

## Xylem vulnerability to cavitation varies among poplar and willow clones and correlates with yield

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**Summary** Xylem vulnerability to cavitation is a promising criterion for identifying trees with high drought tolerance, but traditional techniques for measuring cavitation resistance are unsuitable for screening large numbers of genotypes. We tested the potential of the new Cavitron technique for high throughput screening of cavitation resistance in five poplar (*Populus* spp.) and four willow (*Salix* spp.) clones. The Cavitron technique enabled the screening of three to four clones per day with sufficient accuracy to reveal significant differences between clones. Because intraspecific screening may be better carried out through the identification of correlated and more easily measured traits, we attempted to identify accessible parameters that correlate to cavitation resistance. Variability in vulnerability to cavitation across clones was poorly correlated with anatomical traits such as vessel diameter, vessel wall strength, wood density and fiber wall thickness; however, a striking correlation was established between cavitation resistance and aboveground biomass production, indicating a possible trade-off between xylem safety and growth potential.

**Keywords:** drought resistance, embolism, genotypic variability, xylem physiology.

### Introduction

Willows and poplars are widely cultivated for biomass production throughout Europe, especially in short-rotation coppice systems where crops are harvested every three years over a 15-year lifespan (Grassi et al. 1990). These fast-growing species tend to use much water and show poor tolerance to water stress (Wikberg and Ogren 2004). Global circulation models predict that the occurrence of severe droughts will increase regionally in the future (e.g., Hulme et al. 2002, Barnett et al. 2006). The identification of genotypes that can survive and grow well despite periodic droughts is therefore critical to the future success of these production systems. Tree resilience to drought is conferred by many different physiological traits (Hsiao 1973, Chaves et al. 2003). Breeders have mostly fo-

cused on traits enhancing tree growth under conditions of moderate water stress. Hence, genotypic variability in tree water-use efficiency is well documented (e.g., Zhang et al. 1993, Flanagan and Johnsen 1995, Brendel et al. 2002) and genotypes have been identified that perform well under typical drought conditions. However, the exceptional drought episodes that have recently occurred in Europe highlight the need to select trees that tolerate not only droughts of normal severity, but also occasional extreme droughts. Before tree resilience to severe droughts can be considered in breeding programs, it is necessary to identify relevant screening criteria, because classical selection criteria may be poor predictors of high drought resilience.

Xylem vulnerability to cavitation is correlated with drought tolerance (Pockmann and Sperry 2000, Tyree et al. 2003, Maherali et al. 2004) across a wide range of species with contrasting ecological preferences, suggesting that the maintenance of functional xylem conduits is necessary to survive severe droughts (Hacke and Sperry 2001). However, evidence for significant variability in xylem vulnerability to cavitation among closely related genotypes is scant (Neufeld et al. 1992, Mencuccini and Comstock 1997, Pita et al. 2003, Sangsing et al. 2004a), probably because current techniques for assessing vulnerability to cavitation are unsuitable for such studies. The hydraulic technique (Sperry et al. 1988), for instance, is time consuming and requires large amounts of plant material, whereas the ultrasonic acoustic technique (Tyree and Dixon 1983) is too imprecise for unravelling minute differences between genotypes. Other techniques, such as Cryo-SEM observations (Cochard et al. 2000) are too laborious to be considered for such investigations. Recently, Cochard et al. (2005) modified a centrifuge technique (Alder et al. 1997) in such a way that vulnerability curves can be rapidly obtained. The new Cavitron technique may be suitable for cavitation studies in breeding programs, but the practicability of the technique for such investigations has yet to be evaluated. The first objective of our study was to employ the Cavitron technique to detect variations in cavitation resistance among poplar and willow clones. The second objective was to identify more accessible

parameters that correlate with cavitation resistance. Wood density and other xylem anatomical characteristics meet this criterion (Hacke et al. 2001, Jacobsen et al. 2005); however, these correlations have been established for highly contrasting species only and their ability to discriminate among closely related taxa is unknown. We therefore investigated these correlations for different clones of poplars and willows. Our final objective was to test if increased cavitation resistance among clones came at the cost of reduced growth potential, a hypothesis frequently made for these species (Laureysens et al. 2004, Labrecque and Teodorescu 2005).

