



RESEARCH PAPER

Stem xylem resistance to cavitation is related to xylem structure but not to growth and water-use efficiency at the within-population level in *Populus nigra* L.

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Abstract

Xylem resistance to drought-induced cavitation is a key trait of plant water relations. This study assesses the genetic variation expressed for stem cavitation resistance within a population of a riparian species, the European black poplar (*Populus nigra* L.), and explores its relationships with xylem anatomy, water-use efficiency (WUE), and growth. Sixteen structural and physiological traits related to cavitation resistance, xylem anatomy, growth, bud phenology, and WUE were measured on 33 *P. nigra* genotypes grown under optimal irrigation in a 2-year-old clonal experiment in a nursery. Significant genetic variation was expressed for the xylem tension inducing 50% loss of hydraulic conductivity (Ψ_{50}) within the studied population, as attested by the high value of broad-sense heritability estimated for this trait ($H^2_{ind} = 0.72$). Stem cavitation resistance was associated with xylem structure: the more cavitation-resistant genotypes exhibited lower hydraulic efficiency and higher mechanical reinforcement as assessed from stem xylem cross sections. By contrast, Ψ_{50} was not significantly related to shoot height increment, total above-ground dry mass, or bulk leaf carbon isotope discrimination, a proxy for intrinsic WUE. These findings indicate that the trade-offs between xylem resistance to cavitation, hydraulic efficiency, and mechanical reinforcement can occur at the within-population level. Given that the studied genotypes were exposed to the same environmental conditions and evolutionary drivers *in situ*, the trade-offs detected at this scale are expected to reflect true functional relationships.

Key words: Bud phenology, bulk leaf carbon isotope discrimination, drought-induced cavitation, functional trade-offs, growth, *Populus nigra*, riparian species, water-use efficiency, within-population genetic variation, xylem structure.

Introduction

Long-distance water transport in plants occurs in the xylem, as a consequence of leaf transpiration. Because water is transported under tension in a metastable state, xylem conduits

can be subjected to cavitation events leading to hydraulic dysfunctions. According to the air-seeding hypothesis, drought-induced cavitation results from an air-bubble sucked from an

air-filled conduit into a water-filled conduit through the inter-conduit pit membrane (Sperry and Tyree, 1988). Air-filled (embolized) conduits then lose their functionality in water conduction, thereby decreasing xylem hydraulic conductivity and water transport efficiency (Tyree and Sperry, 1989). Xylem resistance to drought-induced cavitation is classically assessed by constructing vulnerability curves, which represent the per cent loss of hydraulic conductivity in relation to xylem tension. Several parameters are estimated from these curves, the most used being the xylem tension inducing 50% loss of hydraulic conductivity (Ψ_{50}) (Tyree and Ewers, 1991).

Xylem resistance to drought-induced cavitation is a key trait of plant water relations and may be important for plant responses to drought constraints, particularly for perennial species such as trees. This is supported by several lines of evidence. At the individual level, xylem resistance to cavitation has been shown to correlate strongly with the degree of resilience under severe drought both in conifers (Brodribb and Cochard, 2009; Brodribb *et al.*, 2010) and angiosperms (Barigah *et al.*, 2013; Urli *et al.*, 2013). At the species level, Ψ_{50} varies considerably and tends to be related to the minimum seasonal xylem water potential experienced *in situ* (Ψ_{xmin}); species experiencing low Ψ_{xmin} are generally more resistant to cavitation (Hacke *et al.*, 2000; Pockman and Sperry, 2000; Choat *et al.*, 2012). However, most woody species seem to operate close to their cavitation threshold, indicating a global convergence in the optimization of hydraulic functioning (Choat *et al.*, 2012).

Increased xylem resistance to cavitation is supposed to be costly otherwise all species would exhibit a high degree of resistance. Two main functional trade-offs have been proposed to explain the possible cost of increased cavitation resistance. On the one hand, more resistant species have long been thought to be less efficient in water transport (Zimmermann, 1983; Tyree *et al.*, 1994). The 'rare pit' hypothesis (also called the 'pit area' hypothesis) has been proposed as a functional explanation for this relationship in angiosperms (Wheeler *et al.*, 2005; Christman *et al.*, 2009): species with a more efficient xylem are considered more vulnerable to drought-induced cavitation because larger and longer vessels tend to have a greater pitted wall area, which in turn may increase the probability of having a large pore in the pit membrane more prone to air-seeding. On the other hand, increased xylem resistance to cavitation is also thought to come at the expense of a higher mechanical reinforcement of the xylem (Hacke *et al.*, 2001). This relationship lies in the necessity for cavitation-resistant xylem to withstand higher negative pressures to avoid cell wall collapse. The trade-offs between cavitation resistance, hydraulic efficiency, and mechanical reinforcement have, however, not been consistently detected depending on the species sampled, the correction applied for phylogenetic dependencies, and the scales considered (e.g. Maherali *et al.*, 2004; Jacobsen *et al.*, 2007, 2009; Pratt *et al.*, 2007; Fichot *et al.*, 2010, 2011; Lens *et al.*, 2011). Most of our knowledge of the trade-offs linking xylem resistance to cavitation to other physiological traits has come from interspecific comparisons, although the links evidenced may not directly reflect functional relationships. Studies at the intraspecific level may

partly solve this issue because the phylogenetic noise confounding trade-offs at the interspecific level is likely to be minimized. Such studies are rather recent and have mostly focused on population comparison (Kavanagh *et al.*, 1999; Maherali and DeLucia, 2000; Martínez-Vilalta *et al.*, 2009; Corcuera *et al.*, 2011; Lamy *et al.*, 2012; Sterck *et al.*, 2012). However, the comparison of mean population performances may also be questionable if these populations have been subjected to different evolutionary drivers that have shaped the genetic variation at a geographic scale. The comparison of individual performances at the within-population level may therefore provide an alternative way to identify functional relationships.

