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RESEARCH PAPER

Cavitation vulnerability in roots and shoots: does *Populus euphratica* Oliv., a poplar from arid areas of Central Asia, differ from other poplar species?

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Abstract

***Populus euphratica* is a poplar species growing in arid regions of Central Asia, where its distribution remains nevertheless restricted to river-banks or to areas with an access to deep water tables. To test whether the hydraulic architecture of this species differs from that of other poplars with respect to this ecological distribution, the vulnerability to cavitation of *P. euphratica* was compared with that of *P. alba* and of *P. trichocarpa*× *koreana*. The occurrence of a potential hydraulic segmentation through cavitation was also investigated by assessing the vulnerability of roots, stems, and leaf mid-rib veins. Cryo-scanning electron microscopy (cryo-SEM) was used to assess the level of embolism in fine roots and leaf mid-ribs and a low pressure flowmeter (LPFM) was used for stems and main roots. The cryo-SEM technique was validated against LPFM measurements on paired samples. In *P. alba* and *P. trichocarpa*× *koreana*, leaf mid-ribs were more vulnerable to cavitation than stems and roots. In *P. euphratica*, leaf mid-ribs and stems were equally vulnerable and, contrary to what has been observed in other species, roots were significantly less vulnerable than shoots. *P. euphratica* was by far the most vulnerable. The water potential inducing 50% loss of conductivity in stems was close to −0.7 MPa, against ~ −1.45 MPa for the two others species. Such a large vulnerability was confirmed by recording losses of conductivity during a gradual drought. Moreover, significant stem embolism was recorded before stomatal closure, indicating the lack of an efficient safety margin for hydraulic functions in this species. Embolism was not reversed by rewatering. These observations are discussed with respect to the ecology of *P. euphratica*.**

Key words: Drought, embolism, hydraulic architecture, hydraulic segmentation, phreatophyte, *Populus*, stomatal conductance, water relations.

Introduction

Populus euphratica is a poplar species distributed in arid areas of Central Asia that display very hot, dry summers. It is sometimes used for afforestation in semi-arid areas such as in India (Sharma *et al.*, 1999). As a consequence of intensive water use and damming in NW China, the stands of *P. euphratica* have suffered a severe decline, contributing to the numerous changes in vegetation occurring in these regions (Bruelheide *et al.*, 2003; Wang and Cheng, 2000). The growth and survival of this species rely on access to deep water tables such as those occurring on river banks (phreatophytic habit). Gries *et al.* (2003) clearly demonstrated from *in situ* studies in the Taklamakan desert

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Abbreviations: A_L, total leaf area; A_W, functional sapwood area; cryo-SEM, cryo-scanning electron microscopy; g_s, stomatal conductance; K_H, hydraulic conductivity; $K_{H(SAT)}$, saturated hydraulic conductivity; LPFM, low pressure flowmeter; *PLC*, percentage loss of hydraulic conductivity; Ψ, water potential; Ψ_{PLC50}, water potential inducing 50% loss of hydraulic conductivity; *REW*, relative extractable water; VC, vulnerability curve; VP SEM, variable pressure scanning electron microscope.

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(SinKiang, NW China) that the growth rate was dependent on the depth of the water table. This species is also well-known for its ability to cope with high levels of salinity (Chen *et al.*, 2001; Gu *et al.*, 2004). However, to date there is no information about its distribution in arid areas and whether its tolerance to salinity has any consequence on the hydraulic properties of this species that make it differ from other poplars.

Poplars are usually described as highly productive and displaying fast growth, but at the expense of a large water requirement (Tschaplinski *et al.*, 1994; Harvey and Vandendriessche, 1997; Rood *et al.*, 2003). Many poplar species like *P. nigra* in Europe (Lambs and Muller, 2002), and *P. trichocarpa* and *P. deltoides* in North America (Tyree *et al.*, 1994) grow in riparian areas and rely on the presence of a water table. Despite some degree of genetic diversity, all species of the genus *Populus* are known to be rather drought-sensitive (Chen *et al.*, 1997; Brignolas *et al.*, 2000). One important trait contributing to this low tolerance to water deficit is the high vulnerability to drought-induced cavitation: xylem vessels of poplars lose functionality at a rather high water potential (cavitation begins around −1 MPa). Among all investigated woody plants, poplars belong to the most vulnerable species (Tyree *et al.*, 1992, 1994; Hacke and Sauter, 1996; Tognetti *et al.*, 1999).

