

# Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* × *Populus nigra* hybrids

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## ABSTRACT

We examined the relationships between xylem resistance to cavitation and 16 structural and functional traits across eight unrelated *Populus deltoides* × *Populus nigra* genotypes grown under two contrasting water regimes. The xylem water potential inducing 50% loss of hydraulic conductance ( $\Psi_{50}$ ) varied from  $-1.60$  to  $-2.40$  MPa. Drought-acclimated trees displayed a safer xylem, although the extent of the response was largely genotype dependant, with  $\Psi_{50}$  being decreased by as far as 0.60 MPa. At the tissue level, there was no clear relationship between xylem safety and either xylem water transport efficiency or xylem biomechanics; the only structural trait to be strongly associated with  $\Psi_{50}$  was the double vessel wall thickness, genotypes exhibiting a thicker double wall being more resistant. At the leaf level, increased cavitation resistance was associated with decreased stomatal conductance, while no relationship could be identified with traits associated with carbon uptake or bulk leaf carbon isotope discrimination, a surrogate of intrinsic water-use efficiency. At the whole-plant level, increased safety was associated with higher shoot growth potential under well-irrigated regime only. We conclude that common trade-offs between xylem resistance to cavitation and other physiological traits that are observed across species may not necessarily hold true at narrower scales.

**Key-words:** carbon isotope discrimination; photosynthesis; poplars; relative growth rate; stomatal conductance; water deficit; water relations; xylem anatomy; xylem vulnerability to embolism.

## INTRODUCTION

One of the most remarkable aspects of plant water relations is probably that during transpiration, water moves through the xylem under tension (negative pressure). This places xylem under the constant threat of cavitation through air seeding. As xylem tension reaches a critical threshold, air seeding occurs through bordered pits as a result of an air bubble being sucked into a water-filled conduit from an adjacent air-filled conduit. Cavitation events actually lead to vapour-filled (embolized) conduits that no longer carry water, thereby decreasing xylem hydraulic conductivity (Tyree & Sperry 1989). To prevent damaging drops in xylem tension, stomata act as a pressure regulator (Sperry *et al.* 2002), but this may come at the cost of reduced CO<sub>2</sub> uptake and *in fine*, reduced plant growth. Therefore, xylem resistance to cavitation appears as one key trait in understanding the conflicting balance between maximizing carbon uptake and minimizing water loss.

Xylem resistance to cavitation is usually described through vulnerability curves (VCs), that is, the plot of percent loss of xylem hydraulic conductivity versus xylem tension. Cavitation resistance has been widely documented across species and varies greatly (Maherali, Pockman & Jackson 2004). Although reports at the intraspecific level are by far less numerous, there is also evidence that cavitation resistance can vary between populations of the same species (Alder, Sperry & Pockman 1996; Mencuccini & Comstock 1997; Sparks & Black 1999; Choat, Sack & Holbrook 2007) or between closely related genotypes (Neufeld *et al.* 1992; Vander Willigen & Pammenter 1998; Pita, Gascó & Pardos 2003; Sangsing *et al.* 2004; Cochard, Casella & Mencuccini 2007). However, most of our current understandings of how xylem resistance to cavitation is related to other functional traits involved in plant water transport and carbon uptake has come from interspecific comparisons. The comparisons of more or less distantly related taxa growing in contrasting environments tend to indicate that

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increased cavitation resistance is one key component of species growing in drought-prone habitats (Kolb & Sperry 1999; Hacke, Sperry & Pittermann 2000; Pockman & Sperry 2000). A structural trade-off between xylem safety and xylem transport efficiency has for long been hypothesized and several studies have since supported such a trade-off (Martínez-Vilalta *et al.* 2002; Hacke *et al.* 2006; Hacke, Jacobsen & Pratt 2009). This may be explained according to the pit area hypothesis (Wheeler *et al.* 2005), species with more efficient xylem being more susceptible to cavitation because larger and longer conduits tend to have a greater pitted wall area; in turn, a greater pitted area may lead to a greater probability of having large pit membrane pores that are more prone to air seeding. However, results from other studies tend to indicate that the trade-off between xylem safety and efficiency may depend on species considered and life history (Maherali *et al.* 2004; Choat *et al.* 2007; Jacobsen *et al.* 2007; Pratt *et al.* 2007). In addition, several studies focusing on the relationships between cavitation resistance and xylem structural properties showed that increased resistance to cavitation comes at the cost of greater mechanical reinforcement. Such a relationship lies in the necessity for xylem tissue to resist stresses induced by higher negative hydrostatic pressures and has been shown to hold at the cell level (as inferred from conduit wall resistance to collapse), the tissue level (as inferred from wood density) and the organ level (as inferred from modulus of rupture and elasticity) (Hacke *et al.* 2001; Jacobsen *et al.* 2005, 2007; Pratt *et al.* 2007; Jacobsen *et al.* 2009). Finally, although resistance to cavitation may theoretically impose constraints on leaf gas exchange, few studies have directly addressed the relationship between cavitation resistance and leaf physiological traits related to water and CO<sub>2</sub> fluxes. For instance, increased cavitation resistance was found to be weakly associated with lower gas exchange rates and higher water-use efficiency (WUE) in a cross-species comparison of 14 temperate trees (Maherali *et al.* 2006).

Xylem resistance to cavitation has been found to adjust to different levels of irradiance (Cochard, Lemoine & Dreyer 1999; Lemoine, Jacquemin & Granier 2002; Barigah *et al.* 2006; Caquet *et al.* 2009), nutrients (Harvey & Van Den Driessche 1997, 1999; Ewers, Oren & Sperry 2000) or differences in soil porosity (Holste, Jerke & Matzner 2006). However, evidence for a direct effect of soil water availability on cavitation resistance remains surprisingly very poorly documented (Holste *et al.* 2006; Beikircher & Mayr 2009). Although increased xylem resistance to cavitation seems to be adaptive for species growing in drier environments, comparative studies may confound both genetic variability and phenotypic plasticity. In this context, common garden tests are valuable tools to separate the distinct effects of genetics and acclimation.

Poplar species (*Populus* spp.) and especially interspecific hybrids are known to be among the most superior trees under temperate latitudes in terms of growth rates (Heilman *et al.* 1996). However, this comes at the expense of large water requirements, and despite some degree of genetic variability, poplars are known to be very sensitive to

water deprivation (Braatne, Hinckley & Stettler 1992; Tschaplinski, Tuskan & Gunderson 1994; Monclus *et al.* 2006). Xylem resistance to cavitation has already been documented for pure poplar species (Sperry, Perry & Sullivan 1991; Tyree, Alexander & Machado 1992; Hacke & Sauter 1996; Sparks & Black 1999; Tognetti, Longobucco & Raschi 1999; Hukin *et al.* 2005) as well as for various interspecific hybrids (Cochard, Ridolfi & Dreyer 1996; Harvey & Van Den Driessche 1997, 1999; Cochard *et al.* 2007, 2008). All these studies demonstrated that although some variation does exist, poplars are among the most vulnerable tree species with cavitation events beginning as soon as xylem tension reaches the range of  $-1$  to  $-1.5$  MPa.

It is intriguing to test whether common associations between xylem resistance to cavitation and other physiological traits observed when comparing contrasting species still hold true when comparing populations of the same species or closely related genotypes. Here, we examined the relationships between stem xylem resistance to cavitation and 16 structural and functional traits related to water transport and carbon uptake under two contrasting water regimes using eight unrelated poplar genotypes but all issued from a unique hybrid formula *Populus deltoides* Bartr. ex Marsh.  $\times$  *Populus nigra* L. The eight genotypes chosen were already known for differing in water use, growth behaviour and xylem anatomy (Monclus *et al.* 2005, 2006; Fichot *et al.* 2009). Cavitation measurements were performed on 1-year-old shoots using the Cavitron technique (Cochard *et al.* 2005). Saplings of the eight genotypes were grown in a two-plot common garden test, with one plot subjected to a moderate summer water deficit and the other one maintained irrigated throughout the growing season (control). The specific objectives of the study were to determine: (1) the extent of genotypic variation in cavitation resistance and whether water deficit induced acclimation; (2) whether cavitation resistance scaled with xylem traits associated to water transport efficiency and mechanical reinforcement; (3) whether cavitation resistance correlated with leaf traits associated with carbon and water fluxes; and (4) whether cavitation resistance was correlated with individual growth performances.

## MATERIALS AND METHODS

### Plant material and experimental design

Experiments were conducted in 2008, on a 250 m<sup>2</sup> coppice plantation of eight unrelated commercialized *P. deltoides* Bartr. ex Marsh  $\times$  *P. nigra* L. genotypes ('*Agathe\_F*', '*Cima*', '*Eco28*', '*Flevo*', '*I45-51*', '*Luisa\_Avanzo*', '*Pannonia*' and '*Robusta*'). These eight genotypes were already previously selected for contrasting water use, growth performances and xylem anatomy (Monclus *et al.* 2006; Fichot *et al.* 2009). The plantation was located at the Institut National de la Recherche Agromanique (INRA) station of Genetic and Forest Research of Orléans, France (47°46' N, 1°52' E, 110 m above sea level). The field trial was set in June 2006 on a loamy sand soil (pH = 5.9) from 0.25-m-long hardwood

**Table 1.** Monthly climate characteristics of the study site during the period of active growth

Month	Mean T (°C)	Cumulative P (mm)	Cumulative PET (mm)	P/PET	VPD (kPa)
May	15.86	60.0	75.0	0.80	1.57
June	17.53	13.0	66.2	0.20	1.63
July	18.29	36.5	80.9	0.45	2.00
August	18.34	76.5	48.9	1.56	1.58
September	13.59	8.0	35.9	0.22	1.51

Climate data were obtained from a nearby meteorological station located on the field trial. Mean T, mean temperature; cumulative P, cumulative precipitations; cumulative PET, cumulative potential evapotranspiration; P/PET, ratio of cumulative precipitation to cumulative potential evapotranspiration; VPD, water vapour pressure deficit.

cuttings without fertilizer addition. Two twin plots with north–south oriented rows were established 15 m apart from each other. Each plot consisted in five complete randomized blocks with three individuals of each genotype per block. Spacing within and between rows was 0.75 m × 1.20 m, respectively, accommodating an overall density of about 11 000 plants per hectare. A border row of the cv. *Mellone\_Caro* was planted around each plot to minimize edge effects. Throughout each growing season, the plantation management included mechanical and manual weed control as well as irrigation. At the end of 2006 and 2007, all saplings were cut back to create a coppice system. In 2007 and 2008, bud flush occurred within the first two weeks of April for all individuals.