Long-term responses of natural populations to environmental changes will partly depend on the level of standing genetic variation for key functional traits (Alberto *et al.*, 2013), such as xylem resistance to cavitation (Lamy *et al.*, 2014). Estimates of the genetic variation expressed for xylem resistance to cavitation in natural populations of forest tree species remain, however, scarce. Two recent studies conducted in provenance-open-pollinated progenies of *Pinus pinaster* Ait. (maritime pine) evidenced a low level of population differentiation, as estimated by the coefficient of genetic differentiation (Q_{ST}), for xylem resistance to cavitation ($Q_{ST} = 0.027$) (Lamy *et al.*, 2011, 2014). This, combined with a low coefficient of additive genetic variation ($CV_A \approx 5\%$), was interpreted to mean the trait has limited evolvability. Otherwise, substantial variation has been reported for xylem resistance to cavitation both within and among natural populations of *Fagus sylvatica* L. (Wortemann *et al.*, 2011) and *Pinus canariensis* L. (López *et al.*, 2013). However, only one replicate of each genotype was included in these studies, precluding estimates of the genetic variation expressed for xylem resistance to cavitation.

The aim of this study was to assess the extent of genetic variation for xylem resistance to cavitation within one natural population of a riparian species, the European black poplar, *Populus nigra* L., and determine whether the common trade-offs between cavitation resistance and other xylem traits can be revealed at the within-population level. Poplars are among the most vulnerable tree species to drought-induced cavitation in the Northern Hemisphere, although variation has been reported across species (Hukin, 2005) and interspecific hybrids (Harvey and van den Driessche, 1997, 1999; Cochard *et al.*, 2007; Fichot *et al.*, 2010; Schreiber *et al.*, 2011). The genetic variation expressed for xylem resistance to cavitation within natural populations of poplars remains, however, poorly documented with studies conducted on a limited number of genotypes per population ($n \leq 5$; Sparks and Black, 1999; Schreiber *et al.*, 2011). The European black poplar is a major pioneer tree species of riparian ecosystems in Europe, Northern Africa, and Western Asia (Dickmann, 2006). This species covers a wide range of pedoclimatic conditions and expresses a large amount of genetic variation for growth, bud phenology, and water-use efficiency (WUE) (Chamaillard *et al.*, 2011; Rohde *et al.*, 2011). It is, however, intriguing to determine whether natural populations of riparian tree species, such as black poplar, maintain genetic variation for

cavitation resistance and how this relates to the variation observed for other functional traits. The specific objectives of the present study were therefore to evaluate: (i) the amplitude of genetic variation expressed for xylem resistance to cavitation within one natural population of black poplar comprising 33 genotypes; (ii) the relationships between cavitation resistance and xylem structural properties related to hydraulic efficiency and mechanical reinforcement; and (iii) the relationships between xylem resistance to cavitation and growth, bud phenology, and WUE.

Materials and methods

Plant material and experimental design

This study makes use of a natural population of black poplar originating from a Natural National Reserve located along the Loire river (Saint-Pryvé Saint-Mesmin, Loiret, 47°51'N 1°48'E, 90 m above sea level). This population was chosen to be representative of the genetic variation expressed for growth and WUE under non-limiting conditions in black poplar (Chamaillard *et al.*, 2011; Guet *et al.*, unpublished results). In 2000, branch cuttings were sampled on 33 adult trees distributed along a linear distance of 1.8 km in the population. The cuttings were propagated and kept in a clonal archive as a source of material. The experimental plantation was established in May 2012 at Orléans within the National Institute of Agronomic Research (INRA) research station of Forest Genetics (France, Loiret, 47°49'N 01°54'E, 110 m above sea level); all measurements were carried out in 2013, i.e. during the second growing season. The clonal test was set up in a nursery on a loamy-sand soil (8.6% clay, 18.6% silt, and 72.8% sand, pH 6.5) from 25 cm hardwood cuttings and consisted of five randomized complete blocks with three adjacent copies of each genotype per block. The initial spacing within and between rows was 1 × 2 m. A double border row comprising a mix of the 33 genotypes was set up around the experimental plot to reduce border effect. During the growing season, the experimental plantation was weed- and pest-controlled and regularly irrigated with overhead sprinklers to meet evaporative demand (i.e. 4.5 mm of water was sprinkled every time cumulative evapotranspiration reached 4 mm).

Meteorological data of the experimental site were obtained from a meteorological station located close to the field site. In 2013, the year of experiments, the monthly mean temperature ranged from 2.4°C (February) to 21.7°C (July) and the mean annual temperature was 10.7°C. The cumulative annual precipitation was 801 mm with 52% occurring during the growing season (from April to September).

Spring and autumn phenology

Bud flush and bud set were scored on all trees approximately twice a week in 2013 from 27 April to 09 May and from 09 August to 10 September, respectively. At each date of measurement, bud flush and bud set were assessed by visual inspection of the main terminal bud and applying a score describing six [from bud dormancy (stage 0) to active growth (stage 5)] and seven [from active growth (stage 3) to bud dormancy (stage 0)] discrete stages, respectively (Castellani *et al.*, 1967; Rohde *et al.*, 2011). Observed scores of bud set were fitted to local polynomial regressions of degree 2, and the date of stage 1.5 of bud set (in day of the year, DOY) was retrieved as described by Fabbrini *et al.* (2012). The same procedure was applied for bud flush and used to estimate the date of stage 3 of bud flush (DOY).