Vulnerability to drought-induced cavitation may vary within a tree. Diversity in organ vulnerability has, up to now, received relatively little attention compared with inter-specific diversity, despite the fact that it may play a major role in the response to drought at the whole tree level. Tyree *et al.* (1993) detected a larger cavitation vulnerability of petioles compared with 1-year-old shoots in walnut trees and suggested this may lead to an efficient hydraulic segmentation through the induction of leaf shedding in response to drought. Poplars might display similar features since leaf shedding is a common occurrence in several clones in response to unfavourable microclimate and particularly to drought (Cochard *et al.*, 1996*b*). Below ground, root vulnerability to cavitation has less frequently been measured and was usually found to be larger than that of shoots (Alder *et al.*, 1996; Hacke and Sauter, 1996; Froux *et al.*, 2005). Since water potential in a transpiring plant is less negative in roots than in shoots, a higher vulnerability in roots may be of limited consequence for overall plant hydraulic functions. Moreover, as put forward by Froux *et al.* (2005), lateral roots may be more prone to cavitation than main roots. This would lead to hydraulic segmentation protecting root systems against reverse water flow from the main roots to the lateral roots and ultimately to dry soil layers, and therefore from net water losses.

The present work was aimed at assessing the vulnerability to cavitation of *P. euphratica*, in order to ascertain whether this arid-zone species could display a smaller vulnerability than other poplars. Two poplar clones from different species were compared: *P. alba* (clone 2AS11, southern Italy) and *P. trichocarpa*×*koreana* (cv. Peace), a cultivar in which the stomata are insensitive to abscisic acid, shedding leaves as soon as water availability decreases (Cochard *et al.*, 1996*b*). It was also assessed whether significant differences in vulnerability to cavitation occurred among different organs; in particular, there was interest in the vulnerability of lateral roots and leaf veins that may constitute large resistances to water flow with respect to main roots and stems. As small roots and leaf mid-rib veins were not accessible to direct conductivity measurement, the cryo-scanning electron microscopy (cryo-SEM) imaging method developed by Canny (1997*a*, *b*) and adapted by Cochard *et al.* (2000, 2004) was used. The cryo-SEM technique was validated with small branch and root segments on which two adjacent segments were either used to measure loss of conductivity with the technique of Sperry *et al.* (1988) or to count the fraction of embolized vessels from cryo-SEM images. Finally, in order to test for the functional consequences of the vulnerability assessed on severed shoots and roots by pressurization, the loss of hydraulic conductivity was monitored in roots and stems of *P. euphratica* during the course of a gradually increasing drought due to soil water depletion.

Materials and methods

Plant material

Vulnerability curves were established on three clones of poplar: *Populus alba* L. (cv. 2AS11 provided by Maurizzio Sabatti, Universita della Tuscia, Viterbo, Italy), *P. euphratica* Oliv. (originating from seeds imported from China by Andrea Polle, University of Goettingen, Germany) and *P. trichocarpa*×*koreana* (cv. Peace) grown at Nancy. For *P. alba* and *P. trichocarpa*×*koreana*, stem cuttings were planted in a 2/1 v/v mix of sand and peat in 5.0 l pots and grown in a greenhouse at Champenoux, close to Nancy, Eastern France. After 14 d, the plants were fertilized with a slow-release fertilizer (Nutricote 100, N/P/K 13/13/13 plus oligo-elements, 4 g l^{-1} substrate). For *P. euphratica*, cuttings were rooted for one year before they were pruned, transplanted, and grown and fertilized in similar conditions to the two other species. Irrigation was provided daily via drip irrigation, maintaining the substrate close to field capacity. Plants were pruned to keep a maximum of two shoots per plant. Plants with new-growth shoots measuring at least 60 cm were used for investigations after a growing period of 8–16 weeks.