## Environmental conditions and drought induction

Daily environmental conditions in terms of temperature (minimum, mean and maximum, T, °C), relative humidity (RH, %), potential evapotranspiration (PET, mm) and precipitations (P, mm) were recorded at a nearby meteorological station. A monthly vapour pressure deficit (VPD) was calculated using mean monthly temperature and RH to estimate actual vapour pressure, and mean monthly maximum temperature to estimate saturation vapour pressure as described in Martínez-Vilalta *et al.* (2009). Monthly values for climate data are given in Table 1 for the time period May–September, which corresponded roughly to the growing season.

In 2007 and 2008, from May to September, irrigation was performed using overhead sprinklers and was designed to meet evaporative demand (i.e. 4.5 mm of water were sprinkled every time cumulative PET reached 4 mm). In 2008, irrigation was withheld from one of the two plots from June 18 to the end of the growing season, while the second one was maintained irrigated (control). Thus, the not-irrigated plot only received rainfall precipitations.

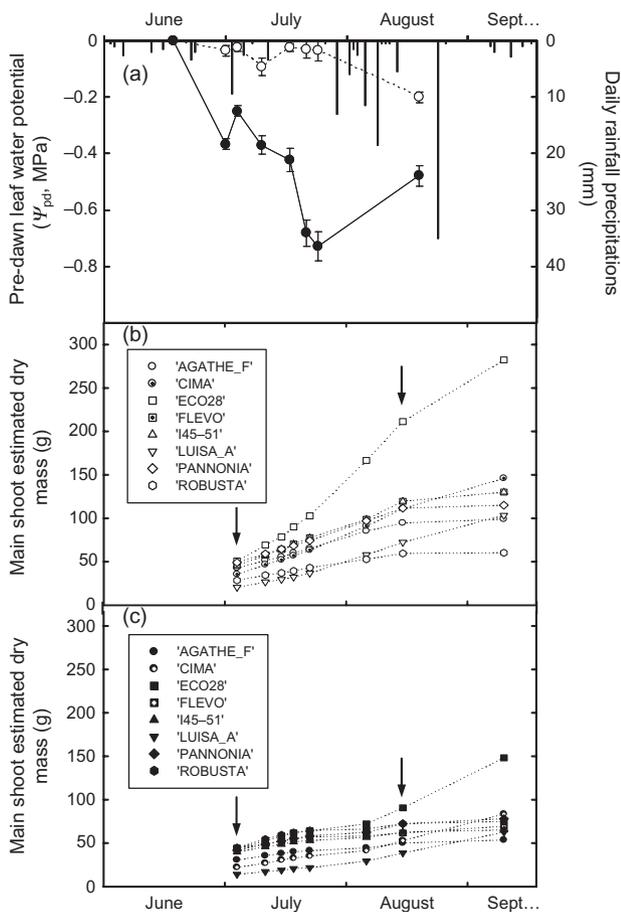
## Soil and plant water status

Water potentials were measured using a pressure chamber (PMS Instruments, Albany, OR, USA). Pre-dawn leaf water potential was used as an index of soil water potential and

was monitored over each plot from 18 June to 18 August ( $\Psi_{pd}$ , MPa) (see Table 2 for the definition of trait abbreviations used in this article). Measurements were performed on one individual per block for the three genotypes ‘*Agathe\_F*’, ‘*Flevo*’ and ‘*Robusta*’ ( $n = 5$  per genotype per plot). Minimum xylem water potential ( $\Psi_{xmin}$ , MPa) was measured on the main shoot of one individual of each genotype per block ( $n = 5$  per genotype per plot) on 23 July 2008, which corresponded to the summer drought peak experienced by the plants in the field (see Fig. 1). To do this, fully mature leaves (foliar index 13–16) were covered with plastic film and a final layer of aluminium foil the evening preceding measurements. On the day of measurements, leaves were removed from plants between 11.00 AM and 13.00 PM local time, put in small plastic bags with moist

**Table 2.** List of abbreviations used in the text

Symbol	Definition	Units
Functional traits		
$A$	Leaf net CO <sub>2</sub> assimilation rate	$\mu\text{mol m}^{-2} \text{s}^{-1}$
$g_s$	Stomatal conductance to water vapour	$\text{mmol m}^{-2} \text{s}^{-1}$
$\Delta$	Bulk leaf carbon isotope discrimination	‰
$\Psi_{50}$	Water potential causing 50% loss of hydraulic conductance	MPa
$\Psi_{12}$	Water potential causing 12% loss of hydraulic conductance	MPa
$\Psi_{88}$	Water potential causing 88% loss of hydraulic conductance	MPa
$\Psi_{pd}$	Pre-dawn leaf water potential	MPa
$\Psi_{xmin}$	Minimum xylem water potential experienced	MPa
RGR	Relative growth rate	$\text{g g}^{-1} \text{day}^{-1}$
Structural traits		
$A_v$	Percentage of vessel lumen area per cross-sectional area	%
$d_h$	Hydraulic vessel diameter	$\mu\text{m}$
$d$	Mean vessel diameter	$\mu\text{m}$
$K_{S(t)}$	Theoretical xylem specific hydraulic conductivity	$\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$
$n_v$	Vessel density per cross-sectional area	$\text{mm}^{-2}$
$\rho_{\text{wood}}$	Wood density	$\text{g cm}^{-3}$
$t_h$	Double vessel wall thickness	$\mu\text{m}$
$(t/b)_h^2$	Thickness-to-span ratio	



**Figure 1.** Seasonal dynamics of drought stress. (a) Summer time course of pre-dawn leaf water potential ( $\Psi_{pd}$ , MPa; circles) and daily rainfall precipitations (mm; vertical bars). Values of pre-dawn leaf water potential are means  $\pm$  SE from 15 well-watered trees (open circles) and 15 drought-exposed trees (closed circles). (b) and (c) Summer growth kinetics of the main shoot in terms of estimated dry mass for well-watered and water deficit trees, respectively. Symbols are genotype means ( $n = 5$ ); SE are not shown for figure clarity. Summer kinetics in terms of shoot dry mass were established from summer kinetics of shoot height increment and from allometric relationships established between shoot height and shoot dry mass for each genotype and each irrigation condition at the end of the growing season (see the Materials and Methods section for details). Arrows indicate the time interval chosen for the calculation of the main shoot relative growth rate.

towel, placed on ice and transported to a near laboratory where they were rapidly processed. The water potential of the wrapped (non-transpiring) leaves was assumed to equal the minimum xylem water potential.

### Leaf gas exchange and carbon isotope discrimination

Net  $\text{CO}_2$  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 measured using a LI-6200 portable gas exchange system (Li-Cor Biosciences Inc., Lincoln, NE, USA). Measurements were

carried out over the two plots on 23 July, a cloudless day (average photosynthetic photon flux density during measurements of  $1350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), between 11.00 AM and 13.00 PM local time. Measurements were made on a fully illuminated young leaf, adjacent to the one used for  $\Psi_{\text{min}}$  ( $n = 5$  per genotype per plot). Leaf temperature, VPD and average  $\text{CO}_2$  concentration inside the chamber matched ambient conditions. The leaves were allowed to equilibrate inside the chamber for 20 s before measurements were taken.

Carbon isotope discrimination against  $^{13}\text{C}$  ( $\Delta$ ) was used as a time-integrated surrogate of intrinsic WUE (Farquhar & Richards 1984). Samplings for  $\Delta$  analyses were performed on 23 July on the same leaves that were used for leaf gas exchange measurements ( $n = 5$  per genotype per plot). Six calibrated disks of leaf lamina ( $2 \text{ cm}^2$ ) were taken from each leaf and oven-dried at  $60^\circ \text{C}$  for 48 h. The six calibrated disks were ground to a fine powder for the analysis of leaf carbon isotope composition ( $\delta^{13}\text{C}$ ). All analyses were performed at the technical platform of functional ecology at the INRA-Nancy ([http://www.nancy.inra.fr/les\\_plateaux\\_techniques/ptef](http://www.nancy.inra.fr/les_plateaux_techniques/ptef)). One milligram subsamples of ground material were enclosed in tin capsules and combusted. The  $\text{CO}_2$  produced by combustion was purified, and its  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratio was analysed with a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS) (Bremen, Germany) coupled to a Carlo Erba NA1500 elemental analyser (Carlo Erba Instruments, Milan, Italy). Carbon isotope composition was expressed relative to the Pee Dee Belemnite standard and was calculated as:

$$\delta^{13}\text{C} = [(R_{sa} - R_{sd})/R_{sd}] \times 1000 (\text{‰}) \quad (1)$$

where  $R_{sa}$  and  $R_{sd}$  are the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  ratios of the sample and the standard, respectively (Farquhar, Ehleringer & Hubick 1989). The accuracy of  $\delta^{13}\text{C}$  over the measurements was  $\pm 0.14\text{‰}$ . The discrimination between atmospheric  $\text{CO}_2$  ( $\delta_{\text{air}}$ ) and plant material ( $\delta_{\text{plant}}$ ) was then calculated as:

$$\Delta = (\delta_{\text{air}} - \delta_{\text{plant}})/(1 + (\delta_{\text{plant}}/1000)), \quad (2)$$

assuming  $\delta_{\text{air}}$  equals  $-8\text{‰}$  (Farquhar *et al.* 1989).