Whole-plant growth

Whole-plant growth was described for each genotype in 2013 by estimating the annual shoot height increment and the total

above-ground dry mass produced over the two growing seasons, 2012 and 2013. Total stem height was measured to the nearest centimetre on all available trees in January and December 2013 and was then used to calculate the shoot height increment in 2013. Biomass measurements were performed on all available trees in December 2013; the fresh mass of each collected tree was measured to the nearest 0.5 g before branches were removed to measure stem fresh mass. Samples of stem and branches were then collected from each tree and weighed before and after being oven-dried at 103°C for 3 days to compute stem and branches dry/fresh mass ratio, which were then used to estimate the total above-ground dry mass.

Bulk leaf carbon isotope discrimination and leaf gas exchange

Bulk leaf carbon isotope discrimination ($\Delta^{13}\text{C}$) (see Table 1 for the definition of trait abbreviations used in this article) was measured on the 33 genotypes and used as a time-integrated value of intrinsic water-use efficiency (WUE_i). For these measurements, one mature and fully illuminated leaf was collected in early July on the 2013 terminal shoot of each tree ($n = 15$ per genotype). Leaves were oven-dried at 60°C for 72 h before being ground to a fine powder. Carbon isotope composition ($\delta^{13}\text{C}$) was measured from 1 mg homogeneous leaf dry powder using a continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) coupled with an elemental analyser (Carlo Erba, Milan, Italy) and was expressed according to the Vienna Pee Dee Belemnite standard as described by Craig (1957). Bulk leaf carbon isotope discrimination was then determined according to Farquhar and Richards (1984).

In order to validate the functional relationship between $\Delta^{13}\text{C}$ and WUE_i , leaf gas exchange measurements were performed on a subset of ten genotypes. The ten genotypes were chosen to cover the range of genetic variation expressed for $\Delta^{13}\text{C}$ within the studied population, under irrigated conditions at Orléans, based on previously collected data from a distinct experimental design (Chamaillard *et al.*, 2011). Measurements were performed in mid-August on a cloudless day, between 11:00 and 15:00 local time, using a LI-6400 portable gas exchange system (Li-Cor Biosciences Inc., Lincoln, NE, USA). Net CO_2 assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance to water vapour (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) were recorded from one mature and fully illuminated leaf of one tree per genotype and block ($n = 5$ per genotype). The temperature of the chamber block was maintained at 25°C and the photosynthetic photon flux density was set to $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ using the 6400–02 LED light source; preliminary measurements indicated that this irradiance was sufficient to reach saturation for all genotypes. Average CO_2 concentration inside the chamber was set to 400 ppm and water vapour pressure deficit matched ambient conditions (1.4 ± 0.1 kPa). Measurements were taken once A and g_s had stabilized (typically 1 min after the leaf was enclosed in the chamber); the whole leaf was then sampled for $\Delta^{13}\text{C}$ determination as described above. WUE_i was calculated as the ratio between A and g_s .

Stem xylem resistance to drought-induced cavitation

Stem xylem resistance to cavitation was evaluated from one tree per block and genotype (5 blocks × 33 genotypes) using the 2013 terminal shoot. Measurements were performed in early October 2013, once primary growth had stopped but before the first autumn frosts occurred. Remaining leaves were first removed to limit transpiration and avoid embolism induction; the shoot was then severed, immediately wrapped in a moist towel and enclosed in black plastic bags to minimize dehydration. Stem xylem resistance to cavitation was measured using the Cavitrone technique (Cochard *et al.*, 2005). This technique uses centrifugal force to generate negative pressures in a calibrated stem sample while measuring its hydraulic conductance. Calibrated samples of 0.4–0.8 cm in diameter and 28 cm in length were re-cut under water from each shoot and processed as described by Fichot *et al.* (2010). Stem vulnerability curves were established

Table 1. List of trait abbreviations used in the text

Symbol	Definition	Units
Functional traits		
A	Net CO ₂ assimilation rate	μmol m ⁻² s ⁻¹
g _s	Stomatal conductance to water vapour	mmol m ⁻² s ⁻¹
WUE _i	Intrinsic water-use efficiency	mmol mol ⁻¹
Δ ¹³ C	Bulk leaf carbon isotope discrimination	‰
Ψ ₁₂	Stem xylem tension causing 12% loss of hydraulic conductivity	MPa
Ψ ₅₀	Stem xylem tension causing 50% loss of hydraulic conductivity	MPa
Ψ ₈₈	Stem xylem tension causing 88% loss of hydraulic conductivity	MPa
Structural traits		
A _v	Percentage of vessel lumen area	%
d _h	Hydraulic vessel diameter	μm
d _{mean}	Mean vessel diameter	μm
K _{s(t)}	Theoretical xylem specific hydraulic conductivity	kg s ⁻¹ m ⁻¹ MPa ⁻¹
t _h	Double vessel wall thickness	μm
(t/b) ² _h	Thickness-to-span ratio	
V _G	Vessel grouping index	
ρ _x	Xylem density	g cm ⁻³

from the percent loss of hydraulic conductivity (PLC) measured for 7–11 steps of xylem tension depending on sample's cavitation resistance. The following sigmoid function was fitted to each curve (Pammenter and Willigen, 1998):

$$PLC = 100 / [1 + \exp (s / 25 \times (\Psi - \Psi_{50}))] \tag{1}$$

where Ψ₅₀ is the stem xylem tension causing 50% loss of hydraulic conductivity (MPa) and *s* is the slope of the curve at Ψ₅₀ (% MPa⁻¹). A high quality of fit was overall observed with *R*² values ranging from 0.955 to 0.999. Values of Ψ₅₀ were used to compare the resistance to cavitation of the different genotypes. Stem xylem tensions inducing 12% and 88% loss of hydraulic conductivity (Ψ₁₂ and Ψ₈₈ respectively) were calculated for each sample according to Domec and Gartner (2001) as: Ψ₁₂ = Ψ₅₀ - 50/*s* and Ψ₈₈ = Ψ₅₀ + 50/*s*. Values of Ψ₁₂ and Ψ₈₈ represent thresholds of xylem tension at the onset and offset of cavitation, respectively (Sparks and Black, 1999; Domec and Gartner, 2001).