## Materials and methods

### Plant material

Measurements were conducted in 2006, which was the fourth growing season in the fourth rotation of a 1-ha coppice plantation of 16 poplar (*Populus* spp.) and 16 willow (*Salix* spp.) clones (Armstrong 1997). The plantation was located at Farnham, U.K. (51°11' N, 0°51' E; 115 m a.s.l.). Hardwood cuttings, 0.25 m in length, were planted in April 1996 in a double-row design with alternating 0.75- and 1.5-m inter-row distances and 0.9-m within row spacing to yield a planting density of 10,000 plants ha<sup>-1</sup>. A randomized block design with 32 clones (i.e., 16 per spp.) × 3 replicated plots was studied according to standard protocols suggested by the British Forestry Commission (Armstrong 1997). Individual monoclonal plots were 9 × 11.5 m in size, containing 10 north-south oriented rows of 10 hardwood cuttings each. In January 1997, all plants were cut back to a height of 0.05 m to create a coppice system.

The diameter at 1 m above the point at which a shoot (i.e., a first-order axis) joins a stool was measured with a digital caliper on all live shoots of each plant at the end of each growing season from 1997 to 2002 (with a total aboveground harvest every three years). Measurements were taken on the 36 plants (i.e., a 6 × 6 array) at the center of each plot. Concomitantly, three shoots per plot were harvested from the 64 surrounding plants, spanning a representative range of the diameter distribution. Harvested shoots (including branches) were oven dried at 95 °C, and allometric relationships between shoot diameter and shoot dry mass (DM) were computed for each clone to estimate standing biomass at the plot level by scaling-up. For each clone, a potential yield (Mg DM ha<sup>-1</sup> year<sup>-1</sup>) was computed over all plots and assessed years.

In 2006, experiments were conducted on five and four mostly interspecific clones of poplar and willow, respectively (Table 1). Clones were selected to obtain a wide range of growth performances (Table 1). At the beginning of September 2006, 0.5-m-long segments were sampled on dominant shoots from the apical current- and previous-year growth increments on different plants for each clone. Samples were immediately wrapped in wet paper, enclosed in plastic bags to minimize dehydration and transported to Edinburgh University for analysis.

Table 1. Name, sex, code number, parentage, place of origin, potential productivity (as estimated for the 1997–2002 period,  $n = 18$ ) and maximal shoot height (measured at the end of the 2006 growing season,  $n = 3$ ) of the poplar (*Populus*) and willow (*Salix*) clones. Yield values and shoot heights with different letters are statistically different at  $P < 0.05$ . Standard deviations are given in parenthesis.

Name	Sex	Code no.	Parentage	Parent code no.	Provenance	Potential yield (Mg ha <sup>-1</sup> year <sup>-1</sup> )	Shoot height (m)
Fritzi Pauley	F	V.235	<i>P. trichocarpa</i> Torr. & A. Gray	–	USA	8.8 (3.9) cd	8.9 (0.08) e
Trichobel	M	S.724–101	<i>P. trichocarpa</i> × <i>P. trichocarpa</i>	V.235 × V.24	USA	8.1 (3.9) cd	7.9 (0.06) d
Balsam Spire	F	TT32	<i>P. trichocarpa</i> × <i>P. balsamifera</i> L.	Hastata × Michauxii		3.1 (1.5) ab	5.6 (0.02) c
Gaver	M	S.688–22	<i>P. deltoides</i> Bartr. ex Marsh. × <i>P. nigra</i> L.	S.71–3 × Gibbecq	USA × Belgium	3.7 (1.7) b	5.0 (0.03) b
Ghoy	F	S.682–68	<i>P. deltoides</i> × <i>P. nigra</i>	S.9–2 × Ghoy	USA / Canada × Belgium	2.2 (0.8) a	4.4 (0.03) a
Q83 (= ST2481/55)	F	113/13	<i>S. triandra</i> L. × <i>S. viminalis</i> L.	–	England	6.1 (1.4) c	5.8 (0.19) c
Bjorn	M	Bjorn	<i>S. viminalis</i> × <i>S. schwerinii</i> E. Wolf	–	Sweden	8.7 (3.2) cd	6.1 (0.08) c
Tora	F	Tora	<i>S. viminalis</i> × <i>S. schwerinii</i>	–	Sweden	11.0 (3.3) d	5.6 (0.30) bc
Delamere	F	026/01	<i>S. caprea</i> L. × <i>S. cinerea</i> L. × <i>S. viminalis</i>	–	England	9.6 (6.3) cd	5.8 (0.11) c