### Growth performances

The growth performance of the eight genotypes was described in terms of vigour of the main shoot [relative growth rate (RGR,  $\text{g g}^{-1} \text{day}^{-1}$ ) and annual dry mass (g)] and in terms of total potential yield [annual total above-ground coppice dry mass (g)] which is both a function of the number of shoots per individual and of their vigour. The calculation of the main shoot RGR was performed by combining seasonal growth kinetics in terms of shoot height increment and allometric relationships established between shoot height and shoot dry mass at the end of the growing season. To this purpose, the height of the main shoot of each individual was measured to the nearest centimetre using a graduated height pole eight times from 4 July to 8

September 2008. At the end of the growing season, all living shoots of all individuals were harvested, measured to the nearest centimetre and oven dried (50 °C) until constant mass. The dry mass of each shoot was recorded to the nearest 0.5 g and was used to calculate annual total above-ground coppice dry mass at the individual level for each genotype. Allometric relationships between shoot height and shoot dry mass were then established for each genotype and each irrigation condition. The best fit was obtained with the following cubic equation:  $Shoot_{DM} = aH^3 + bH^2 + cH$ , where  $Shoot_{DM}$  is the shoot dry mass and  $H$  is the shoot height; for all genotypes, regressions were significant at  $P < 0.001$ , and  $r^2$  lied between 0.95 and 0.99. From these measurements (height increment and allometric relationships), seasonal growth kinetics (July–September) of the main shoots were established in terms of estimated dry mass (Fig. 1b,c). The main shoot RGR was then calculated as  $RGR = [\ln(m_2) - \ln(m_1)]/\Delta t$ , where  $m_1$  and  $m_2$  are the estimated shoot dry mass on 4 July and 14 August, respectively, and  $\Delta t$  is the time interval between the two dates (Cernusak, Winter & Turner 2009). This time interval was selected based on the fact that the trees from the irrigated plot were still under a period of active growth (Fig. 1b). Please note that the use of the term RGR throughout the paper actually refers to the RGR of the main shoot.

### Xylem resistance to cavitation

Xylem resistance to cavitation was assessed during October 2008 from the main shoots already used for growth kinetics,  $\Psi_{xmin}$ , leaf gas exchange and  $\Delta$  measurements ( $n = 5$  per genotype per plot). An 80-cm-long stem segment was sampled in the half top part of each shoot, immediately wrapped in moist towel and enclosed in a black plastic bag to minimize dehydration. Stem segments were brought to the laboratory where they were stored for a maximum of 6 d at 4 °C until measurements were completed. We used the Cavitrone technique (Cochard *et al.* 2005) to measure the vulnerability of stem xylem to water stress-induced cavitation. This technique uses the centrifugal force to increase the tension in a calibrated xylem segment while measuring variations in its hydraulic conductance at the same time. Calibrated samples of 0.7–0.8 cm in diameter and 28 cm in length were cut in air from the 80-cm-long segments and were air perfused at a pressure of 0.1 MPa (Cochard *et al.* 2005) before being set on the custom-built rotor and spun at given velocities. Maximal conductance ( $k_{max}$ ) was determined by setting the xylem pressure to a reference value (–0.75 MPa); preliminary experiments showed that there was no significant difference in  $k_{max}$  when beginning at less negative  $\Psi$ -values than –0.75 MPa (data not shown). Afterward, xylem pressure was set to gradually more negative pressures by 0.25–0.50 MPa steps. For each new xylem pressure, the hydraulic conductance ( $k_{\Psi}$ ) was measured at equilibrium and the percent loss of conductance (PLC) calculated as  $PLC = 100 \times (1 - k_{\Psi}/k_{max})$ . This procedure was repeated until PLC reached at least 90%. Rotor velocity was monitored with an electronic tachymeter (10 rpm

resolution). The dependence of PLC upon xylem pressure was used to generate VCs for each stem segment, which were fit to the following sigmoid function (Pammenter & Vander Willigen 1998):

$$PLC = 100/(1 + \exp(s/25 \times (\Psi - \Psi_{50}))), \quad (3)$$

where  $\Psi_{50}$  is the xylem tension causing 50% loss of conductance and  $s$  is the slope parameter. Values of  $\Psi_{50}$  were used to compare resistance to cavitation among genotypes, while  $s$  is representative of the range of xylem tension over which cavitation occurs. Using the Eqn 3 and the values of  $\Psi_{50}$  and  $s$ , we derived the xylem tension causing 12 and 88% loss of conductance for each sample ( $\Psi_{12}$  and  $\Psi_{88}$ , respectively), assumed to represent the xylem tension at the onset and the offset of cavitation, respectively (Sparks & Black 1999; Domec & Gartner 2001). Values of  $\Psi_{12}$ ,  $\Psi_{50}$ ,  $\Psi_{88}$  and  $s$  were then averaged for each genotype. To assess the degree of xylem safety against drought-induced embolism, a safety margin was calculated as the difference between  $\Psi_{12}$  and  $\Psi_{xmin}$ . Please note that  $\Psi_{12}$ ,  $\Psi_{50}$  and  $\Psi_{88}$  are reported as negative values throughout the paper.

### Vessel anatomy and hydraulic efficiency

Measurements of anatomical parameters were performed on the same stem segments which were already used for cavitation measurements ( $n = 5$  per genotype per plot). Stem cross sections 30  $\mu\text{m}$  thick were obtained with a hand microtome (RM 2155, Leica Microsystems, Vienne, Austria), stained with toluidine blue 0.1% (w : v), flushed with clean acetic water to improve contrast and avoid staining diffusion, and embedded in a glycerol-like medium. Stained sections were examined under a light microscope (Leica DMR) coupled with a Leica DFC 320 digital camera. For vessel diameter and vessel density analyses, observations were made on two opposite radial sectors that were most representative for the whole cross section. Images at 100 $\times$  magnification were taken of wedge-shaped sectors, using vascular rays as the borders, to sample for vessels from pith to cambium. All of the vessels in sectors were counted so that at least 150 vessels per stem were measured. Image analysis was performed using the image analysis software Visilog 6.3 (Noesis, St Aubain, France). Vessel diameters ( $d$ ,  $\mu\text{m}$ ) were calculated from vessel areas as the diameter of an equivalent circle. Vessel density ( $n_v$ ,  $\text{mm}^{-2}$ ) was calculated as the ratio of vessel number to xylem area. From all vessel counts, a mean vessel diameter ( $d_{mean}$ ,  $\mu\text{m}$ ) was calculated for each stem. The percentage transverse area of vessel lumen ( $A_v$ , %) was also estimated for each stem. To take into account the disproportionate contribution of large vessels to total flow, a hydraulic vessel diameter ( $d_h$ ,  $\mu\text{m}$ ) was derived from the formula  $d_h = (\sum d^5)/(\sum d^4)$  (Sperry *et al.* 1994). The theoretical xylem specific hydraulic conductivity ( $K_{S-(t)}$ ,  $\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$ ) was calculated based on the sampled vessels using Hagen–Poiseuille law for ideal capillaries and assuming laminar flow as  $K_{S-(t)} = (\pi\rho/128\eta A_{image}) \times \sum d^4$  where  $\rho$  is the density of water at 20 °C

( $998.2 \text{ kg m}^{-3}$ ),  $\eta$  is the viscosity of water at  $20^\circ\text{C}$  ( $1.002 \times 10^{-9} \text{ MPa s}$ ) and  $A_{\text{image}}$  is the area of the images analysed for vessel counts ( $\text{m}^2$ ) (Santiago *et al.* 2004). Experiments have shown that the xylem specific hydraulic conductivity is accurately modelled by the Hagen–Poiseuille equation, although overestimated (Santiago *et al.* 2004; Choat *et al.* 2007).