A drought experiment was conducted on a *P. euphratica* clone originating from the Ein Avdat natural park (provided by A Altman, Rehovot University, Israel). After *in vitro* multiplication, plantlets were acclimated to greenhouse conditions, transferred into 7.0 l pots filled with a peat/sand mix (1/1 v/v), grown for 2 months, and then subjected to a controlled water deficit. Soil volumetric water content was controlled by TDR probes (Trase, Soilmoisture Equipement Corp., Goleta, CA, USA) and pot weighing. Controls were irrigated to field capacity twice a day. Control and droughted plants were harvested at four levels of soil volumetric water content (10, 7.5, 5, and 4%) and after recovery (10 d of full rewatering). Pieces of stem and main root were collected under water and loss of hydraulic conductivity was measured using a low pressure flowmeter as described below. Stomatal conductance to water vapour (g_s) was monitored every second day on a separate batch of plants submitted

to a similar drought course, with a portable gas exchange chamber Li-Cor 6200 (Li-Cor; Lincoln, USA) on leaf 15, a young fully expanded leaf.

Vulnerability to cavitation

Prior to measurements, plants were placed in black plastic bags and kept in the dark overnight to ensure zero transpiration and full hydration. Shoots and whole root systems were subsequently harvested under water and placed in a pressure chamber. Dehydration of plant material to the target values of water potential (between 0 and −2.5 MPa) was performed by the air injection method (Cochard *et al.*, 1992). When the balance pressure reached within 0.05 MPa of the desired water potential (demonstrated by the cessation of sap exudation from the protruding end), shoots or roots were removed from the chamber and dissected under water into samples for embolism determination. Embolism was estimated in 2 cm internode stem segments (taken at 10, 20, and 30 cm from the branch apex) and 2 cm main root segments as the percentage loss of conductivity (PLC) measured using a low pressure flowmeter (Sperry *et al.*, 1988). Leaf mid-rib veins and lateral roots were harvested for visual analysis using cryo-scanning electron microscopy (cryo-SEM) imaging (Canny, 1997*a*; Cochard *et al.*, 2000). Paired samples were also harvested from stems and roots over a range of water potentials to allow a comparison of LPFM and cryo-SEM methods of measuring embolism.

Embolism by low pressure flowmeter (LPFM)

Conductivity measurements of samples were made using a xylem embolism meter (XYL'EM, Instrutec, France) and the percentage loss of conductivity was recorded following the procedure described previously (Sperry *et al.*, 1988; Cruiziat *et al.*, 2002). Initial state conductivity (K_H: mol MPa^{-1} m s^{-1}) was measured by gravitation perfusion with 10 mM KCl solution at low pressure (1.5 kPa). Afterwards, samples were flushed at high pressure (150 kPa) with 10 mM KCl solution to remove emboli from conduits. Saturated state conductivity ($K_{H(SAT)}$: mol MPa^{-1} m s^{-1}) was then measured and *PLC* computed as described by Sperry *et al.* (1988).

Embolism by cryo-scanning electron microscopy (Cryo-SEM)

Following dissection under water, samples to be examined by cryo-SEM imaging were frozen immediately in liquid nitrogen and stored at −80 °C. It was important that samples were not allowed to thaw, so all handling during preparation was carried out in liquid nitrogen. Frozen samples were placed on a loading arm and inserted into a preparation cryo-chamber (model Alto 2100, Gatan, Oxford, UK). Samples were freeze-fractured and, once the vacuum achieved, loaded in the scanning electron microscope (model 1450VP, Leo, Cambridge, UK) onto a cryo-stage for sublimation. The specimen was freeze-etched under a vacuum of 50 Pa and equilibrated to −90 °C for about 1 min to eliminate contaminant frost accumulated during sample preparation. The samples were then recooled to −120 °C and backscattered secondary electron images were observed at an accelerating voltage of 12 kV, a probe intensity of 500 pA and at a working distance of 10 mm. Digital images of the sample vasculature were captured. After optimizing the clarity of images by adjusting brightness and contrast with the software Adobe Photoshop (v. 5.0 LE, Adobe), images were printed with a high resolution and vessels identified on the prints. Analysis was carried out by visually counting the total number of ice-filled (conductive) and empty (embolized) vessels (Fig. 1). Embolism was thereafter computed as the ratio of empty vessels versus the total number of vessels.