Xylem density and anatomy

Xylem density (ρ_x, g cm⁻³) was evaluated from the same stem segments used for cavitation measurements (*n* = 5 per genotype; 33 genotypes). Stem samples 4 cm long were placed in a vial of deionized water and allowed to equilibrate under vacuum at room temperature for five days. Xylem density was then determined following the protocol developed by Hacke et al. (2000) using the Archimedes' principle to measure fresh volume of the stem samples.

Xylem anatomical properties were evaluated on a subset of two groups of five genotypes contrasted for their mean value of Ψ₅₀: five genotypes were selected for their relatively low resistance to cavitation (mean Ψ₅₀ = -1.77 ± 0.02 MPa, hereafter referred to as 'less resistant' group), while five other genotypes were selected for their relatively high resistance to cavitation (mean Ψ₅₀ = -2.20 ± 0.01 MPa, hereafter referred to as 'more resistant' group). All anatomical measurements were made on 30 μm-thick stem cross sections obtained from a rotary microtome (RM 1225, Leica Microsystems, Vienne, Austria) from each stem sample used for cavitation measurements (*n* = 5 per genotype). Cross sections were stained for 45 s in a Safranin O solution (1

% (w: v) in ethanol 50%) before being flushed with absolute ethanol and permanently mounted on slides using Canada Balsam. Stained cross sections were observed under a light microscope (Leica DMR) coupled with a Leica DFC 320 digital camera, and image analysis was performed using the ImageJ software (<http://imagej.nih.gov>).

Anatomical traits related to hydraulic efficiency were measured from observations made on three radial sectors per stem section. Images of radial sectors were taken with an objective ×10. All of the vessels in the sectors were counted from pith to cambium, such that at least 465 vessels were counted per stem section. Vessel diameter (*d*, μm), mean vessel diameter (*d*_{mean}, μm), the percentage of vessel lumen area (*A*_v, %), the hydraulically weighted vessel diameter (*d*_h, μm), and the theoretical xylem specific hydraulic conductivity (*K*_{s(t)}, kg s⁻¹ m⁻¹ MPa⁻¹) were determined following the same protocol developed by Fichot et al. (2010). The total number of vessel groupings was calculated from the same images as the sum of solitary vessels and vessel clusters and was then used to estimate a vessel grouping index (*V*_G), defined as the ratio between the total number of vessels and the total number of vessel groupings (Carlquist, 1984; Scholz et al., 2013a). Values of the three radial sectors were averaged to give a single value per stem cross section.

The 'thickness to wall span ratio' [(*t/b*)_h²] was evaluated from images taken with an objective ×20. The (*t/b*)_h² was used as an anatomical surrogate of vessel wall reinforcement against implosion (Hacke et al., 2001) and was determined for vessel clusters in which at least the diameter of one vessel fell within ±8 μm of *d*_h (Fichot et al., 2010). The double-wall thickness between two clustered vessels (*t*_h, μm) was measured directly on images, while the width of the conduit (*b*, μm) was calculated as the diameter of the vessel obtained from the equivalent area.

Statistical analyses

Statistical analyses were performed using the R software (R Development Core Team). All tests were considered significant at *P* < 0.05. Means are expressed with their standard errors.

All the variables measured on the 33 genotypes were analysed using the following mixed linear model: *Y*_{ij} = μ + *B*_{*i*} + *G*_{*j*} + *e*_{ij}, where *Y*_{ij} refers to tree individual performance, μ is the general mean, *B*_{*i*} is the effect of block *i* considered as fixed, *G*_{*j*} is the effect of the genotype *j* considered as random and *e*_{ij} is the residual error. Data required no transformation to meet the assumption of homoscedasticity and normal distribution of residuals. Variance components were estimated using the restricted maximum likelihood method. Broad-sense heritability was then calculated on an individual basis (*H*²_{ind}) according to the following equation: *H*²_{ind} = σ²_G / (σ²_G + σ²_e), where σ²_G and σ²_e are genetic and residual variance components. The coefficient of genetic variation (CV_G) was calculated as the ratio of genetic standard deviation to the mean and was expressed as a percentage. Given that phenological traits and Δ¹³C were respectively expressed according to an arbitrary scale (i.e. in DOY) and a standard (i.e. the Vienna Pee Belemnite standard), CV_G values were not estimated for these traits because they would be meaningless (Brendel, 2014).

Prior to exploring the differences between the two groups of five genotypes for traits related to xylem anatomy, a Levene's test was performed and indicated that variances were not homoscedastic between the two groups. In addition, the distribution of individual data did not follow a normal distribution within each group. The differences between the two groups of genotypes were therefore tested using the non-parametric Mann–Whitney–Wilcoxon test (*W*). Phenotypic relationships between traits were analysed on a genotypic mean basis and described using linear regressions and Pearson's correlation coefficients (*r*_p).