### Xylem mechanical properties

Measurements of xylem mechanical properties were performed on the same stem segments as those used for cavitation measurements ( $n = 5$  per genotype per plot). Xylem density was measured following an adapted protocol from Hacke *et al.* (2000). Samples 4-cm-long were taken from the base of each stem segment, placed in a vial of deionized water and allowed to equilibrate at room temperature for c. 48 h. The samples were split longitudinally, and the pith, phloem and epidermis were removed with a razor blade. Fresh volume of the sample was determined by Archimedes' principle. The samples were submerged, displacement weight was recorded on an analytical balance and was converted to stem fresh volume, correcting for the density of water at  $20^\circ\text{C}$  ( $0.998 \text{ g cm}^{-3}$ ). The samples were then oven-dried at  $70^\circ\text{C}$  until constant mass (typically 48 h), and wood density ( $\rho_{\text{wood}}$ ,  $\text{g cm}^{-3}$ ) was calculated as the ratio of dry mass to fresh volume. The vessel 'thickness to span ratio'  $(t/b)_h^2$ , was used as anatomical proxy for vessel wall reinforcement against collapse (Hacke *et al.* 2001). Estimates of  $(t/b)_h^2$  were obtained from images taken at  $200\times$  magnification from the cross sections used for xylem anatomy. The  $(t/b)_h^2$  was determined for vessels pairs in which at least one of the vessels fell within  $\pm 8 \mu\text{m}$  of the calculated  $d_h$  ( $n = 10\text{--}30$  per stem); we chose a margin of  $\pm 8 \mu\text{m}$  rather than the commonly used margin of  $\pm 3 \mu\text{m}$  (Hacke *et al.* 2001) because of the low number of vessel pairs that fell within  $\pm 3 \mu\text{m}$  of the  $d_h$ . The  $t$  represented the double wall thickness of the vessel pair ( $\mu\text{m}$ ) and was measured directly on the images; the  $b$  represented the width of the conduit ( $\mu\text{m}$ ) and was taken as the diameter of the vessel obtained from the equivalent area. Please note that  $t$  is thereafter indexed  $t_h$  as it refers to measurements made on vessel pairs falling in the range of  $d_h$ .

### Statistical analyses

Data management and statistical analyses were carried out with the statistical software SPSS 11.0 (SPSS, Chicago, IL, USA). Data were found to meet the assumptions of homoscedasticity and normality distribution of residuals. Statistical tests were considered significant at  $P < 0.05$ . Means are expressed with their standard error.

For the analysis of block effects, the following model of analysis of variance was used:  $Y_{ij} = \mu + B_i + \varepsilon_{ij}$  where  $\mu$  is the general mean,  $B_i$  is the effect of block  $i$  considered as fixed and  $\varepsilon_{ij}$  is the residual. For each trait,  $B_i$  was calculated as the difference between the mean of each block and the general mean over the eight genotypes (Marron *et al.* 2006). Global

genetic variations and effect of water deficit were then evaluated using the following full-factorial model:  $Y_{ijk} = \mu + G_i + T_j + (G_i \times T_j) + \varepsilon_{ijk}$  where  $Y_{ijk}$  refers to individual values adjusted to the block effects,  $\mu$  is the general mean,  $G_i$  is the effect of genotype  $i$  considered as random,  $T_j$  is the effect of treatment  $j$  considered as fixed,  $(G_i \times T_j)$  is the interaction between the two factors and  $\varepsilon_{ijk}$  is the residual.

Linear regression analysis and Pearson's correlation coefficients were used to characterize relationships between pairs of continuous variables. Correlations were computed at the genotype level and under the two separate irrigation regimes to investigate the effect of water deficit on trait relationships.

## RESULTS

### Time course of the drought experiment

From mid-June to mid-August,  $\Psi_{\text{pd}}$  remained above  $-0.20 \text{ MPa}$  on the control plot, while a progressive drop was recorded on the non-irrigated plot with a peak reaching  $-0.75 \text{ MPa}$  on 24 July (Fig. 1a). The rainfall precipitations that occurred by the end of July should have been responsible for the increase in  $\Psi_{\text{pd}}$  within non-irrigated plants. The comparison of  $\Psi_{\text{pd}}$  values between blocks of each plot revealed no significant spatial differences for each date of measurement. From these observations, we concluded that the water deficit was moderate, long-lasting (several weeks) and homogeneous.

Seasonal growth kinetics in terms of dry mass for the main shoot (Fig. 1b,c) were established for each genotype and each irrigation condition by combining seasonal height kinetics (data not shown) and allometric relationships between shoot height and shoot dry mass established at the end of the growing season (data not shown). The main shoots of all genotypes on the well-irrigated plot underwent a phase of approximately linear growth between 4 July and 14 August (Fig. 1b); growth was progressively slowed down on the not-irrigated plot (Fig. 1c).

### Xylem resistance to cavitation, $\Psi_{\text{xmin}}$ and safety margins

Regardless of the irrigation regime, the VCs established using the Cavitron technique were well fitted to the logistic equation ( $r > 0.99$ ) with the exception of 'I45-51' ( $r > 0.96$ ; Fig. 2). Under well-watered conditions,  $\Psi_{12}$ ,  $\Psi_{50}$  and  $\Psi_{88}$  differed significantly among the eight genotypes ( $P < 0.001$ ). The xylem tension corresponding to 50% loss in hydraulic conductance ( $\Psi_{50}$ ) varied from  $-1.6 \text{ MPa}$  to  $-2.4 \text{ MPa}$  for 'Robusta' and 'Eco28', respectively (Fig. 2). The slope value exhibited less genotypic variations ( $P = 0.045$ ) than did  $\Psi_{12}$ ,  $\Psi_{50}$  and  $\Psi_{88}$ ; variations were mainly driven by 'I45-51' which exhibited a steadier increase in PLC than the other genotypes (Fig. 2). There was no trend for more negative  $\Psi_{50}$  to be associated with lower slopes ( $r = -0.35$ ,  $P = 0.396$ ). A significant 'genotype by treatment' interaction was recorded for  $\Psi_{12}$ ,  $\Psi_{50}$  and  $\Psi_{88}$  ( $P < 0.010$ ): 'Agathe\_F',

'Pannonia' and 'Robusta' were found to exhibit more negative  $\Psi_{50}$  values in response to drought ( $P < 0.01$ ), while the other five genotypes did not respond significantly (Fig. 2). Water deficit did not affect significantly the slopes of the VCs regardless of the genotype (Fig. 2).

The minimum xylem water potential ( $\Psi_{\text{min}}$ ) varied from  $-0.8$  to  $-1.0$  MPa for well-irrigated trees and from  $-1.2$  to  $-1.3$  MPa for drought-exposed trees, so that the differences between genotypes were not significant within each irrigation regime ( $P = 0.757$  and  $0.975$ , respectively, Fig. 3). As a consequence, variations observed in safety margins were essentially driven by differences in  $\Psi_{12}$  ( $r > 0.97$ ,  $P < 0.001$  in both water regimes). Under well-watered conditions, the safety margins ranged from  $0.98$  MPa for 'Eco28' to  $-0.02$  MPa for 'I45-51'. Under water deficit, the safety margins were significantly reduced for 'Eco28' and 'Luisa\_Avanzo' only, and ranged from  $0.66$  MPa for 'Cima' to  $-0.05$  MPa for 'I45-51'.

### Growth performances, leaf gas exchange and carbon isotope discrimination

Under well-watered conditions, the main shoot RGR, the main shoot annual dry mass and the annual total above-ground coppice dry mass varied significantly among the eight genotypes from  $0.016$  to  $0.034$  g g<sup>-1</sup> day<sup>-1</sup>, from  $60$  to  $282$  g and from  $211$  to  $670$  g, respectively ( $P < 0.001$ ; Figs 1b & 4). Noticeably, 'Eco28' displayed higher RGR and main shoot dry mass than its counterparts (Fig. 4a,b). A significant 'genotype by treatment' interaction was recorded for RGR only ( $P < 0.001$ ). Except for 'Robusta', genotypes exhibited a drought-induced decrease in RGR ( $P < 0.050$ ) that ranged from  $22\%$  for 'Luisa\_Avanzo' to  $68\%$  for 'Flevo' (Figs 1c & 4a). Although most of the genotypes exhibited a reduced RGR and a reduced main shoot annual dry mass in response to water deficit, the annual total above-ground coppice dry mass was not significantly affected. A significant and positive correlation was detected between the RGR and the main shoot annual dry mass under well-watered conditions ( $r = 0.76$ ,  $P = 0.028$ ), but this relationship did not hold anymore under water deficit ( $r = 0.17$ ,  $P = 0.693$ ). RGR tended to scale positively with annual above-ground coppice dry mass under well-watered conditions ( $r = 0.65$ ,  $P = 0.081$ ), but the relationship broke down under water deficit ( $r = 0.50$ ,  $P = 0.211$ ). In both water regimes, the main shoot annual dry mass did not correlate with the annual total above-ground coppice dry mass ( $r \leq 0.32$ ,  $P > 0.400$ ). Regardless of the irrigation regime, the number of shoots per individual correlated negatively with the main shoot annual dry mass ( $r > -0.94$ ,  $P < 0.050$ ) but not with the RGR or the annual total above-ground coppice dry mass ( $r \leq 0.18$ ,  $P > 0.650$ ).