Vulnerability curves

Vulnerability curves were constructed based on the relationship between xylem water potential (equivalent to the opposite pressure applied to the sample) and embolism. Data were fitted to a sigmoïd equation (equation 1) as exposed by Pammenter and Willigen (1998):

$$PLC = 100/(1 + \exp(a(\Psi - b))) \quad (1)$$

where *PLC* is the percentage loss of conductivity, Ψ the water potential, and *a* and *b* are the slope of the curve and the water potential at 50% loss of conductivity (Ψ_{PLC50}), respectively.

Parameters *a* and *b* for each combination [species×organ] and their confidence interval were estimated by fitting a non-linear model with *gnls( )* function of *R* software (The R Foundation for Statistical Computing Version 2.0.1 (2004-11-15), ISBN 3-900051-07-0).

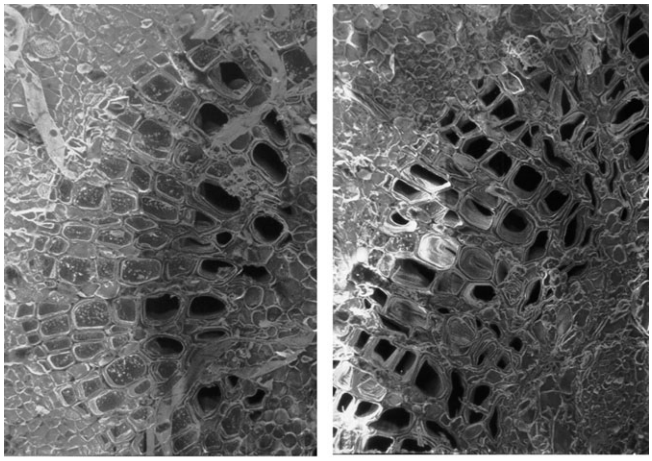


Fig. 1. Cryo-SEM images of leaf mid-rib veins of *Populus alba* after submitting the shoot to a pressure of 0.5 MPa (left) and 2.5 MPa (right). Embolized vessels are empty, while the functional ones are filled with ice.

Statistical analysis

Contrasts were used for testing the equality of *b* estimates (Ψ_{PLC50}), (i) among species within each organ and (ii) among organs within each species. Pairwise comparison error rates were adjusted for an overall error rate $\alpha < 0.05$, taking into account the joint *t* distribution of contrasts estimated from the parameters of the covariance matrix. The function *csimtest( )* from the multcomp R package (version 0.4–8) was used for this purpose.

Results

An example of the cryo-SEM images obtained from mid-rib veins of *P. euphratica* following exposure to 0.5 MPa and 2.5 MPa pressure is given in Fig. 1. The technique allowed easy and clear-cut distinction between empty (embolized) and full (functional) vessels. The validation of this technique with paired estimates of embolism in stems and roots using either hydraulic conductivity measurements (LPFM) or cryo-SEM imaging resulted in a very close relationship over the whole range of induced embolism as shown in Fig. 2. The relationship was linear (r^2=0.96) and close to the 1:1 regression line. Based on this calibration, the two techniques were used concurrently to construct and compare vulnerability curves, LPFM for large samples (stems and main roots) and cryo-SEM for small samples (leaf mid-rib veins and lateral roots).

