007), *lmerTest* (Kuznetsova, Brunn Brockhoff & Haubo Bojesen Christensen 2014), *multcomp* (Hothorn, Bretz & Westfall 2008), *lsmeans* (Lenth & Hervé 2015), *e1071* (Meyer *et al.* 2014) and *diptest* (Maechler 2013). Boxplots of the raw data at the provenance level are provided in Appendix S3. A linear mixed effects model was carried out using the *lmer()*

Table 1. Test site location and climate averages for the period from 1998 to 2012 (age of the test sites)

	Latitude	Longitude	Elevation	MAT	MCMT	MWMT	MAP	MSP
BC	58.32	−122.2	335	1.1	−17.8	17.4	470	314
nAB	56.46	−117.28	525	1.3	−17.9	16.7	407	271
cAB	54.53	−113.18	570	2.2	−15.8	17.2	494	350
ABf	52.44	−114.47	970	2.8	−11.9	15.9	530	395

MAT, mean annual temperature (°C); MCMT, mean coldest month temperature (°C); MWMT, mean warmest month temperature (°C); MAP, mean annual precipitation (mm); MSP, mean summer (May–September) precipitation (mm).

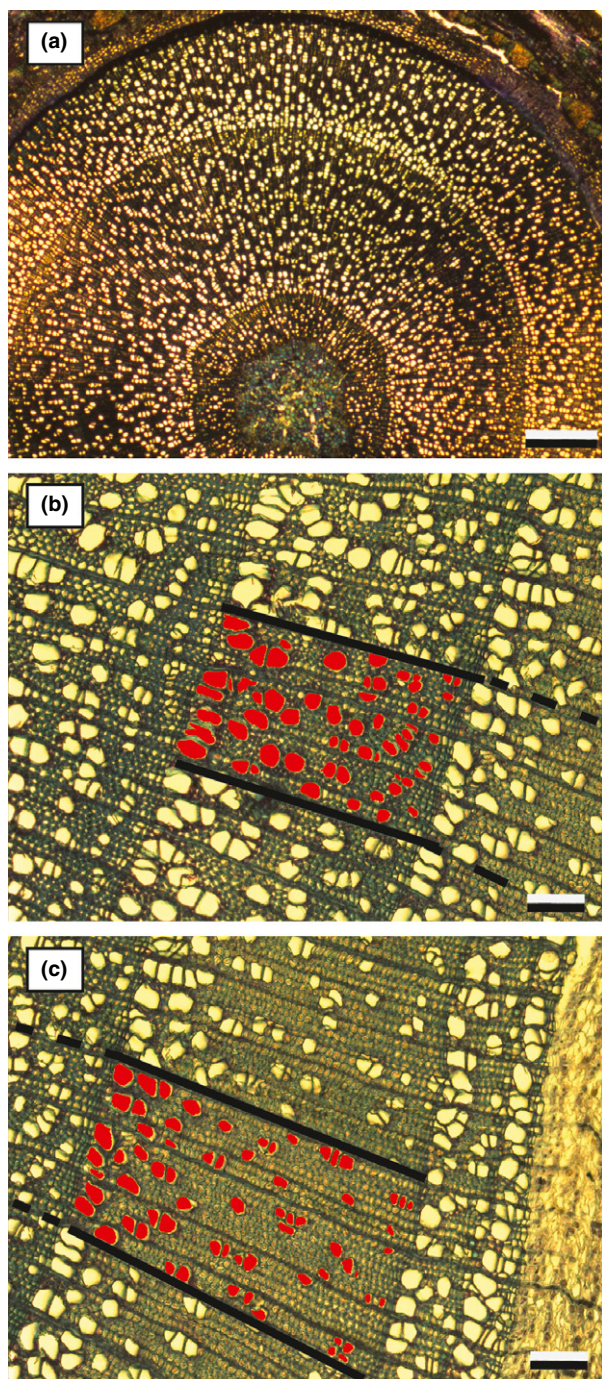


Fig. 1. Branch cross sections of *Populus tremuloides*. (a) Overview of a 3-year old branch of a Minnesota tree growing at the central Alberta test site; (b) and (c) represent a 4-year old Minnesota tree growing at the British Columbia test site. All vessels that fall between two rays in two consecutive growth rings were measured. Scale bars are 500 µm (a) and 100 µm (b–c).