For well-irrigated trees,  $g_s$  and net CO<sub>2</sub> assimilation rate ( $A$ ) ranged from  $359$  to  $690$  mmol m<sup>-2</sup> s<sup>-1</sup> and from  $13.5$  to  $18.1$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Fig. 5a,b). In response to summer water deficit, no significant 'genotype by treatment' interactions were detected for  $g_s$  and  $A$ , which were reduced on average by  $87$  and  $56\%$ , respectively ( $P < 0.001$ ).  $g_s$  and

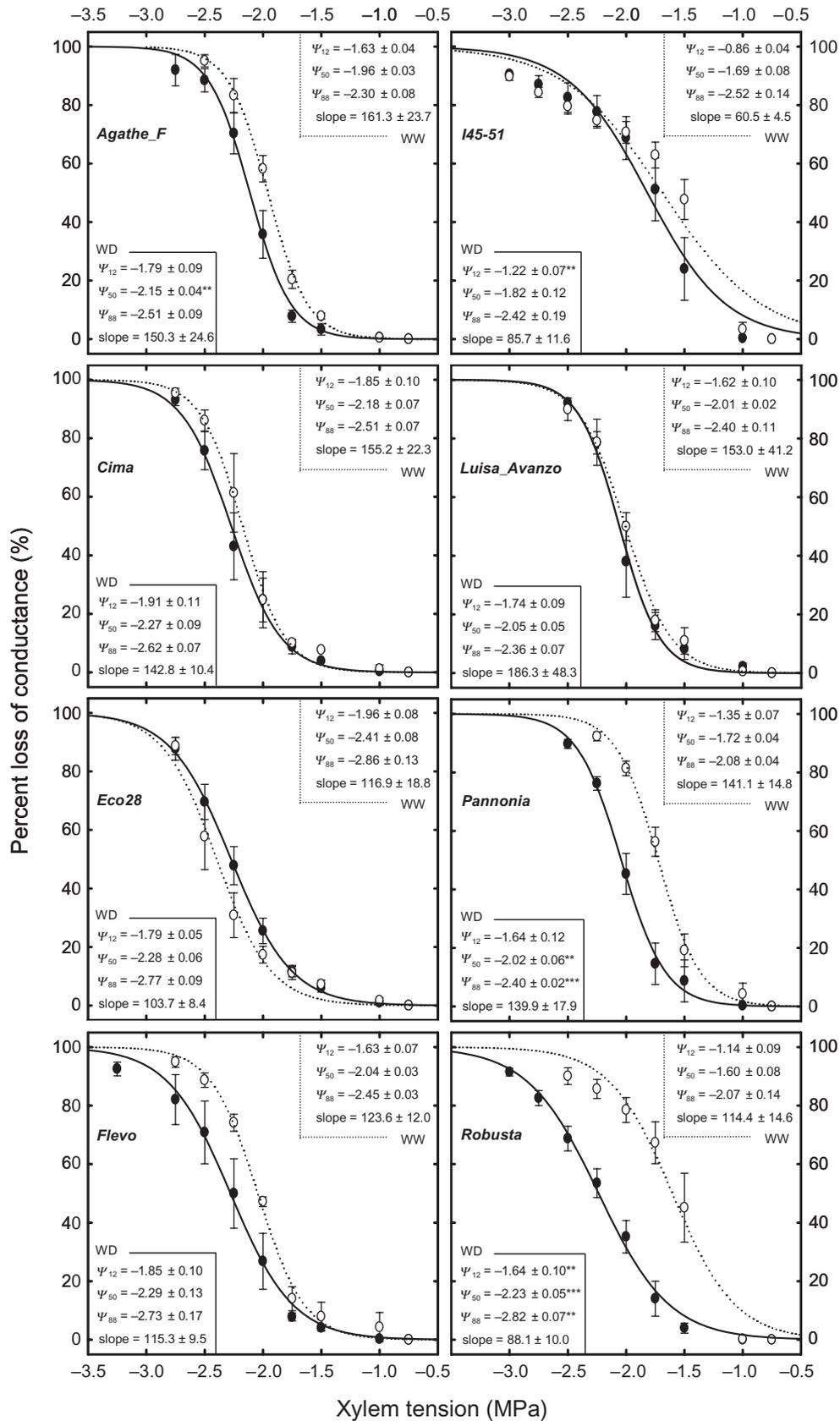
$A$  were not significantly correlated under well-watered conditions ( $r = 0.45$ ,  $P = 0.259$ ), while they scaled positively under water deficit conditions ( $r = 0.86$ ,  $P = 0.006$ ). Bulk leaf carbon isotope discrimination ( $\Delta$ ) spanned from  $19.8$  to  $21.3\%$  for well-watered plants (Fig. 5c). Values of  $\Delta$  were lower under water deficit but the extent of the decrease was genotype dependent as attested by the significant 'genotype by treatment' interaction ( $P = 0.003$ ); for instance,  $\Delta$  was reduced by  $1.3\%$  in 'Luisa\_Avanzo' and by  $3.3\%$  in 'Cima'. Regardless of the irrigation regime, genotypic variations in  $\Delta$  were mainly driven by genotypic variations in  $g_s$  ( $r = 0.62$ ,  $P = 0.103$  under well-watered regime and  $r = 0.73$ ,  $P = 0.039$  under water deficit) rather than by variations in  $A$  ( $r = -0.08$ ,  $P = 0.860$  under well-watered regime and  $r = 0.51$ ,  $P = 0.195$  under water deficit). Whatever the irrigation regime, no significant correlation could be observed between growth variables and either leaf gas exchange rates or  $\Delta$ , as already reported by Monclus *et al.* (2006) on the same set of genotypes under comparable environment.

### Xylem anatomy and mechanical properties

Significant differences between the eight genotypes were found under well-watered conditions for all anatomical and mechanical traits ( $P < 0.020$ ). For instance,  $d_{\text{mean}}$  and  $K_{S(t)}$  varied from  $34$  to  $42$   $\mu\text{m}$  and from  $14.0$  to  $21.1$  kg s<sup>-1</sup> m<sup>-1</sup> MPa<sup>-1</sup> (Fig. 6). Wood density ( $\rho_{\text{wood}}$ ) and  $(t/b)_h^2$  varied in narrow range from  $0.33$  to  $0.41$  g cm<sup>-3</sup> and from  $0.005$  to  $0.008$ , respectively (Fig. 6d,e). A significant 'genotype by treatment' interaction was recorded for all variables ( $P < 0.050$ ). When genotypes were found to respond significantly, the expected adjustments occurred, that is, traits related to hydraulic capacity ( $d_{\text{mean}}$ ,  $d_h$  and  $K_{S(t)}$ ) were decreased, while vessel density ( $n_v$ ) and traits related to mechanical reinforcement ( $\rho_{\text{wood}}$ ,  $t_h$  and  $(t/b)_h^2$ ) were increased (Fig. 6). Vessel lumen area ( $A_v$ ) was not significantly affected by drought regardless of the genotype (Fig. 6). In both water regimes,  $d_{\text{mean}}$  and  $n_v$  varied negatively with each other ( $r = -0.97$  and  $-0.88$ ,  $P < 0.001$  and  $P = 0.004$ , respectively). Under well-watered conditions only,  $\rho_{\text{wood}}$  increased with decreasing  $d_h$  and  $K_{S(t)}$  ( $r = -0.80$  and  $-0.86$ ,  $P = 0.017$  and  $0.004$ , respectively). In both water regimes, variations in  $(t/b)_h^2$  were mainly driven by differences in  $d_h$  ( $r = -0.64$  and  $-0.93$ ,  $P = 0.086$  and  $0.001$ , respectively) rather than in  $t_h$  ( $r = 0.37$  and  $-0.47$ ,  $P = 0.362$  and  $0.240$ , respectively). Regardless of the irrigation regime,  $\rho_{\text{wood}}$  correlated neither with  $t_h$  nor with  $(t/b)_h^2$ .

### Relationships between xylem cavitation resistance, structural and functional traits

At the tissue level, no significant correlation could be detected between  $\Psi_{50}$  and vessel traits related to hydraulic efficiency ( $K_{S(t)}$ ,  $d_{\text{mean}}$ ,  $A_v$ ), regardless of the irrigation regime (Fig. 6a–c). A negative correlation was detected between  $\Psi_{50}$  and  $t_h$  under well-watered condition, while no correlation could be observed with other traits related to



**Figure 2.** Stem xylem vulnerability curves (percent loss of hydraulic conductance versus xylem tension) for well-watered trees (WW, dotted lines, open circles) and drought-exposed trees (WD, solid line, closed circles) of the eight *Populus deltoides* × *Populus nigra* genotypes. Lines are logistic fits to the data and error bars correspond to SE ( $n = 3$  to 5). Values of  $\Psi_{12}$ ,  $\Psi_{50}$ ,  $\Psi_{88}$  (xylem tension at 12, 50 and 88% loss of hydraulic conductance, respectively) and slope are means  $\pm$  SE for each irrigation regime. Asterisks indicate significant differences between well-watered and water deficit conditions. Levels of significance are: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

xylem biomechanics ( $\rho_{\text{wood}}$  and  $(t/b)_h^2$ , Fig. 6d–f). At the leaf level,  $\Psi_{50}$  scaled significantly and positively with  $g_s$  under well-watered regime; the relationship broke down under water deficit (Fig. 5a). No relationship could be found between  $\Psi_{50}$  and  $A$  whatever the irrigation condition (Fig. 5b). Similarly, no significant correlation could be detected between  $\Psi_{50}$  and  $\Delta$ , except under water deficit conditions where  $\Psi_{50}$  tended to scaled positively with  $\Delta$  ( $P = 0.062$ , Fig. 5c). At the whole-plant level,  $\Psi_{50}$  varied negatively with the main shoot RGR and the main shoot annual dry mass under optimal irrigation, but no significant correlation was observed between  $\Psi_{50}$  and annual total above-ground coppice dry mass (Fig. 7). Under water deficit,  $\Psi_{50}$  did not correlate with any of the three traits related to growth performance (Fig. 7).