### Xylem vulnerability to cavitation

Xylem cavitation was assessed by the Cavitrone technique (Cochard et al. 2005). The principle of the technique is to lower the pressure in a xylem segment by centrifugal force and, at the same time, measure the variation in its hydraulic conductance. The percent loss of xylem conductance (PLC) versus the xylem pressure represents the sample vulnerability curve. Vulnerability curves were determined on five samples of each clone. To obtain information on variation in cavitation resistance within a tree, two samples were taken from the current-year growth unit, and three from the previous-year growth unit. Samples were cut in air to obtain 0.28-m-long segments, i.e., longer than the longest vessels as indicated by the air perfusion method. When segment diameter was larger than 0.01 m, the segment was split longitudinally with a knife into two to six parts. Bark was removed from all segments and pith was removed from the split segments. Xylem pressure ( $P$ ) was first set to a reference pressure ( $-0.5$  or  $-1.0$  MPa) and maximal conductance ( $K_{\max}$ ) determined. The xylem pressure was then set to a more negative pressure for 30 s and subsequently returned to the reference pressure to determine the new conductance ( $K$ ). Percent loss of conductance was then computed as  $PLC = 100(1 - K/K_{\max})$ . The procedure was repeated for more negative pressures (typically in  $-0.25$  MPa step increments) until PLC reached at least 95%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution) and xylem pressure was adjusted at about  $\pm 0.02$  MPa. A total of 45 vulnerability curves were obtained for nine clones over three days. The following sigmoid function was fitted to each curve (Pamenter and Vander Willigen 1998):

$$PLC = \frac{100}{1 + e^{\frac{s}{25}(P - P_{50})}}$$

where  $P_{50}$  is the pressure causing 50% loss of conductance and  $s$  is a slope parameter. Values of  $P_{50}$  and  $s$  were averaged for each clone, and values for poplar and willow clones were compared by  $t$ -tests.

### Wood density

Wood xylem density was determined according to Archimedes's principle. A 0.03-m-long segment was cut from one end of each sample, its fresh volume measured by water displacement with an analytical balance and its dry mass determined. Additionally, three dominant shoots per clone were harvested in December 2006 to estimate wood density on bigger segments. Branches and bark were removed from each shoot after its length was recorded. The fresh volume of each shoot was then estimated by measuring the end-diameters of all consecutive 50-cm-long segments from the bottom to the top with a caliper. Sampled shoots were dried at 95 °C and their dry masses determined.

### Anatomical traits

Anatomical observations were made on all 2-year-old segments used for cavitation analysis. Cross sections were ob-

tained from one end of the sample and stained with Congo red. Sections were observed with a light microscope (Wild M11, Heerbrugg, Switzerland) equipped with a Camera Lucida and a digital measuring device. The resolution of the system was about 0.2  $\mu\text{m}$ . The following parameters were measured with at least 60 observations for each sample. On cross sections, we measured the lumen diameter ( $D$ ) and wall thickness of the vessels, the thickness ( $t$ ) of walls between adjacent vessels (bordered pit field wall), and lumen diameter and wall thickness of fibres. On one representative segment of each clone, we measured pit diameter and pit aperture dimensions on tangential sections. Following Hacke et al. (2001), we derived the mean vessel hydraulic diameter ( $D_h = \Sigma D^5 / \Sigma D^4$ ) and the mechanical strength of the bordered pit fields at  $D_h$  as  $(t^2/D_h^2)_h$ .