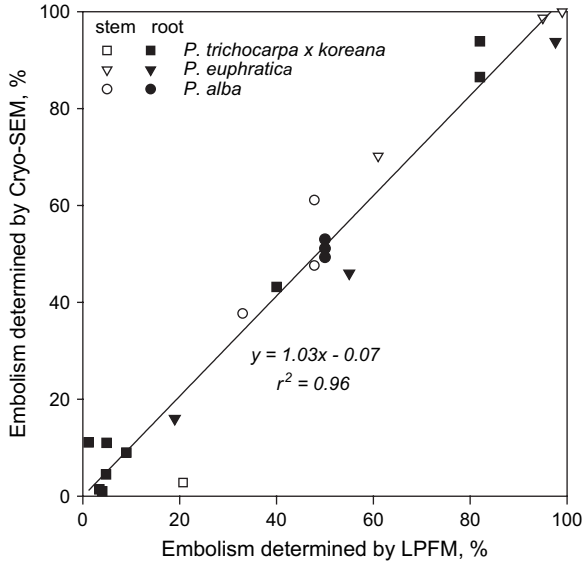


Fig. 2. Relationship between estimates of embolism obtained on adjacent stem and root segments with a low pressure flowmeter (LPFM) measuring the percentage loss of hydraulic conductivity or with cryo-SEM records of the fraction of embolized vessels. Each point represents a paired measurement on stems or roots from rooted cuttings of *Populus alba* cv. 2AS11, *P. euphratica* or *P. trichocarpa*×*koreana* cv. Peace. The linear regression between the two estimates is represented.

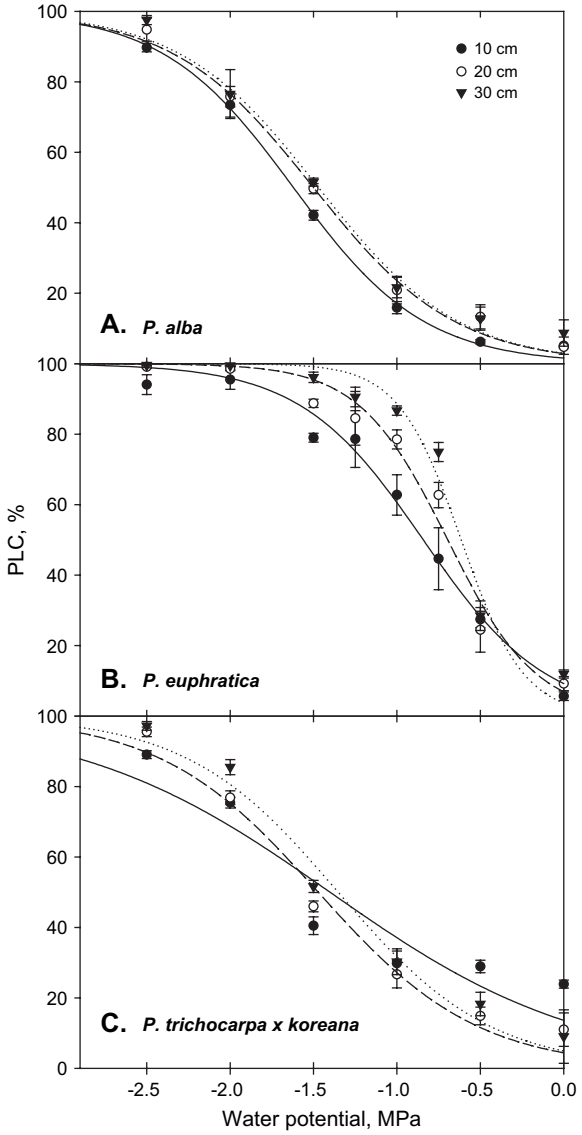


Fig. 3. Percentage loss of conductivity (*PLC*) as a function of the imposed pressure (water potential) along main stems of rooted cuttings of poplar, as estimated on small segments at a distance of 10, 20, and 30 cm from the terminal apex. (A) *Populus alba* cv. 2AS11, (B) *Populus euphratica*, and (C) *Populus trichocarpa*×*koreana* cv. Peace. Whole shoots were pressurized to the corresponding indicated water potential. Vertical bars correspond to standard errors (n=3).