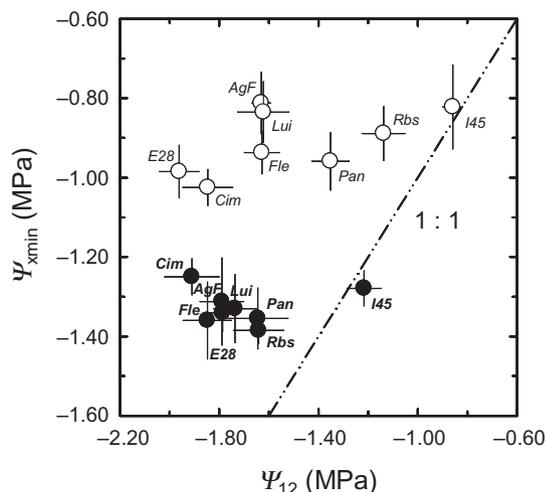
## DISCUSSION

### Genotypic variation and drought-induced plasticity in xylem resistance to cavitation

Poplar species are known to be extremely vulnerable to drought-induced cavitation, a trend consistent with their

pioneer behaviour and their tight requirements upon water availability. None of the eight *P. deltoides* × *P. nigra* genotypes appeared to be an exception to this paradigm since all genotypes had a fully embolized xylem as soon as xylem tension approached  $-3$  MPa. This is in line with data already published for pure poplar species (Sperry *et al.* 1991; Hacke & Sauter 1996; Tognetti *et al.* 1999; Hukin *et al.* 2005) and various hybrids (Cochard *et al.* 1996; Harvey & Van Den Driessche 1997, 1999; Cochard *et al.* 2007; Coleman *et al.* 2008). However,  $\Psi_{50}$  (a proxy of cavitation resistance) varied significantly among the eight genotypes from  $-1.60$  MPa for the least resistant ('*Robusta*') to  $-2.40$  MPa for the most resistant one ('*Eco28*'). It may be worth mentioning that this consistent range of variation cannot be attributed to intraspecific variation strictly speaking since the eight genotypes resulted from unrelated interspecific hybridization.

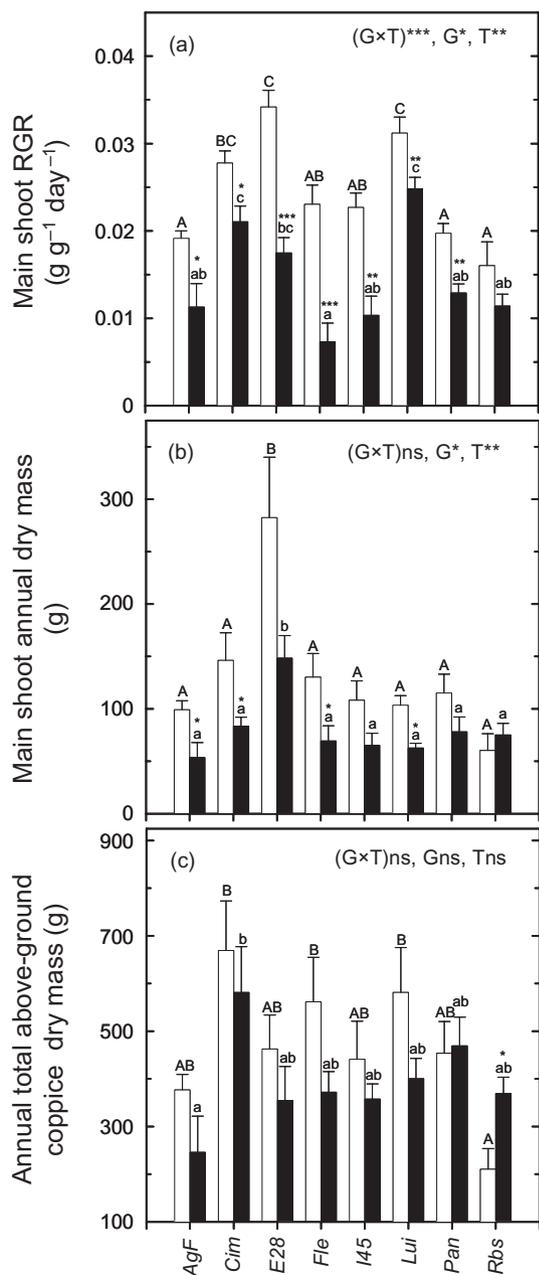
We found direct evidence for drought-induced acclimation of xylem resistance to cavitation, trees grown under limited water availability exhibiting a lower  $\Psi_{50}$  and thus a safer xylem. This is consistent with other very few reports (Holste *et al.* 2006; Beikircher & Mayr 2009; Brodrribb & Cochard 2009). This is also consistent with interspecific comparisons showing that taxa growing in drier habitats tend to exhibit a safer xylem (Hacke *et al.* 2000; Pockman & Sperry 2000), although such studies may confound both genetic variations and phenotypic plasticity. However, it must be noted that acclimation was clearly apparent for only three genotypes, suggesting the extent of phenotypic plasticity is largely genotype dependent. Among the genotypes that were found to respond significantly,  $\Psi_{50}$  was decreased by as much as 0.60 MPa in the genotype '*Robusta*'. Interestingly, this range of variation was almost as high as that observed when comparing genotypes under adequate water supply.



**Figure 3.** Relationship between minimum xylem water potential experienced in the field ( $\Psi_{x\text{min}}$ ) and water potential at 12% loss of hydraulic conductance ( $\Psi_{12}$ ) for well-watered trees (open circles) and drought-exposed trees (close circles) of *Populus deltoides* × *Populus nigra*. Values are means  $\pm$  SE ( $n = 3$  to 5). The difference between  $\Psi_{12}$  and  $\Psi_{x\text{min}}$  was taken as a safety margin against drought-induced xylem embolism. The dashed line indicates the 1:1 relationship. Genotype abbreviations (well-watered, not bold; water deficit, bold): AgF, '*Agathe\_F*'; Cim, '*Cima*'; E28, '*Eco28*'; Fle, '*Flevo*'; I45, '*I45-51*'; Lui, '*Luisa\_Avanzo*'; Pan, '*Pannonia*'; Rbs, '*Robusta*'.

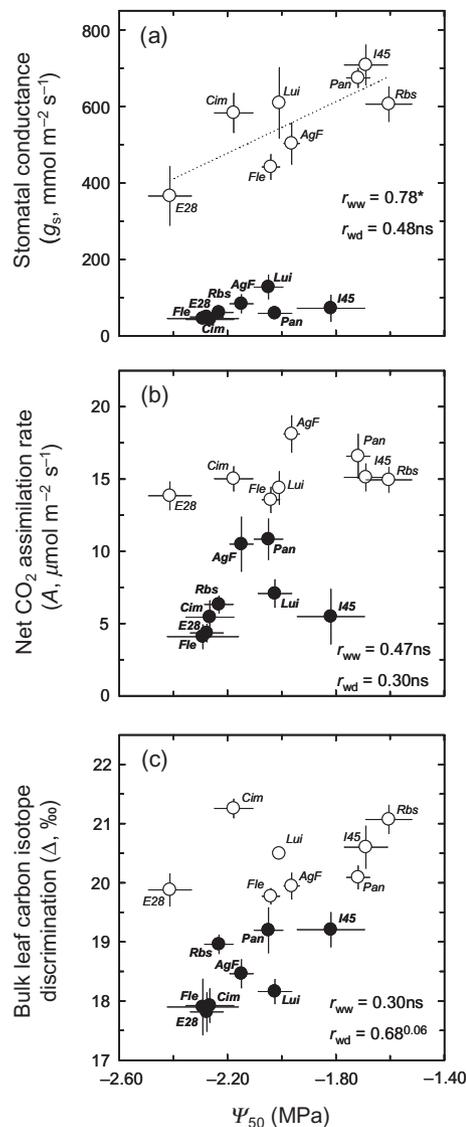
### Xylem resistance to cavitation and structural properties

The xylem tension corresponding to the onset of embolism (the  $\Psi_{12}$  in our study) is primarily dictated by the frequency of rare, leaky pits in the pit membrane (Christman, Sperry & Adler 2009), while the distribution of cavitation events across a range of xylem tensions (estimated through the slope parameter) may be more determined by the range of pore sizes. Except for '*I45-51*', the genotypes differed more in  $\Psi_{12}$  than in the slope of their VCs, so that we can hypothesize that differences in xylem resistance to cavitation must originate mainly from size differences in these rare big pores at inter-vessel junctions. In addition, drought-acclimated plants tended to be more resistant to cavitation