function with *vessel diameter* representing the response variable. *Population* (genotype response of two provenances per ecoregion), *test site* as well as their interaction (*test site* × *population*) were representing the fixed effects. *Provenance* within *population* was specified as the random effect to test for within-population variation using restricted maximum likelihood (REML) estimation. Significant fixed effects were followed up using the *ghlt()* and *lsm()* functions to perform Tukey-adjusted multiple mean comparisons.

Table 2. Vessel diameter (µm) averaged by test site and by site and population. Standard errors of the mean are given in parentheses. Significant differences after multiple mean comparisons are indicated by different letters. All mean comparisons were adjusted using Tukey's HSD test with a family-wise confidence level set at 0.95. Comparisons were made between test sites, between identical populations across test sites and among populations within test sites (letters in parentheses)

Site	VD (by site, $n = 48$)	Population	VD (by site and population, $n = 16$)
BC	25.62 (0.37) ^A	BC	26.66 (0.54) ^{A, (A)}
		cAB	25.52 (0.61) ^{AB, (A)}
		MN	24.69 (0.68) ^{A, (A)}
nAB	23.88 (0.25) ^B	BC	24.66 (0.30) ^{A, (A)}
		cAB	23.92 (0.47) ^{A, (A)}
		MN	23.07 (0.45) ^{A, (A)}
cAB	27.06 (0.39) ^C	BC	29.35 (0.57) ^{B, (A)}
		cAB	27.03 (0.50) ^{B, (B)}
		MN	24.81 (0.41) ^{A, (C)}
ABf	27.75 (0.42) ^C	BC	25.40 (0.37) ^{A, (A)}
		cAB	27.96 (0.62) ^{B, (AB)}
		MN	29.90 (0.69) ^{B, (B)}

Gaussian kernel density estimation was used to compare vessel diameter distributions among populations and environments. Kernel density estimation is a nonparametric data smoothing procedure that results in probability density functions based on a finite sample data set (Silverman 1986; Venables & Ripley 2002). All individual vessel measurements were included in this analysis. The chosen bandwidth for the kernel density estimator was calculated according to Silverman's 'rule of thumb' (Silverman 1986, page 48, eqn. 3.31), which also represents the default bandwidth calculation in the R *density()* function.

Testing for multimodality was performed using the R package *dipTest*, which calculates Hartigan's dip statistic (*D*) using the *dipTest()* function. A significant result indicates a non-unimodal distribution, that is a distribution that is at least bimodal. To further describe the shape of these distributions, coefficients for kurtosis and skewness were calculated using the *kurtosis()* and *skewness()* functions of the R package *e1071*. A negative coefficient for kurtosis indicates a flat data distribution (platykurtic), and a positive coefficient indicates a peaked distribution (leptokurtic). The normal distribution has zero kurtosis (mesokurtic). A negative value for skewness indicates a left-skewed distribution (longer left tail), and a positive value indicates a right-skewed distribution (longer right tail).

Results

GENOTYPE BY ENVIRONMENT INTERACTIONS

In this experiment, the selection of planting sites reflects an environmental gradient from continental boreal conditions with a cold and dry climate to a wetter and milder sub-boreal environment (Table 1, see Appendix S2 and S4). At the boreal test sites, that is north-east British Columbia, northern Alberta and central Alberta, mean vessel diameter varied within test sites but shared the same ranking from largest to smallest (British Columbia, central Alberta and Minnesota) but only significant at the central Alberta test site (Table 2). A significant change in vessel diameter ranking was observed at the sub-boreal Alberta foothills