### Results

The annual yields of selected poplar and willow clones ranged from 2.2 to 11.0 Mg DM ha<sup>-1</sup> year<sup>-1</sup>, and willows were more productive on average than poplars (Table 1). At the end of the 2006 growing season, dominant shoot heights ranged from 4.4 to 8.9 m for poplars and from 5.6 to 6.1 m for willows (Table 1).

Xylem vulnerability curves (VCs) were typically sigmoid and were well-fitted ( $r^2 > 0.98$ ) by the Pamenter Vander Willigen (1998) equation. Splitting stem segments into several pieces so they would fit the Cavitrone had no substantial effect on the resulting VCs (Figure 1). Values of  $P_{50}$  for current- and previous-year shoots were closely correlated in all clones ( $r^2 = 0.82$ ,  $P < 0.01$ ), but current-year shoots were significantly less vulnerable to cavitation (Student's paired  $t$ -test,  $P = 0.014$ ). The difference was more pronounced for poplar clones (0.12 MPa, SE = 0.04;  $n = 5$ ) than for willow clones (0.04 MPa, SE = 0.01,  $n = 4$ ). Hence, the variation in Figure 1 probably reflects the greater vulnerability of bigger stems, which were

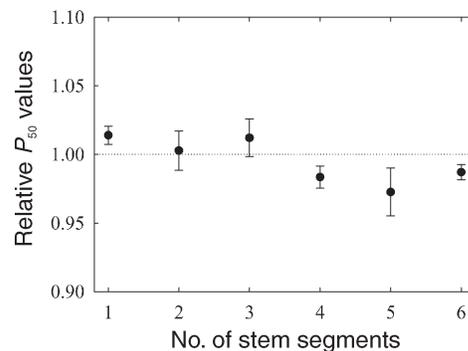


Figure 1. Effect of splitting shoots longitudinally into several segments on their vulnerability to cavitation as assessed by the Cavitrone technique. The x-axis represents the number of segments into which the stem was split (1 is for an entire segment, 2 for a shoot split in half, etc.). Larger stems were split into more segments. The y-axis represents the xylem pressure causing 50% loss of conductance ( $P_{50}$ ) of the different samples relative to the mean  $P_{50}$  value for the same clone. Error bars are 1 SE ( $n = 3-13$ ).

split into more segments. Poplar and willow clones were relatively sensitive to cavitation, with mean  $P_{50}$  values of  $-2.0$  and  $-1.8$  MPa, respectively. Figure 2 shows the vulnerability curves for the most and least vulnerable clones of each genus. Differences between clones were small but highly significant, because variation within clones was small (Figure 3A). The slope parameter exhibited less variation than  $P_{50}$  among clones. Only the more vulnerable willow clones had significantly steeper slopes (Figure 3B).

Xylem vulnerability to cavitation was correlated to several macroscopic and microscopic traits. A striking, highly significant, and positive correlation was found between  $P_{50}$  and yield (Figure 4A), the most productive clones being the most vulnerable to cavitation. By contrast, wood density, whether measured on terminal shoots or whole trees, had no correlation with  $P_{50}$  (Figure 4B). Xylem anatomical traits were obtained for 2-year-old shoots and correlated with the mean  $P_{50}$  values of the same segments (Figure 4). The correlation between  $P_{50}$  and vessel dimensions was not significant, but there was a trend for clones with the largest vessels to be less vulnerable to cavitation (Figure 4C). Similarly, the correlation with pit field wall strength was not significant, i.e., clones more resistant to cavitation did not have thicker walls (Figure 4D). There was a negative but weak ( $P = 0.08$ ) correlation between xylem cavitation and fiber wall thickness (Figure 4E), with clones more resistant to cavitation having thicker fiber walls. Pit dimensions were unrelated to cavitation resistance (Figure 4F).