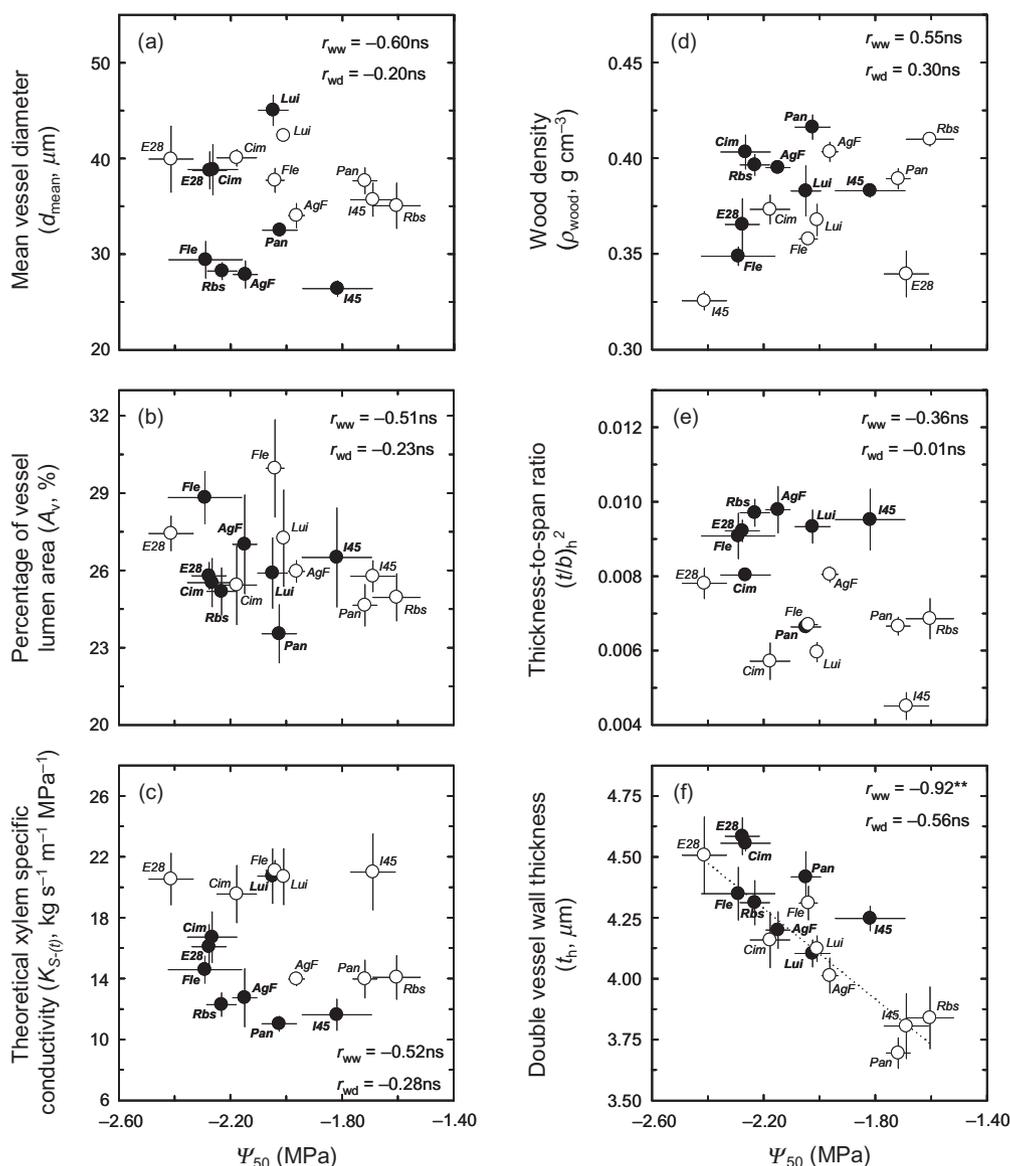


**Figure 4.** Growth characteristics for the eight *Populus deltoides* × *Populus nigra* genotypes grown under well-watered (open bars) or water deficit (close bars) conditions. Values are shown for the main shoot relative growth rate (RGR) (a), the main shoot annual dry mass (b) and the annual total above-ground coppice biomass (c). Values are means ± SE (*n* = 5). Significance of the main effects of the two-way analysis of variance is given (G, genotype effect; T, treatment effect; G × T, genotype by treatment interaction). Different letters indicate significant differences (Tukey’s post hoc test); upper case letters are for well-watered trees, lower case letters are for drought-exposed trees. Levels of significance are: \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. ns, non significant. Genotype abbreviations are as in Fig. 3.

(more negative  $\Psi_{12}$  and  $\Psi_{50}$ ), but the slopes of the VCs were not significantly affected. The exact mechanism by which xylem resistance to cavitation acclimates to soil water deficit remains to be explained, but it is likely that water deficit exerts primarily its control through the alteration of the developmental pattern of the primary cell wall deposition during vessel ontogeny. Investigating pit membrane characteristics of a limited number of genotypes with



**Figure 5.** Relationships between xylem resistance to drought-induced embolism estimated as the tension causing 50% loss in hydraulic conductance ( $\Psi_{50}$ ) and leaf functional traits for well-watered trees (open circles, dashed lines) and drought-exposed trees (close circles, solid lines). (a) Relationships with stomatal conductance; (b) relationships with net CO<sub>2</sub> assimilation rate; (c) relationships with bulk leaf carbon isotope discrimination. Values are means ± SE (*n* = 3 to 5). Pearson’s coefficients are given for each irrigation regime ( $r_{ww}$ , well-watered;  $r_{wd}$ , water deficit). Levels of significance are: \**P* < 0.05, ns, non significant. Pearson’s coefficients with *P*-value comprised between 0.05 and 0.1 are given in exponent. Genotype abbreviations are as in Fig. 3.



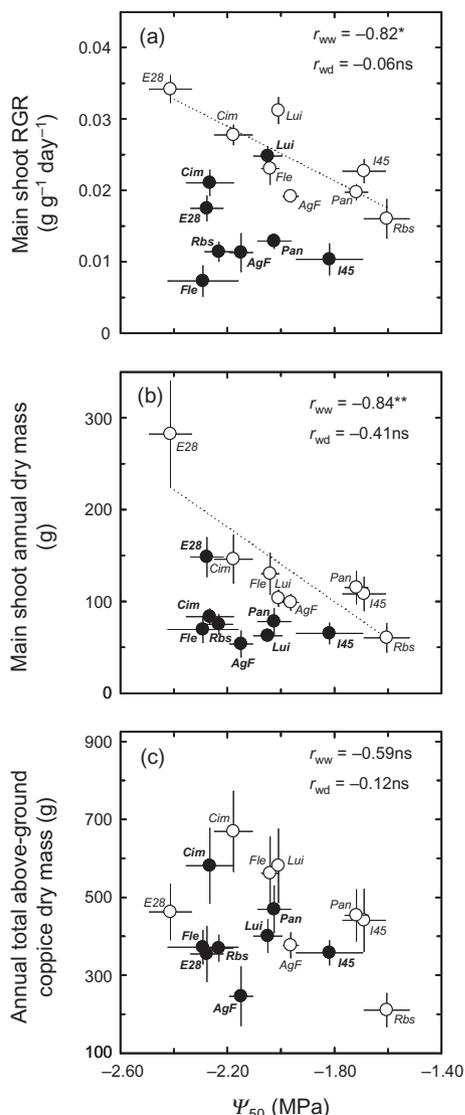
**Figure 6.** Relationships between xylem resistance to drought-induced embolism estimated as the tension causing 50% loss in hydraulic conductivity ( $\Psi_{50}$ ) and xylem structural traits related to water transport capacity (a to c) or mechanical reinforcement (d to f) for well-watered trees (open circles, dashed lines) and drought-exposed trees (close circles, solid lines). Values are means  $\pm$  SE ( $n = 3$  to 5). Pearson's coefficients are given for each irrigation regime ( $r_{ww}$ , well-watered;  $r_{wd}$ , water deficit). Levels of significance are: \*\* $P < 0.01$ , ns, non-significant. Genotype abbreviations are as in Fig. 3.

contrasting response to drought would be valuable to yield insights into the anatomical basis of drought-induced plasticity of cavitation resistance.

There did not appear to be a trade-off between xylem safety and xylem transport efficiency (estimated through  $d_{\text{mean}}$ ,  $d_h$ ,  $A_v$  and  $K_{S(t)}$ ) across the eight genotypes. The absence of a safety versus efficiency trade-off is consistent with the air-seeding hypothesis which relates drought-induced embolism to pit membrane properties and contact area between vessels rather than directly to the diameter of the xylem conduits (Choat *et al.* 2003; Wheeler *et al.* 2005; Jansen, Choat & Pletsers 2009). However, we estimated xylem transport efficiency by measuring vessel features

from xylem cross sections so that possible hydraulic limitations resulting from pit membrane resistance were not accounted for, possibly masking a trade-off between hydraulic safety and efficiency.

In addition to transporting water efficiently while avoiding cavitation, the xylem conduit network must also bear mechanical stresses imposed by negative sap pressures. Values of  $\rho_{\text{wood}}$  and  $(t/b)_h^2$  were in the range of those already found for poplars and willows (Cochard *et al.* 2007). As expected from theory,  $\rho_{\text{wood}}$  should be a function of  $(t/b)_h^2$  (Hacke *et al.* 2001), but the two parameters were not found to correlate in our data set which may be partly explained by the rather low range of variations found for these traits.



**Figure 7.** Relationships between xylem resistance to drought-induced embolism estimated as the tension causing 50% loss in hydraulic conductance ( $\Psi_{50}$ ) and growth determinants for well-watered trees (open circles, dashed lines) and drought-exposed trees (close circles, solid lines). (a) Relationships with the main shoot relative growth rate (RGR); (b) relationships with the main shoot annual dry mass; (c) relationships with the annual total above-ground coppice biomass. Values are means  $\pm$  SE ( $n = 3$  to  $5$ ). Pearson's coefficients are given for each irrigation regime ( $r_{ww}$ , well-watered;  $r_{wd}$ , water deficit). Levels of significance are: \* $P < 0.05$ , \*\* $P < 0.01$ , ns, non-significant. Genotype abbreviations are as in Fig. 3.

There was no clear evidence for increased cavitation resistance to be related to a greater degree of mechanical strength at the tissue level (as inferred from  $\rho_{wood}$ ); this finding contrasts with the trade-off frequently observed at the interspecific level between cavitation resistance and bulk xylem density (Hacke *et al.* 2001; Jacobsen *et al.* 2005, 2007; Pratt *et al.* 2007; Jacobsen *et al.* 2009). There was no more clear evidence for increased cavitation resistance to

be related to a greater degree of mechanical strength at the cellular level (as inferred from  $(t/b)_h^2$ ). Thus, although xylem resistance to cavitation and  $(t/b)_h^2$  have been found to co-vary both across gymnosperm and angiosperm species (Hacke *et al.* 2001), our results suggest that the two parameters can be at least partially uncoupled at narrower scales. Actually, the two parameters are not functionally related since air seeding of cavitation and collapsing stresses relate to different wall regions. Because vessel collapse is a threshold-type response, vessels should be safe from implosion as long as  $(t/b)_h^2$  remains above the implosion threshold. Therefore, the maintenance of a constant safety factor from implosion may not be required among all genotypes. The additional role of the fibre matrix for supporting vessel walls against implosion should further complicate the analysis (Jacobsen *et al.* 2005). For instance, increased fibre wall thickness has been shown to correlate with cavitation resistance across distantly related taxa (Jacobsen *et al.* 2005, 2007) as well as across willow and poplar clones (Cochard *et al.* 2007).