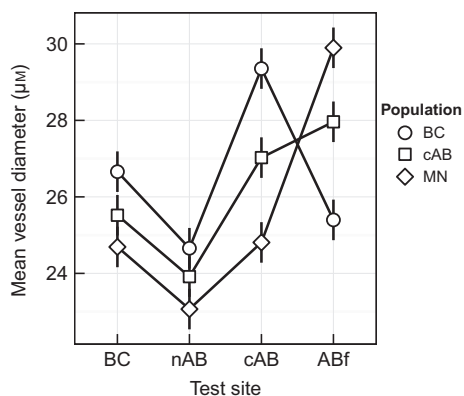


Fig. 2. Interaction plot of the average differences in vessel diameter among populations and across test sites. Population means are represented by the pooled values of two provenances per eco-region with error bars given as the standard error of the mean. $n = 16$. BC, British Columbia; nAB, northern Alberta; cAB, central Alberta; ABf, Alberta foothills; MN, Minnesota.

test site where vessel diameter from Minnesota provenances ranked largest and British Columbia provenances smallest (Fig. 2, Appendix S3). Trees belonging to the central Alberta population always ranked intermediately. The linear mixed effects model revealed significant differences for *test site* (d.f. = 3, $F = 31.3$, $P < 0.0001$) as well as the interaction between *population* \times *test site* (d.f. = 6, $F = 13.1$, $P < 0.0001$), while *population* was not significant

(d.f. = 2, $F = 2.9$, $P = 0.06$). The random effect estimate was zero indicating that no within-population variation was detected. The biggest difference in mean vessel diameter across different test sites was observed for trees originating from Minnesota (Alberta foothills versus northern Alberta test site = $6.8 \mu\text{m}$), followed by British Columbia (central Alberta versus northern Alberta test site = $4.7 \mu\text{m}$) and central Alberta (Alberta foothills versus northern Alberta test site = $4.0 \mu\text{m}$) (Table 2).

CHANGES IN VESSEL DIAMETER DISTRIBUTIONS ACROSS SITES

The observed genotype by environment effect was also reflected in vessel diameter distributions among provenances and test sites. Gaussian kernel density estimates for vessel diameter distributions were bimodal in shape at the coldest test site (British Columbia) and changed to unimodal distributions at the warmest test site (Alberta foothills), with northern and central Alberta test sites showing intermediate distributions (Fig. 3, Table 3). The first peak consistently occurred at around $16 \mu\text{m}$ (Fig. 3, dotted line). The second peak was much more variable falling between 20 and $30 \mu\text{m}$, but then decreasing quickly (Fig. 3, dashed line). The largest vessel diameters fell between 40 and $55 \mu\text{m}$. The transition from boreal to sub-boreal is also reflected in a symmetry change from left-skewed to right-skewed distributions (Table 3).