Results

Stem xylem resistance to drought-induced cavitation, xylem density and xylem anatomy

Significant differences (*P* < 0.001) were detected between the 33 genotypes for Ψ₁₂, Ψ₅₀, and Ψ₈₈. The xylem tension

inducing 50% loss of hydraulic conductivity (Ψ_{50}) ranged from -1.72 to -2.31 MPa between extreme genotypic means; the same amplitude of variation was observed for Ψ_{12} and Ψ_{88} (Supplementary Fig. S1). Significant differences ($P < 0.001$) were also detected between genotypes for the slope parameter, which ranged from 141 to 270% MPa $^{-1}$. However, strong and positive relationships were detected between Ψ_{12} , Ψ_{50} , and Ψ_{88} ($0.86 \leq r_p \leq 0.98$, $P < 0.001$), indicating that the shape of the curves slightly varied between genotypes. High values of broad-sense heritability on an individual basis were recorded for Ψ_{12} ($H^2_{\text{ind}} = 0.57$), Ψ_{50} ($H^2_{\text{ind}} = 0.72$), and Ψ_{88} ($H^2_{\text{ind}} = 0.70$) and were associated with low coefficients of genetic variation ($\text{CV}_G = 7.0$, 7.3 , and 7.6% for Ψ_{12} , Ψ_{50} , and Ψ_{88} respectively).

Xylem density (ρ_x) varied significantly ($P < 0.001$) among the 33 genotypes from 0.353 to 0.468 g cm $^{-3}$ (Fig. 1A). The value of H^2_{ind} and CV_G estimated for ρ_x ($H^2_{\text{ind}} = 0.73$ and $\text{CV}_G = 7.1\%$) were comparable to those obtained for Ψ_{50} . A significant and negative relationship was detected between ρ_x and Ψ_{50} ($r_p = -0.44$, $P = 0.010$; Fig. 1A).

Significant differences ($P < 0.001$) were detected between the two groups of five genotypes for all anatomical traits related to mechanical reinforcement at the vessel level (Fig. 2A,B): on average, the 'more resistant' group exhibited higher $(t/b)_h^2$ (0.023 ± 0.001 vs. 0.013 ± 0.001) and t_h (6.60 ± 0.04 μm vs. 5.3 ± 0.2 μm) than the 'less resistant' group (Fig. 2A,B). Traits related to hydraulic efficiency also differed significantly ($P < 0.01$) between the two groups of genotypes: on average, the 'more resistant' group exhibited lower d_{mean} (35.0 ± 0.9 μm vs. 36.9 ± 0.3 μm), A_v ($18.1 \pm 0.4\%$ vs. $19.7 \pm 0.5\%$) and $K_{s-(t)}$ (9.5 ± 0.3 kg s $^{-1}$ m $^{-1}$ MPa $^{-1}$ vs. 11.7 ± 0.5 kg s $^{-1}$ m $^{-1}$ MPa $^{-1}$) than the 'less resistant' group (Fig. 2C,D,E). A significant difference was also detected between the two groups of genotypes for vessel grouping (V_G): the 'more resistant' group exhibited a lower value of V_G than the 'less resistant' group (1.65 ± 0.04 vs. 1.76 ± 0.05 ; Fig. 2F).

Carbon isotope discrimination and leaf gas exchange

Significant differences ($P < 0.001$) were detected between the 33 genotypes for $\Delta^{13}\text{C}$, which ranged from 20.6 to 23.0% between extreme genotypic means (Fig. 1B). This range of variation was comparable to those previously reported for $\Delta^{13}\text{C}$ in the same population under non-limiting water conditions (Chamaillard *et al.*, 2011; Guet *et al.* unpublished results). A linear and negative relationship was detected between $\Delta^{13}\text{C}$ and WUE_i as expected from theory ($r_p = -0.75$, $P = 0.012$; Supplementary Fig. S2). The variations observed for WUE_i were mainly driven by variations in g_s ($r_p = -0.77$, $P = 0.008$) rather than in A ($r_p = -0.14$, $P = 0.685$). A high H^2_{ind} (0.70) was recorded for $\Delta^{13}\text{C}$. There was no significant relationship between $\Delta^{13}\text{C}$ and ρ_x ($r_p = 0.13$, $P = 0.456$) or Ψ_{50} ($r_p < 0.01$, $P = 0.991$; Fig. 1B).

Bud phenology and growth

Significant differences ($P < 0.001$) were detected between the 33 genotypes for the timing of phenological events. Bud

flush and bud set respectively occurred in a range of 24 days between 14 April (DOY 104) and 08 May (DOY 128) and between 17 August (DOY 229) and 10 September (DOY 253) for all genotypes. The 33 genotypes also differed for growth. The shoot height increment in 2013 varied from 54 to 145 cm and total above-ground dry mass spanned from 78 to 352 g between extreme genotypic means. Bud flush exhibited the highest value of broad-sense heritability ($H^2_{\text{ind}} = 0.94$) among the different studied traits, while bud set and growth variables exhibited low to moderate H^2_{ind} values ($0.20 \leq H^2_{\text{ind}} \leq 0.58$). Shoot height increment and total above-ground dry mass exhibited comparable CV_G values ($\text{CV}_G = 23.3\%$ and 25.7% , respectively).

Significant relationships were detected between shoot height increment and the date of bud flush ($r_p = -0.50$, $P = 0.003$) and bud set ($r_p = 0.38$, $P = 0.030$). However, these relationships were mainly explained by the fact that the genotypes that flushed late or set bud early achieved a low shoot height increment. Total above-ground dry mass did not correlate with the date of bud flush ($r_p = -0.03$, $P = 0.856$) or bud set ($r_p = 0.07$, $P = 0.682$). No significant relationship could be detected between $\Delta^{13}\text{C}$ and growth performances, as inferred from shoot height increment ($r_p = -0.17$, $P = 0.333$) or total above-ground dry mass ($r_p = -0.14$, $P = 0.451$). A positive relationship was detected between xylem density and the date of bud flush ($r_p = 0.40$, $P = 0.020$), but was principally explained by the fact that the genotypes that flushed bud later exhibited the highest wood density. No significant relationship was detected between xylem density and the date of bud set ($r_p = -0.29$, $P = 0.097$). Xylem density (ρ_x) was negatively correlated with shoot height increment ($r_p = -0.46$, $P = 0.008$) and tended to scale negatively with total above-ground dry mass ($r_p = -0.30$, $P = 0.086$). A significant and positive relationship was evidenced between Ψ_{50} and the date of bud set ($r_p = 0.46$, $P = 0.008$; Fig. 1C), while no correlation could be observed with bud flush ($r_p = -0.26$, $P = 0.144$). There was no relationship between Ψ_{50} and shoot height increment ($r_p = 0.20$, $P = 0.249$) or total above-ground dry mass ($r_p < -0.01$, $P = 0.995$; Fig. 1D).