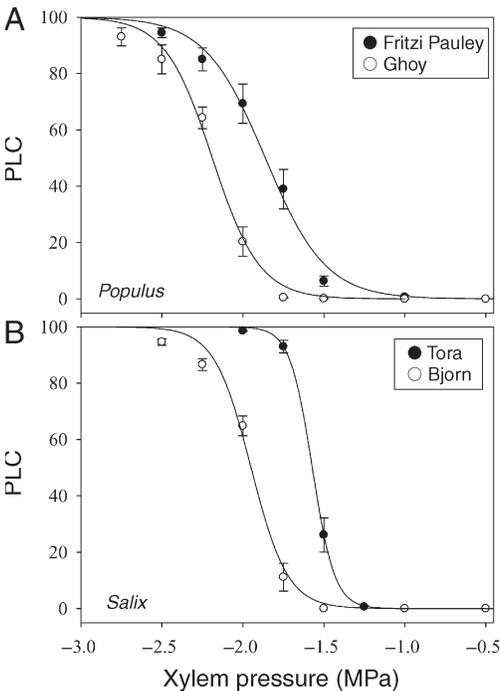


Figure 2. Xylem vulnerability curves for the most contrasted (A) poplar and (B) willow clones. Lines are logistic fits to the data and error bars are 1 SE ( $n = 5$ ). Abbreviation: PLC, percent loss of conductance.

**Discussion**

Xylem vulnerabilities to cavitation reported in this study are in the range of values previously reported for poplar (e.g., Sperry et al. 1991, Hacke and Sauter 1996, Cochard et al. 1996,

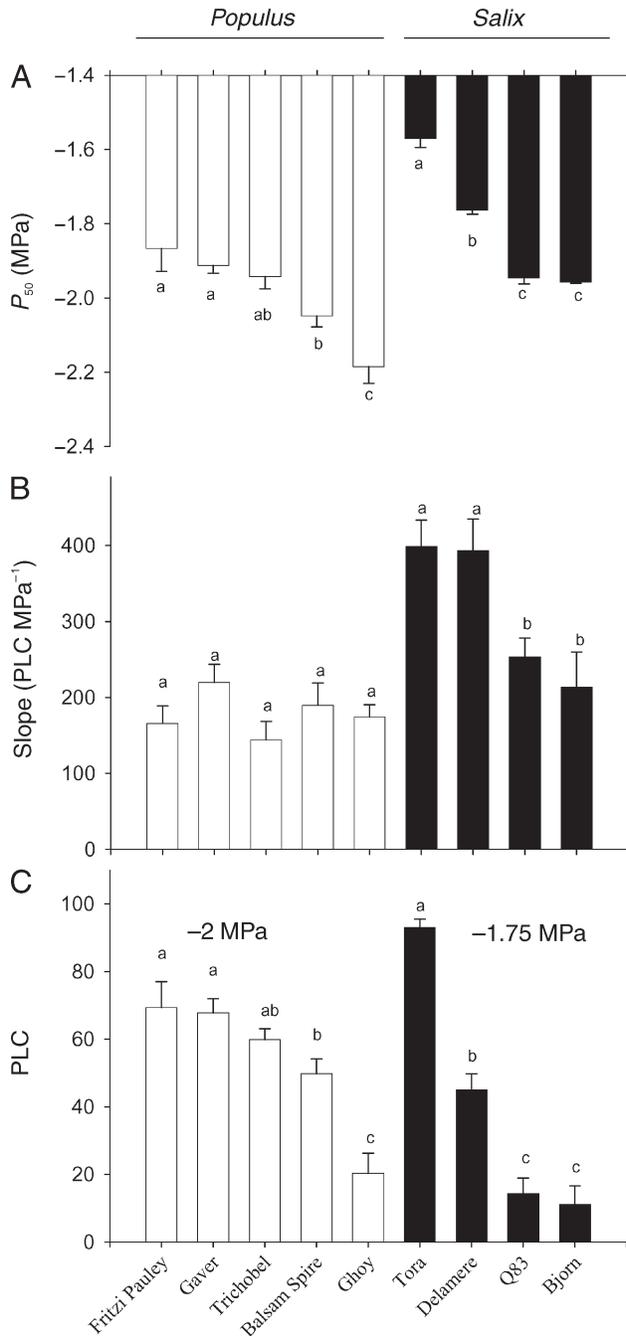


Figure 3. Inter-clonal variation in xylem vulnerability to cavitation. The graphs show the variation of the two parameters of the logistic fits to the vulnerability curves, i.e., the pressure causing (A) 50% loss of conductance ( $P_{50}$ ) and (B) the slope at the inflection point. (C) Mean percent loss of conductance (PLC) at  $-2.0$  and  $-1.75$  MPa for *Populus* and *Salix* clones, respectively. Error bars are 1 SE ( $n = 5$ ). Clones with different letters have statistically different values at  $P < 0.05$ .