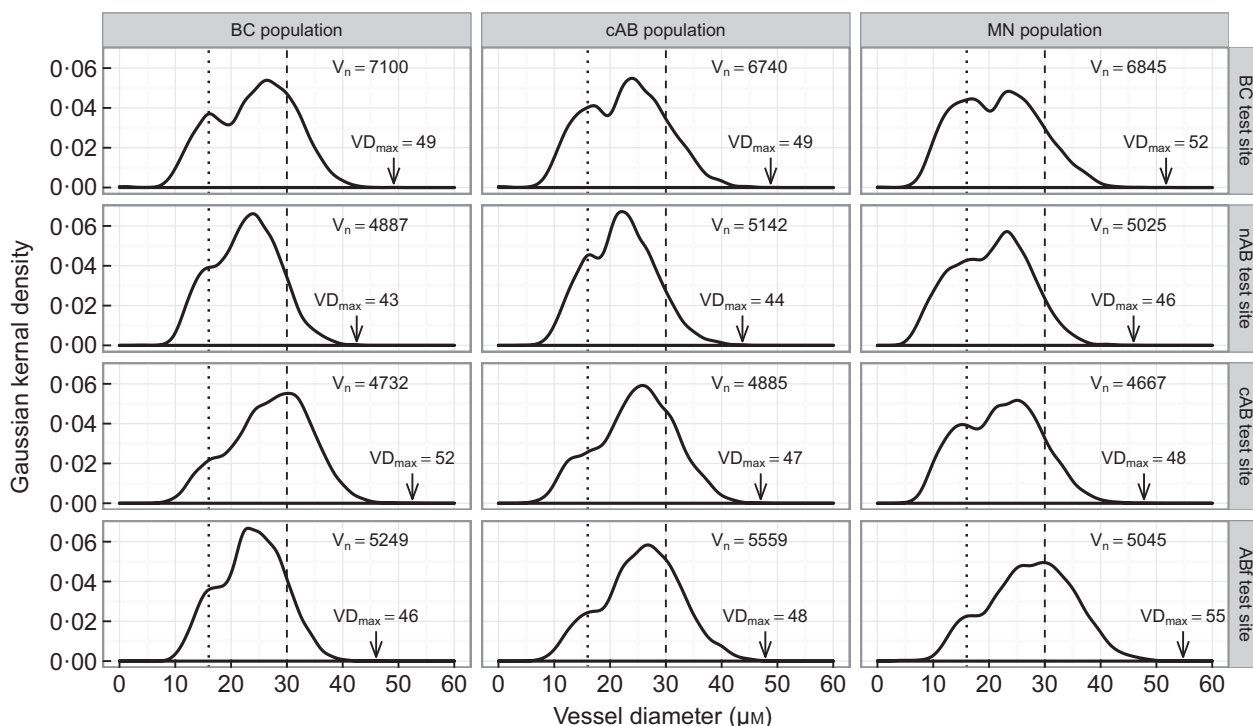


Fig. 3. Gaussian kernel density estimates of vessel diameter faceted by population and test site. Arrows point at the largest measured vessel diameter (VD_{max}). V_n represents the total number of vessels measured per population and test site. The dotted lines intersect the x -axis at $16 \mu\text{m}$; the dashed lines intersect at $30 \mu\text{m}$. BC, British Columbia; nAB, northern Alberta; cAB, central Alberta; ABf, Alberta foothills; MN, Minnesota.

Table 3. Hartigan's *D* for testing multimodality and its statistical significance, as well as kurtosis and skewness, to assess peakedness and symmetry, respectively. Significant non-unimodal distributions are indicated in bold face ($\alpha = 0.05$)

Site	Population	Hartigan's <i>D</i>	Kurtosis	Skewness
BC	BC	0.0072 (0.02)	-0.656	-0.117
	cAB	0.0076 (0.01)	-0.533	0.097
	MN	0.0079 (0.01)	-0.628	0.211
nAB	BC	0.0028 (0.99)	-0.468	0.035
	cAB	0.0043 (0.82)	-0.279	0.176
	MN	0.0037 (0.69)	-0.607	0.045
cAB	BC	0.0038 (0.97)	-0.427	-0.198
	cAB	0.0027 (0.99)	-0.447	-0.147
	MN	0.0044 (0.87)	-0.632	0.090
ABf	BC	0.0027 (0.99)	-0.446	-0.042
	cAB	0.0026 (0.99)	-0.330	-0.087
	MN	0.0039 (0.94)	-0.398	-0.086

Coefficients for kurtosis indicate flat distributions (platykurtic) across all populations and environments. Vessel diameter distributions appear to be more dependent on environmental conditions than on genetics (cf. columns representing genotypes versus rows representing environments in Fig. 3, respectively).