Discussion

Genetic variation for stem xylem resistance to cavitation

Compared with other forest tree species of the Northern Hemisphere, poplars are particularly vulnerable to drought-induced cavitation (Maherali *et al.*, 2004). The results obtained on the *P. nigra* population studied here (mean Ψ_{50} across the 33 genotypes = -2.01 MPa) confirm this trend and are consistent with data already reported for other poplar species ($-0.70 \leq \Psi_{50} \leq -2.13$ MPa) and hybrids ($-1.07 \leq \Psi_{50} \leq -2.19$ MPa) (Fichot *et al.*, 2015).

Broad-sense heritability recorded for Ψ_{50} in the present study was high ($H^2_{\text{ind}} = 0.72$), indicating that micro-environmental variations were well controlled. This enabled the detection of significant genetic variation for xylem resistance to cavitation within the population. Large-scale phenotyping

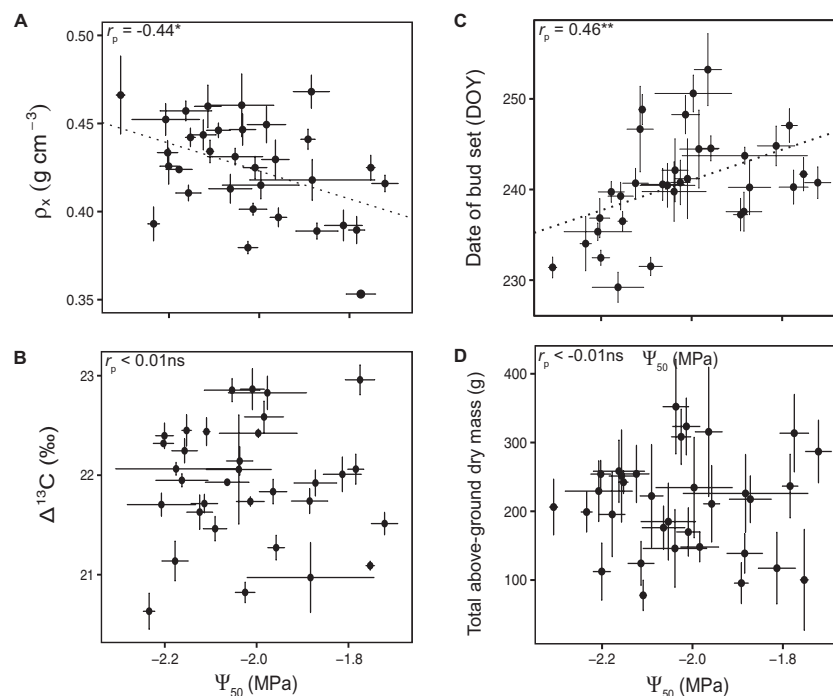


Fig. 1. Relationships between xylem resistance to drought-induced cavitation estimated as the stem xylem tension inducing 50% loss of hydraulic conductivity (Ψ_{50}) and (A) xylem density (ρ_x), (B) bulk leaf carbon isotope discrimination ($\Delta^{13}\text{C}$), (C) date of bud set and (D) total above-ground dry mass. Each point represents genotypic means $\pm$ standard error. Dashed lines represent linear regressions fitted to the data. Pearson's correlation coefficients (r_p) were calculated on a genotypic mean basis ($n = 33$). ** $P < 0.01$; * $P < 0.05$; ns, non-significant at a 5% risk level. DOY, day of the year.

of cavitation resistance has already been conducted on natural populations of *Pinus* species (Corcuera et al., 2011; Lamy et al., 2011, 2014; Sterck et al., 2012; López et al., 2013) and *F. sylvatica* (Wortemann et al., 2011), but such studies are lacking for riparian tree species. This study is believed to be the first reporting estimates of the genetic variation expressed for this trait within a natural population of a riparian tree species. A low CV_G value was found for Ψ_{50} (7.3%), which is consistent with results previously reported in natural populations of maritime pine ($\text{CV}_A \approx 5\%$; Lamy et al., 2011, 2014).

Although black poplar and maritime pine widely differ in their ecological preferences, these species seem to maintain a low level of genetic variation for cavitation resistance. In maritime pine, the low level of genetic variation for cavitation resistance was associated with a low level of phenotypic plasticity, which led Lamy et al. (2014) to suggest that facing drought through cavitation resistance-related traits may have limited adaptive potential in maritime pine as compared to other morphological and growth-related traits. Black poplar is, however, much more vulnerable to drought-induced cavitation than maritime pine [mean Ψ_{50} over six populations = -3.93 MPa (Lamy et al., 2011)]. Therefore, the level of genetic variation expressed for stem cavitation resistance in the *P. nigra* population may not have the same physiological significance as in maritime pine because CV_G do not refer to the same means. The 0.59 MPa difference observed between extreme genotypic means could actually be physiologically relevant for enabling evolutionary responses in such vulnerable species, for which cavitation events begin as soon as xylem tension reaches -1.5 to -2.0 MPa. In addition, stem xylem resistance to cavitation was only evaluated under non-limiting

water conditions in the present study. The capacity for a plastic response of cavitation resistance in the *P. nigra* population studied has been ignored; however, previous results obtained on different hybrid poplars have indicated that xylem resistance to cavitation is responsive to moderate water deficit and in a genotype-dependent manner, with decreases in Ψ_{50} down to 0.60 MPa (Fichot et al., 2010).