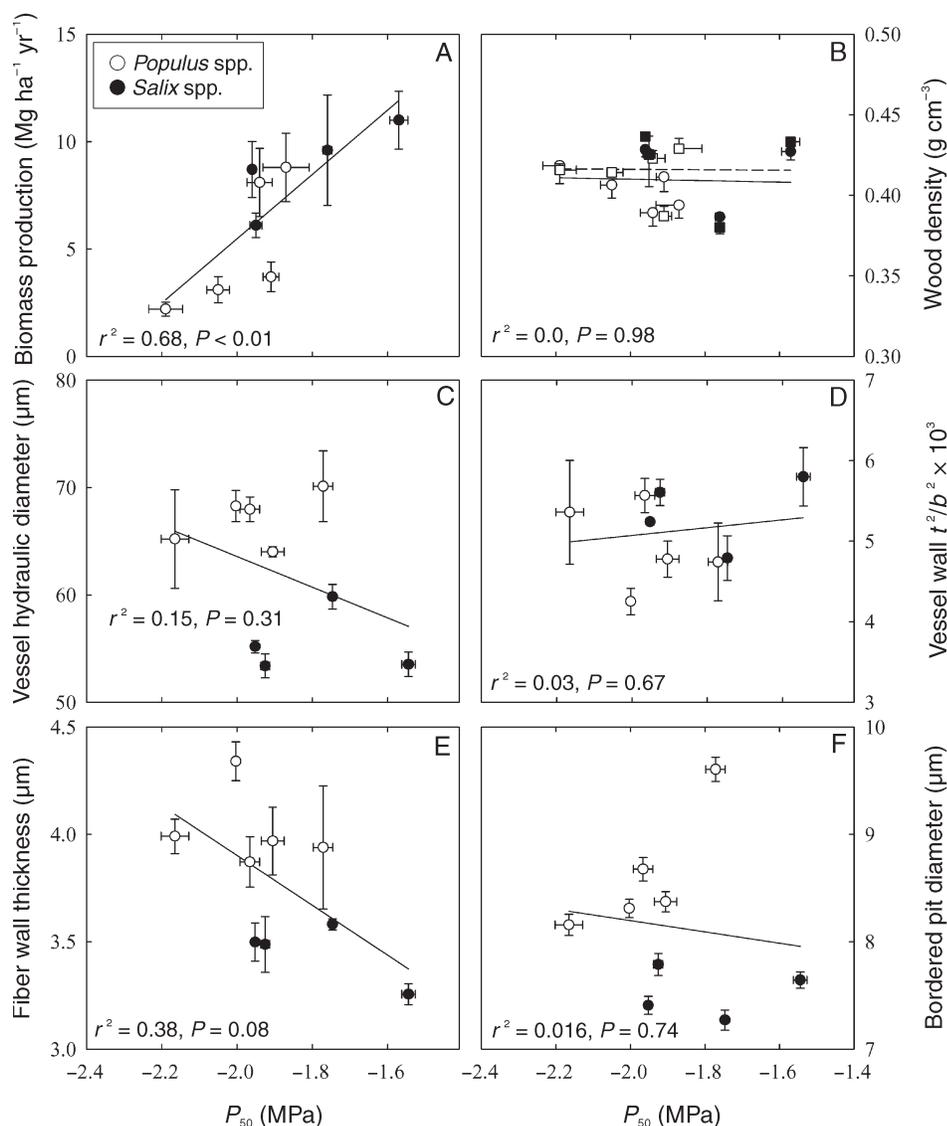


Figure 4. Correlations between xylem vulnerability to cavitation measured as the xylem pressure causing 50% loss of conductance ( $P_{50}$ ) and (A) mean clone annual yield, (B) wood density measured on terminal shoots (○, ●) or whole trees (□, ■), (C) vessel hydraulic diameter, (D) mechanical strength of pit field walls, (E) fiber wall thickness and (F) pit diameter. For panels A and B, the x-axis represent the mean  $P_{50}$  values for each clone ( $n = 5$ ). For panels C–F,  $P_{50}$  values are for 2-year-old shoots only ( $n = 3$ ). Lines are linear regressions, and error bars are 1 SE.