ASSOCIATION OF PHENOTYPES WITH ENVIRONMENTS

A comparison with average site-specific vessel diameters and site-specific climate parameters for a 5-year period leading up to and including the years for which growth rings were measured showed strong relationships primarily with mean annual precipitation and mean summer precipitation and less associations with temperature variables (Fig. 4, Table 4). Similar results were observed for a 2-year and 10-year period (Appendix S5 and S6). Drought indices such as the summer heat moisture index or the Hargreaves climatic moisture deficit index also showed strong significant relationships with vessel diameter. Site-specific vessel diameters were not associated with frost or extreme minimum temperatures. A 10-year, 5-year and 2-year average for mean annual temperature, mean coldest month temperature, mean warmest month temperature, mean annual precipitation and mean summer precipitation showed similar variations across test sites irrespective of the chosen period, for example the Alberta foothills test site is always the wettest, whereas the northern Alberta test site is always the driest (Appendix S4).

HEIGHT AND VESSEL DIAMETER CORRELATIONS

A comparison of the average vessel diameter and tree height at the provenance level showed negative relationships with vessel diameter and tree height at the central Alberta test site (d.f. = 5, $R^2 = 0.87$, $P = 0.007$), the British Columbia test site (d.f. = 5, $R^2 = 0.66$, $P = 0.051$) and the northern Alberta test site (d.f. = 5, $R^2 = 0.5$, $P = 0.115$) (Fig. 5). At the wetter and milder sub-boreal Alberta foothills test site, the height/vessel diameter

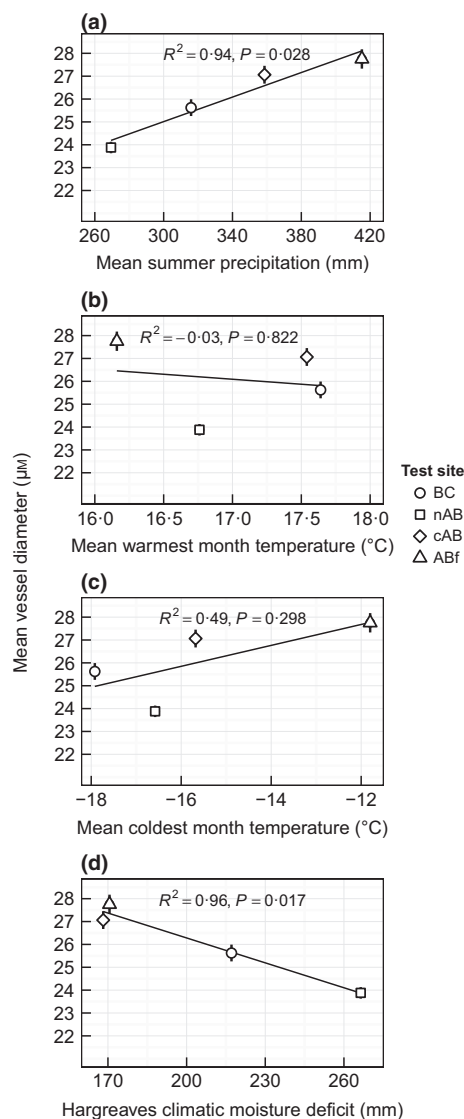


Fig. 4. Relationship between average site vessel diameter and a selected set of site-specific annual climate variables. Annual climate variables were averaged over a 5-year period leading up to and including the years of the analysed growth rings. cAB and ABf: 2004–2008; nAB: 2006–2010; BC: 2009–2013. Error bars represent the standard error of the mean. $n = 48$. BC, British Columbia; nAB, northern Alberta; cAB, central Alberta; ABf, Alberta foothills.

relationship was reversed (d.f. = 5, $R^2 = 0.65$, $P = 0.051$), that is the tallest trees showed the largest average vessel diameters.