Stem cavitation resistance and xylem structure

Trade-offs between xylem resistance to cavitation, hydraulic efficiency, and mechanical reinforcement have long been hypothesized from interspecific comparisons, but have not been systematically detected depending on the species sampled and the correction applied for phylogenetic dependencies (e.g. Pockman and Sperry, 2000; Maherali et al., 2004; Pratt et al., 2007; Jacobsen et al., 2009; Markesteijn et al., 2011). At the intraspecific level, comparisons of population performances have generally failed to evidence relationships between xylem structure and function (Kavanagh et al., 1999; Choat et al., 2007; Martínez-Vilalta et al., 2009; Schreiber et al., 2011; Lamy et al., 2012; Sterck et al., 2012; López et al., 2013). The present study is the first one addressing the question within a single population, and the results obtained here on *P. nigra* support the idea that trade-offs between xylem structure and function can actually occur at such scale. Given that the *P. nigra* genotypes originated from the same population, and were exposed to the same environmental conditions and evolutionary drivers *in situ*, it can be concluded that the trade-offs evidenced in this study reflect true functional relationships between traits. The apparent trade-off between

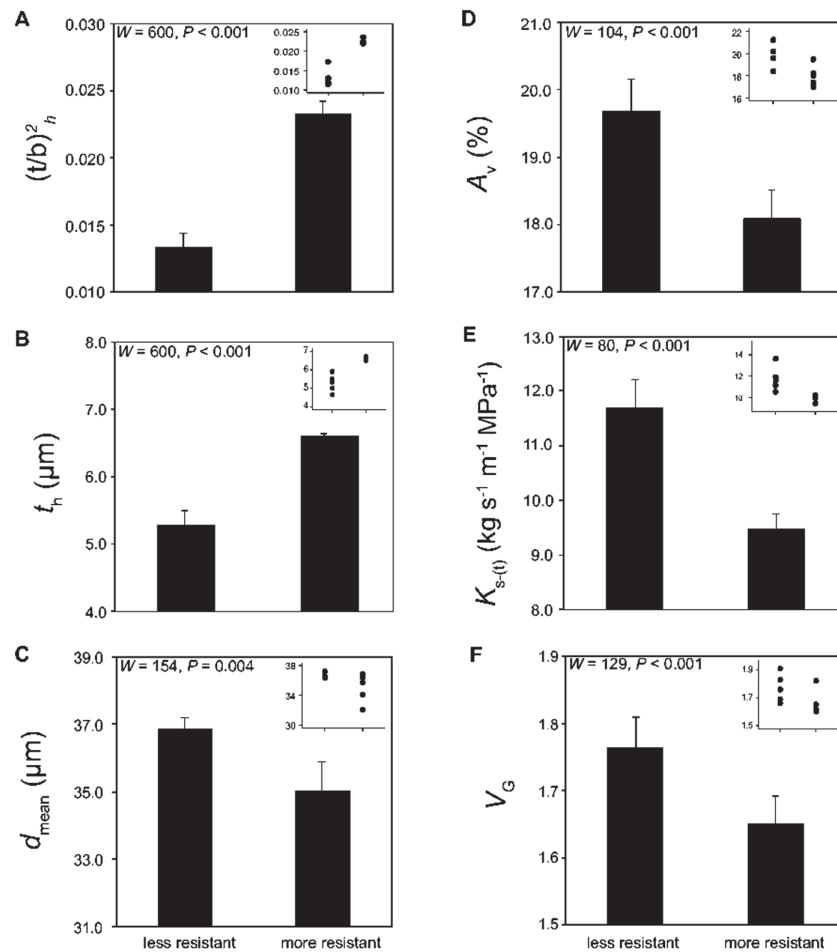


Fig. 2. Differences in xylem anatomical traits between the group of five genotypes with a relatively low and a relatively high resistance to drought-induced cavitation ('less resistant' and 'more resistant', respectively). Data are shown for: (A) thickness to wall span ratio $[(t/b)_h^2]$, (B) double vessel wall thickness (t_h), (C) mean vessel diameter (d_{mean}), (D) percentage of vessel lumen area (A_v), (E) theoretical xylem specific hydraulic conductivity ($K_{s(t)}$), and (F) vessel grouping index (V_G). Each bar represents means of each group $\pm$ standard error. The differences between means of the two groups were tested according to a Mann–Whitney–Wilcoxon test (W). Each inset shows the distribution of genotypic means within each group.

hydraulic efficiency and safety could be explained by the 'rare pit' hypothesis (also called the 'pit area' hypothesis) (Wheeler *et al.*, 2005; Christman *et al.*, 2009). Genotypes with smaller vessels tend to be less efficient in water transport, but may also have a lower pitted wall area, which in turn decreases the probability of having a large pore in the pit membrane more prone to air-seeding. The relationship between cavitation resistance and mechanical reinforcement has been generally interpreted as a result of the need for cavitation-resistant genotypes to withstand the strong mechanical load induced by high negative pressures on vessel wall to avoid vessel wall collapse (Hacke *et al.*, 2001). An alternative hypothesis has been proposed to explain the relationship between cavitation resistance and mechanical reinforcement at the vessel level and relates to the co-variation between vessel wall thickness and pit membrane properties. Anatomical observations of pit membrane properties using electron microscopy evidenced that the porosity and the degree to which pit membranes are damaged by mechanical deformation decrease with an increase of pit membrane thickness, which is positively related to vessel wall thickness (Jansen *et al.*, 2009; Tixier *et al.*, 2014).