We found a strong relationship between the double vessel wall thickness ( $t_h$ ) and resistance to cavitation, genotypes exhibiting a thicker double wall being more resistant. This relationship tended to be conserved under water deficit. A recent study across *Prunus* species reported similar results (Cochard *et al.* 2008). The reason for such a relationship may relate to co-variation between pit membrane properties and wall thickness. For instance, recent anatomical observations using electron microscopy indicated that reduced porosity (and possibly increased resistance to cavitation) may be achieved through greater pit membrane thickness, which in turn is correlated with greater wall thickness (Jansen *et al.* 2009).

### Xylem resistance to cavitation, minimum xylem water potential and leaf function

Within each separate water regime,  $\Psi_{xmin}$  varied in a narrower range compared to  $\Psi_{12}$  so that predicted safety margins varied considerably among genotypes. Thus, the genotypes did not systematically exploit the gain in xylem safety by operating at more negative  $\Psi_{xmin}$ . The more general comparison of minimum  $\Psi_{12}$  along with  $\Psi_{xmin}$  showed that  $\Psi_{xmin}$  was always constrained to values close to or less negative than predicted cavitation thresholds. This was true regardless of the water regime, although values of minimum  $\Psi_{xmin}$  were more negative under water deficit. These results are consistent with the general view that when soil water availability is not excessively low, adequate stomatal regulation along with the intrinsic properties of the soil-to-leaf hydraulic pathway contributes to the maintenance of  $\Psi_{xmin}$  within a range of functional water potentials (Sparks & Black 1999; Brodribb *et al.* 2003; Meinzer *et al.* 2009). A tight control of  $\Psi_{xmin}$  in order to prevent the risk of catastrophic runaway embolism must be particularly relevant for the genotypes studied since most of them exhibited VCs with steep slopes. It is noteworthy that the only

one genotype that was found to operate at xylem water potentials close to cavitation thresholds ('I45-51') was the one that exhibited the least steep increase in PLC. In this case, loss of a proportion of the conducting tissue may be less catastrophic and may even be beneficial in terms of maximizing total plant water flux and short-term carbon uptake (Jones & Sutherland 1991; Pammenter & Vander Willigen 1998; Sparks & Black 1999).

Our results revealed that  $g_s$  increased with decreasing xylem safety under well-watered conditions, consistently with what was observed across 14 temperate species (Maherali *et al.* 2006). However, there is *a priori* no functional reason for cavitation resistance to directly impose limits to gas exchange when water is not limiting, unless increased cavitation resistance co-varies with other plant hydraulic parameters that constrain gas exchange. Considering the well-supported Ohm's law analogy for water flow in plants, the maximum  $g_s$  that plants can achieve is ultimately constrained by the leaf area-specific whole-plant hydraulic conductance ( $k_{\text{plant}}$ ) (Tyree & Ewers 1991; Meinzer 2002; Tyree & Zimmermann 2002). Therefore, a more general trade-off between vulnerability to cavitation and water transport efficiency at the whole-plant level, rather than just within specific organs, could explain the relationship observed between xylem resistance to cavitation and  $g_s$  (Maherali *et al.* 2006).

Provided that photosynthetic capacity is determined by stomatal limits to CO<sub>2</sub> diffusion (Wong, Cowan & Farquhar 1979), a relationship between  $\Psi_{50}$  and photosynthetic rate would be expected under well-watered conditions as a consequence of an indirect  $g_s$ -mediated effect. However,  $A$  and  $g_s$  did not correlate significantly in our data set so that variations in  $\Psi_{50}$  were not perfectly mirrored by variations in  $A$ . This merely resulted from the fact that, under optimal irrigation,  $A$  was close to  $A_{\text{max}}$  and was no longer constrained by  $g_s$ . Nevertheless, the lack of relationship between cavitation resistance and photosynthetic capacity was further confirmed by the fact that other leaf traits associated with carbon uptake such as specific leaf area and nitrogen content did not correlate with  $\Psi_{50}$  (data not shown).

There was no clear relationship between  $\Psi_{50}$  and  $\Delta$ , although variations in  $\Delta$  were clearly driven by  $g_s$  rather than by  $A$ . Martínez-Vilalta *et al.* (2009) reported a weak positive relationship between  $\Psi_{50}$  and  $\Delta$  across populations of Scots pine, while Maherali *et al.* (2006) found no significant correlation across 14 temperate species. In contrast, Ducrey *et al.* (2008) reported a strong and negative relationship between  $\Psi_{50}$  and  $\Delta$ . Irrespective of the study scale (interspecific versus intraspecific), it seems therefore that the functional coordination between cavitation resistance and the economics of leaf gas exchange is unclear. In fact, the direction and the strength of the correlation between the two parameters are likely to depend on (1) which of photosynthetic capacity versus  $g_s$  drives the variations in  $\Delta$  and (2) to what extent and in which way cavitation resistance can be related to other aspects of whole-plant hydraulics that constrain leaf water fluxes.

## Xylem resistance to cavitation and growth performances

Increased xylem cavitation resistance is often thought to come at the expense of reduced plant growth. Such a trade-off is expected if increased cavitation resistance lies in the necessity to build a denser wood with thicker cell walls, a feature supposed to be costly in terms of carbon allocation (Enquist *et al.* 1999). Several recent studies have provided evidence in support to this hypothesis across willow and poplar hybrids (Wikberg & Ögren 2004; Cochard *et al.* 2007). However, our results do not fit at all with this concept since the more cavitation-resistant genotypes grew faster, as attested by the negative correlation between  $\Psi_{50}$  and the main shoot growth potential (as estimated by the RGR and the annual dry mass). This finding highlights the importance of considering species and genetic background before general conclusions are to be drawn. Actually, the reason(s) for such a relationship remain(s) only hypothetical. We know from other studies that in poplar species and related hybrids, including *P. deltoides* × *P. nigra*, the most important factor promoting juvenile growth is the total canopy area (Marron *et al.* 2005; Monclus *et al.* 2005, 2006; Marron & Ceulemans 2006). If cavitation resistance somehow comes at the cost of reduced  $k_{\text{plant}}$  as suggested above, and assuming that decreased  $k_{\text{plant}}$  is mainly achieved by increasing total canopy area, then a higher cavitation resistance should be associated with a greater shoot growth potential.

It is interesting to note that although cavitation resistance scaled with the main shoot growth potential, no relationship could be found with annual total above-ground dry mass, suggesting that the relationship cannot be extrapolated to the whole genotype potential yield. This may be easily explained by the fact that variations in RGR and annual dry mass of the main shoot on which measurements were performed were only partially matched by variations in annual total above-ground dry mass. This merely resulted from differences between genotypes in the number of shoots per stool and their individual vigour. Therefore, there is good theoretical background for  $\Psi_{50}$  to be better related to the growth potential of individual shoots on which measurements are performed rather than to a whole potential yield which might be biased by genotypic differences in terms of coppice allocation strategies.

The genotypes that performed better under water deficit conditions were not necessarily those exhibiting the safer xylem. Similar results have already been reported by Harvey & Van Den Driessche (1997) on four poplar genotypes issued from an interspecific cross between *P. trichocarpa* × *P. deltoides*. On a general ground, this finding suggests that cavitation resistance can be uncoupled from growth depending on the environmental conditions, but the reason why the two parameters became uncoupled remains unknown. Resistance to cavitation is expected to be crucial for plant growth in the context of drastic water deprivation (Sperry *et al.* 2002). However, the summer water deficit was really moderate in our study ( $\Psi_{\text{pd}}$  never dropped below  $-0.75$  MPa), so that stem embolism was predicted to be

negligible based on  $\Psi_{x_{min}}$  measurements. Therefore, under such moderate droughts that do not pose a threat to tree survival, homeostasis of the plant water balance and growth maintenance is likely to rely on other hydraulic adjustments than cavitation resistance. These may include a combination of adequate short-term stomatal regulation and optimal allocation of resources between transpiring foliage, conductive sapwood area and absorbing roots (Magnani, Grace & Borghetti 2002; Addington *et al.* 2006).

## CONCLUSION

Despite the overall vulnerability to drought-induced xylem cavitation found within the *Populus* genus, we evidenced substantial genotypic variation and phenotypic plasticity for  $\Psi_{50}$  and related traits among unrelated *P. deltoides* × *P. nigra* genotypes. It should be mentioned that some of the variation uncovered in this study was due to possible positive transgressive segregation (i.e. heterosis phenomenon). We were unable to find evidence of associations or trade-offs between xylem safety and other physiological traits that have been commonly reported across species. Some of the particular trends observed here may relate to the fact that the genotypes studied resulted from artificial interspecific hybridization, and were thus not subjected to natural selection process. It is, however, still unclear why such trade-offs tend to only manifest at broad scales, and additional work is needed in order to firmly establish whether common associations between xylem safety and other functional traits actually reflect true mechanistic trade-offs.

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