Discussion

GENETIC AND ENVIRONMENTAL INFLUENCE ON VESSEL DIAMETER

Our results showed that variation in vessel diameter is influenced by environmental factors and the interaction of the genotype with the environment. A potential effect of within-population variation among provenances influencing

Table 4. Pearson's correlation coefficients and their statistical significance between annual and seasonal climate variables with mean vessel diameter at four test sites, that is British Columbia (BC), northern Alberta (nAB), central Alberta (cAB) and Alberta foothills (ABf). Annual and seasonal climate variables were averaged over a 5-year period leading up to and including the years of the analysed growth rings. cAB and ABf: 2004–2008; nAB: 2006–2010; BC: 2009–2013. Significant correlations are indicated in bold face ($\alpha = 0.05$)

Climate variables measured at four test sites	Correlation with vessel diameter: Pearson's r (P -value)
Annual variables	
Mean annual temperature (°C)	0.88 (0.122)
Mean warmest month temperature (°C)	–0.18 (0.822)
Mean coldest month temperature (°C)	0.70 (0.298)
Temperature difference (°C)	–0.61 (0.392)
Mean annual precipitation (mm)	0.98 (0.017)
Mean summer (May–September) precipitation (mm)	0.97 (0.028)
Annual heat:moisture index	–0.60 (0.396)
Summer heat:moisture index	–0.95 (0.048)
Number of frost free days	0.41 (0.589)
Precipitation as snow (mm)	–0.48 (0.519)
Hargreaves climatic moisture deficit (mm)	–0.98 (0.017)
Seasonal variables	
Winter (December–February) mean temperature (°C)	0.87 (0.130)
Spring (March–May) mean temperature (°C)	0.88 (0.118)
Summer (June–August) mean temperature (°C)	–0.32 (0.675)
Autumn (September–November) mean temperature (°C)	0.50 (0.504)
Winter precipitation (mm)	–0.35 (0.655)
Spring precipitation (mm)	0.69 (0.304)
Summer precipitation (mm)	> 0.99 (0.003)
Autumn precipitation (mm)	0.33 (0.670)
Hargreaves climatic moisture deficit (spring)	–0.79 (0.209)
Hargreaves climatic moisture deficit (summer)	–0.97 (0.025)

mean vessel diameters was not detected but could also not be ruled out since the number of provenance within each ecoregion was limited. The ranking from smallest to largest mean vessel diameter among populations remained steady but showed a crossover interaction between the British Columbia and Minnesota populations at the Alberta foothills test site (Fig. 2, Table 2). Despite the apparently opposite response observed at the Alberta foothills test site, plastic response functions of different genotypes were in principle consistent. When northern provenances experienced warmer environments than what they were adapted to (British Columbia provenances transferred to Alberta foothills test site), their vessel diameters decreased although precipitation levels appeared favourable, suggesting a stress response. Similarly, when southern provenances experienced much colder and drier environments than what they were adapted to (Minnesota provenances transferred to the boreal planting sites in British Columbia, northern Alberta and central Alberta), their vessel diameters also decreased as a response to minimize

the impact of drought and freezing-induced cavitation. The significant crossover interactions can therefore be interpreted as a response to unusual environments for the respective locally adapted populations.

VESSEL DIAMETER DISTRIBUTIONS AMONG POPULATIONS AND ENVIRONMENTS

Kernel density estimates showed a bimodal distribution of vessel diameters for all provenances at the north-east British Columbia (BC) test site (Fig. 3), whereas in most other cases the distributions were unimodal. Bimodal distributions are known for ring-porous trees, whereas semi-ring-porous and diffuse-porous trees tend to approach normal distributions (Woodcock 1989a,b; Carlquist 2001). Depending on the literature source, trembling aspen can either be considered diffuse-porous (Mackes & Lynch 2001) or semi-ring-porous (Ilvessalo-Pfäffli 1995). Our results suggest that depending on the experienced climate, vessel diameter distributions in aspen can indeed vary between bimodal and unimodal, trending between semi-ring- and diffuse-porous vessel patterning, and also progressively decreasing in size from early- to latewood (Fig. 1). Furthermore, there was a striking tendency for the first peak in vessel frequency to consistently occur at around 16 μm across all sites and populations. This observation suggests that latewood formation is strongly genetically controlled. In contrast, the second peak was much more variable and occurred between 20 and 30 μm (Fig. 3). This suggests that the larger earlywood vessels are far more responsive to different environmental conditions, compared to the highly conserved diameter of latewood vessels, particularly at the most northern test site in north-east British Columbia.