Besides xylem traits directly related to water transport efficiency and mechanical reinforcement, the more cavitation-resistant genotypes exhibited a lower degree of vessel grouping. These results are consistent with those previously reported by Scholz *et al.* (2013b) across *Prunus* species. They support the hydraulic model of Loepe *et al.* (2007), which suggests that a high degree of vessel connectivity decreases xylem resistance to cavitation by increasing the risk of embolism spreading between adjacent vessels via air-seeding. However, this is in contradiction with the vessel grouping hypothesis postulated by Carlquist (1984), stating that xeric-adapted species have a higher degree of vessel grouping than those growing in mesic conditions to bypass the more frequent embolism. This hypothesis has since been supported by Lens *et al.* (2011) who found a positive relationship between cavitation resistance and the degree of vessel grouping across *Acer* species.

Stem xylem resistance to cavitation, growth, and WUE

Increased xylem resistance to drought-induced cavitation has long been thought to come at the expense of reduced plant

growth. Such a trade-off could be detected if cavitation resistance comes at the cost of greater mechanical reinforcement of the xylem, a feature associated with greater construction costs in terms of carbon allocation (Enquist *et al.*, 1999). However, the relationship between cavitation resistance and growth remains equivocal. These two traits were found to be unrelated in natural populations of different forest tree species (Martínez-Vilalta *et al.*, 2009; Lamy *et al.*, 2011; Schreiber *et al.*, 2011; Sterck *et al.*, 2012; López *et al.*, 2013). Actually, only few studies evidenced a significant relationship between these traits, but with opposite direction. A negative relationship was detected between xylem resistance to cavitation and biomass production across willow genotypes (Wikberg and Ögren, 2004; Cochard *et al.*, 2007) and provenances of cedar (Ducrey *et al.*, 2008). By contrast, a positive relationship was detected between these traits across poplar hybrids (Fichot *et al.*, 2010). In this study, there was no evidence for a trade-off between xylem resistance to cavitation and growth, despite cavitation resistance being positively related to xylem density, which in turn was negatively correlated with shoot height increment. In fact, although the relationships between xylem density and cavitation resistance or shoot height increment were significant, they remained weak ($r_p < 0.50$), indicating that the variation in xylem density only explains a small part of the variation in cavitation resistance and growth. This may partly explain why cavitation resistance and growth were uncoupled in the *P. nigra* population studied.

Growth performances of the *P. nigra* genotypes studied here were significantly influenced by the dates of bud flush and bud set. However, these relationships were mainly explained by the fact that the genotypes that flushed late or set bud early achieved the lowest shoot height increment. Only a weak negative relationship was detected between xylem resistance to cavitation and the date of bud set and was mainly explained by the fact that the genotypes that set bud earlier exhibited the highest degree of resistance to cavitation. The complex relationships linking bud phenology to cavitation resistance and growth indicate that the *P. nigra* population studied maintains a wide diversity of phenotypic combinations for these traits. This may further explain why growth was uncoupled from cavitation resistance in the studied *P. nigra* population.

$\Delta^{13}\text{C}$ is a complex and composite trait generally used as a proxy of WUE_i but also, more generally, as a set point for leaf physiology (Ehleringer *et al.*, 1992). As expected from theory, $\Delta^{13}\text{C}$ was negatively related to WUE_i in the studied *P. nigra* population, but no significant relationship was detected with leaf nitrogen content (data not shown) or growth variables. These results indicate that the variations in $\Delta^{13}\text{C}$ mainly reflected variations in plant water-use as already reported in hybrid poplars (Monclus *et al.*, 2006). Results previously obtained in natural populations of different forest tree species indicate that the relationship between $\Delta^{13}\text{C}$ and Ψ_{50} is species-specific. Martínez-Vilalta *et al.* (2009) found a positive relationship between $\Delta^{13}\text{C}$ and Ψ_{50} across populations of *Pinus sylvestris* L.. By contrast, Lamy *et al.* (2011) evidenced a negative relationship between these traits in natural populations of maritime pine, but the relationship broke down at the

genetic level. $\Delta^{13}\text{C}$ and Ψ_{50} were found to be unrelated across genotypes of *Populus tremuloides* (Schreiber *et al.*, 2011). In the present study, no significant relationship could be detected between $\Delta^{13}\text{C}$ and Ψ_{50} . The lack of relationship between these two traits has a major functional significance because it indicates that stem cavitation resistance and WUE could evolve independently in response to environmental changes.

Conclusions

This study is, as far as can be determined, the first one reporting estimates of genetic variation expressed for stem xylem resistance to cavitation and its relationships with xylem structure, growth, and WUE at the within-population level and in a riparian tree species. Significant genetic variation was recorded for stem xylem resistance to cavitation in the studied *P. nigra* population under non-limiting water conditions. The findings also demonstrate that the trade-offs between xylem resistance to cavitation, hydraulic efficiency, and mechanical reinforcement of the xylem can be evidenced at the within-population level. However, how the expression and the amplitude of genetic variation would be modulated under drier conditions has not been explored. Such information would be valuable to gain insight into the phenotypic plasticity and adaptive potential of natural populations of black poplar.

Supplementary material

Supplementary data can be found at *JXB* online.

Supplementary Fig. S1. Stem xylem vulnerability curves of the 33 *P. nigra* genotypes.

Supplementary Fig. S2. Relationship between intrinsic WUE and bulk leaf carbon isotope discrimination.

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