The longitudinal variability of vulnerability to cavitation was assessed on 60 cm long main stems of the three species, at a distance of 10, 20, or 30 cm from the terminal bud (Fig. 3). A small but significant variability was recorded, the apical segment displaying a lower vulnerability than the more distal ones (Fig. 3; Table 1). In *P. trichocarpa*×*koreana*, the youngest segment apparently displayed a high native level of embolism (*PLC* in excess of 20% at 0 MPa). This segment also contained larger mid-stem pith compared with the two others (data not shown). The observed high native *PLC* was therefore probably an artefact due to the circulation of pressurized water through the central pith during flushing. Indeed, cryo-SEM images of apical stem segments revealed no such native levels of embolism, further suggesting that the LPFM method may have produced artificially high estimates of embolism for these samples.

Cavitation vulnerability curves of diverse plant organs in the three poplar species is shown in Fig. 4. Native state embolism was usually rather small, below 10%, showing that the plants had not undergone a significant stress before the measurements. Again, the larger native embolism in the stem of *P. koreana*×*trichocarpa* may be a measurement artefact due to a large central pith. In all cases, leaf mid-ribs displayed the largest vulnerability, as confirmed by the values of Ψ_{PLC50} shown in Table 2. In *P. alba* and *P. trichocarpa*×*koreana*, roots and stem vulnerabilities were almost confounded and significantly less vulnerable than leaf mid-ribs. In *P. euphratica*, stem and leaf mid-rib vulnerabilities were similar and roots displayed a significantly lower vulnerability than shoots (Fig. 4; Table 2).

Among the three species, *P. euphratica* was the most vulnerable. This clone had already suffered 50% loss of conductivity in the stems at −0.70 MPa compared with −1.41 MPa for *P. trichocarpa*×*koreana* and −1.53 MPa for *P. alba* (Table 2). This large interspecific difference was not detected in the roots, whose vulnerability was similar in the three species ($-1.64 < \Psi_{PLC50} < -1.32$ MPa).

To check *in vivo* whether *P. euphratica* was highly vulnerable to cavitation, embolism development was followed on rooted plants during the course of a slowly

Table 1. *Estimates of water potential inducing 50% loss of hydraulic conductivity (Ψ_{PLC50}, MPa) at three distances from the stem apex of three poplar species*

Values are derived from statistical adjustments of a logistic model to the experimental points of vulnerability curves (estimate ±SE). Different letters in a line indicate significant difference between segments within this species.

	10 cm	20 cm	30 cm
P. alba cv. 2AS11	−1.62±0.09 a	−1.50±0.03 a	−1.47±0.03 a
P. euphratica	−0.84±0.08 c	−0.69±0.03 b	−0.61±0.02 a
P. trichocarpa ×koreana	−1.40±0.10 ab	−1.47±0.02 b	−1.35±0.03 a

imposed drought due to soil water depletion and its recovery (Fig. 5). Indeed, drought-stressed plants displayed significant percentage loss of conductivity (*PLC*) after 12 d, i.e. at a relative extractable soil water of about 38%. *PLC* increased gradually during the course of the drought, while stem and roots of control plants exhibited low *PLC* during the whole experiment (6.1%±1.0 and 4.8%±0.5, respectively, data not shown). In addition, whatever the soil water content, larger levels of embolism were recorded in the stems than in the roots, thereby confirming the higher vulnerability of *P. euphratica* stems compared with roots. Following rewatering for 10 d, *PLC* of stem and roots remained at the same level as during the strongest stress intensity, showing that embolism was not reversed. Interestingly, the onset of cavitation and embolism began before stomatal closure and the stem had already lost 30% of its conductivity when stomatal conductance was still 50% of controls (Fig. 5).