The reason why bimodal distributions of vessel diameter were most pronounced at northern test sites might be that boreal environments generally have a very short growing season with abundant water right after snowmelt and maximum light availability during summer solstice around June 20th. The timing of budbreak in trembling aspen occurs approximately a month prior to summer solstice during mid-May (Schreiber *et al.* 2013a). This is the period when growth conditions are most favourable and biomass can be accumulated. The second half of the season is much drier favouring smaller vessel diameters, which may represent an adaptation to minimize drought-induced cavitation during the growing season while at the same time also minimizing the degree of freezing-induced embolism to ensure functional xylem for subsequent growing seasons. In general, vessel diameters fell below or close to an experimentally established 30 μm threshold below which no freezing-induced embolism were observed (Davis, Sperry & Hacke 1999). Subsequent field experiments confirmed that trees experiencing freezing tend to have vessel diameters below 30 μm (e.g. Fisher *et al.* 2007; Schreiber *et al.* 2013b; Medeiros & Pockman 2014). The high variation of vessel diameter below this threshold may represent a fine-tuning mechanism to adapt to the site-specific climatic conditions.

THE INFLUENCE OF CLIMATIC PARAMETER ON VESSEL DIAMETER

We interpret the variability in vessel diameter in trembling aspen as a fine-tuning mechanism to different environments. This is also supported by site-specific averages of vessel diameter showing a strong relationship with mean annual precipitation, mean summer precipitation and drought indices such as the Hargreaves climatic moisture deficit for different time periods (Fig. 4, Table 4, Appendix S5 and S6). Since cell expansion is a turgor-driven process (cf. Fromm 2013), correlations of vessel diameter and water availability were not unexpected, although we were surprised by the strength of the observed relationships. The reason why we observed strong correlations with summer precipitation, and not spring-, autumn- or winter precipitation (snow) separately, probably reflects the late start of the growing season in the boreal forest and also coincides with the fact that the majority of precipitation occurs during the summer months (Appendix S2 and S4). However, we note that our analysis is based on only four test sites due to logistical limitations of large-scale field research. Nonetheless, our results are in agreement with other studies linking water availability with conduit diameter, underlining the importance of precipitation as a strong contributor to vessel diameter variation (Villar-Salvador *et al.* 1997; Arend & Fromm 2007; Fonti & Garcia-Gonzalez 2008; Plavcová & Hacke 2012; Montwé, Spiecker & Hamann 2014).

VESSEL DIAMETER AND TREE HEIGHT RELATIONSHIPS

Vessel diameter plays a significant role in transport efficiency and safety, which can ultimately impact growth and survival (Sperry, Hacke & Pittermann 2006). A previously established negative relationship between vessel diameter and tree height, both measured in 2009 at a boreal test plantation in central Alberta, suggested that vessel diameter may be used as a proxy for growth potential in trembling aspen and hybrid poplars (Schreiber *et al.* 2011). Trees with the smallest vessel diameters also showed the least amount of freezing-induced embolism, which, over the years, may have contributed to taller trees (Schreiber *et al.* 2013b). This study sought to test this hypothesis by including three other boreal test sites into the analysis. As previously discussed in Schreiber *et al.* (2011, 2013b), the observed negative correlations between tree height and vessel diameter were not just a result of a size effect. In short, since vessel diameter varies with path length (McCulloh & Sperry 2005; Olson *et al.* 2014), one would expect larger vessel diameters in taller trees and smaller vessel diameters in shorter trees when similar aged samples were taken at the same height (Weitz, Ogle & Horn 2006). We observed the opposite vessel diameter-height relationship at the British Columbia, northern Alberta and central Alberta test site (Fig. 5) suggesting that variation in vessel diameter, particularly in the crown, may also be influenced by other