Harvey and van den Driessche 1997, Hukin et al. 2005) and willow (Wikberg and Ogren 2004) species. This confirms that these species are among the most vulnerable temperate tree species, which is consistent with their requirement for high soil water content. With cavitation thresholds as high as  $-1.5$  MPa, the risk for xylem dysfunction is high in drying soils. Therefore, selecting more cavitation-resistant genotypes will substantially lower the risk of drought-induced embolism, and, hypothetically, increase tree resilience to drought.

The Cavitron technique revealed small but significant differences in vulnerability to cavitation among clones. Five replicates were sufficient to detect statistically significant differences. Hence, the plant material required for this analysis is minimal (a few shoots), which is an important consideration in breeding programs. Splitting stems had no substantial effect on their vulnerability to cavitation. Similar results were obtained with *Pinus pinaster* Ait. shoots (Author's unpublished observations). This contrasts with the observations of Kikuta et al. (2003), who used a different technique to detect cavi-

tion. Vulnerability curves were typically obtained in half an hour, which means that between three and four clones could be characterized with the Cavitron technique during a working day. One way to improve the efficiency of the technique might be to measure the PLC value of different clones at a single pressure instead of building a vulnerability curve. Figure 3C shows the mean PLC value for poplar and willow at  $-2.0$  and  $-1.75$  MPa, respectively. This protocol discriminates among genotypes as efficiently as the measurement of  $P_{50}$  values and might double the number of genotypes that could be evaluated during a day. However, screening hundreds of genotypes with this method is probably impossible, indicating the necessity of finding correlated and more accessible parameters if cavitation resistance is to be considered in breeding programs.

Among anatomical traits measured, few were correlated with xylem cavitation. From a deterministic point of view, this is unsurprising, because the mechanism of water-stress-induced cavitation is thought to be determined by the porosity of pit walls. Although several characteristics are correlated with

cavitation, such as wood density and pit field wall strength (Hacke et al. 2001), this was not the case for the poplar and willow clones we analyzed. This suggests that the good correlations observed when highly contrasting species were compared may no longer apply when more closely related genotypes are evaluated, and implies that there is little likelihood of finding suitable anatomical traits for indirect assessments of cavitation resistance among willow or poplar genotypes. The only exception to this rule might be fiber wall thickness, which was negatively correlated to xylem cavitation in our study, in agreement with the recent finding of Jacobsen et al. (2005), who compared more contrasting species.

We identified a striking correlation between yield and xylem vulnerability to cavitation, with the more productive clones being more vulnerable. This is consistent with the recent finding of Wikberg and Ogren (2004) for willow. This correlation may be explained on the basis of a higher construction cost of wood with thicker walled fibers, or by more biomass allocation to roots, or both, for the less productive clones compared with the more productive clones. This finding suggests a possible trade-off between increased cavitation resistance and decreased productivity for these species. However, it is unknown whether there is a causal relationship between the two parameters or if growth can be genetically uncoupled from cavitation resistance. For instance, more productive *Hevea* clones tend to be more resistant to cavitation (Sangsing et al. 2004a, 2004b).

In conclusion, cavitation resistance exhibits substantial variation among willow and poplar clones, but this variation is poorly related to easily assessed anatomical traits. The Cavitrone technique is appropriate for evaluating a moderate number of clones, but either new techniques or improved protocols are necessary for high throughput screening of genotypes for these characteristics. Cavitation resistance seems to come at the cost of reduced growth and yield in both poplar and willow. It would be appropriate to document the progeny of a crossing between contrasting genotypes to investigate whether these traits can be unravelled. More experimental evidence is also required to demonstrate that variation in cavitation resistance among clones correlates with drought resilience in the field.

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