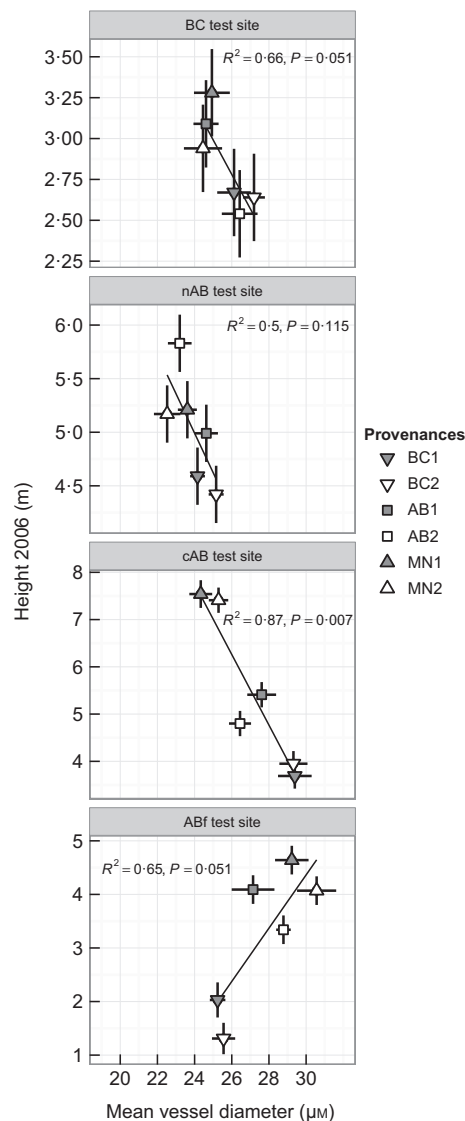


Fig. 5. Relationship between vessel diameter and tree height in 2006. Presented are the selected two provenances from British Columbia (BC1, BC2), the boreal plains of central Alberta (AB1, AB2) and the boreal shield ecoregion in Minnesota (MN1, MN2). Error bars represent the standard error of the mean. $n = 8$. Note: Due to large variation in tree height across test sites, the y-axis scale was adjusted for better data visualization.

factors, such as precipitation, rather than path length. Only at the sub-boreal test site in the Alberta foothills, this relationship reversed into a positive relationship, that is the tallest trees were associated with the largest vessels. We believe that this effect was mainly due to a stress response of the cold-adapted British Columbia provenances at the warmest test site in the Alberta foothills and not necessarily driven by precipitation patterns. In other words, as long as populations are not moved too far south of their respective ecological niche, we would expect a negative relationship with branch vessel diameter and growth potential in the boreal and sub-boreal ecoregions of western Canada.

Another factor to consider is the degree of plasticity in vessel diameters. Although we have not tested all provenances across the full environmental range in which they might survive, it is notable that the Minnesota population showed the highest degree of plasticity with a maximum vessel diameter difference of 6–8 μm between the northern Alberta and the Alberta foothills test site (Table 2). This high degree of plasticity was also associated with the highest growth potential at all test sites (Schreiber *et al.* 2013a). Given the established links between vessel diameter, growth potential and hydraulic safety, genotypes with branch vessel diameters below 30 μm and a high degree of plasticity may represent the safest option when selecting planting stock in genetic resource management programmes for reforestation in boreal environments.

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Data accessibility

Data are available in the Dryad repository <http://dx.doi.org/10.5061/dryad.jn81h> (Schreiber, Hacke & Hamann 2015). Data on growth of trees were provided by a commercial tree breeding cooperative (see acknowledgements), and so are not owned by the authors and thus not available in the archive.

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Supporting Information

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

Appendix S1 Measurements of vessel diameter across growth rings.

Appendix S2 Climate conditions at four test sites for the period from 1998–2012.

Appendix S3 Boxplots of vessel diameter per provenance faceted by test site.

Appendix S4 Annual climate variables averaged over a 2-year, 5-year and 10-year period.

Appendix S5 Correlations of vessel diameter with climate variables over a 2-year period.

Appendix S6 Correlations of vessel diameter with climate variables over a 10-year period.