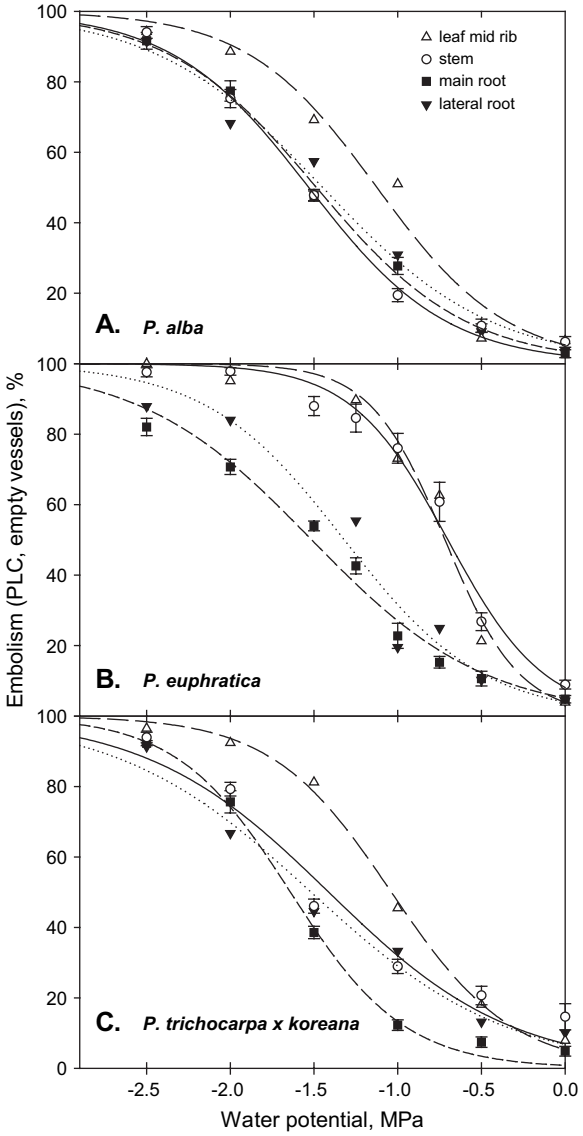


Fig. 4. Degree of embolism as a function of the imposed pressure (water potential) in stems and main roots (*PLC* measured by LPFM) and in leaf mid-ribs and lateral roots (fraction of embolized vessels recorded on cryo-SEM images). (A) *Populus alba* cv. 2AS11, (B) *P. euphratica*, and (C) *P. trichocarppa×koreana* cv. Peace. Cavitation was induced by pressurizing whole shoots and root systems. Vertical bars correspond to standard errors (n=3–9).

Discussion

The cryo-SEM technique yielded very convergent results with respect to low-pressure flowmeter (LPFM) measurements. Cryo-SEM has been used to demonstrate embolized vessels in different organs such as roots (McCully *et al.*, 1998; Pate and Canny, 1999), stems (Utsumi *et al.*, 1999), and leaves (Canny, 1997*b*). It is not known whether there has been any other attempt to compare directly results produced by these two techniques. This study's experiment showed a particularly tight correlation between the fraction of embolized vessels detected by cryo-SEM and the fraction loss of hydraulic conductivity measured with the LPFM. Such a linear and 1:1 relationship may not be the general case for all species, depending on their hydraulic anatomy. Indeed, a linear relationship is expected in diffuse porous wood with homogeneous vessel diameters and with long parallel vessels with limited lateral interconnections. A frequently discussed potential artefact of the cryo-SEM technique is the fact that xylem vessels under tension may cavitate during the freezing process (Cochard *et al.*, 2000, 2001; Richter, 2001). It has been suggested that, in order to avoid such problems, samples should first be excised under water before freezing (as carried out in the present study) rather than by freezing intact samples on transpiring plants. These findings confirmed this point of view and demonstrated the suitability of cryo-SEM to assess the degree of embolism in cavitated organs.

Poplar species in general are known to be extremely vulnerable to cavitation and none of the three clones studied in this investigation proved to be an exception to this paradigm. As compared with many other tree species, the poplars tested were very vulnerable to cavitation, which was induced as soon as the water potential dropped below

Table 2. *Estimates of water potential inducing 50% loss of hydraulic conductivity (Ψ_{PLC50}, MPa) in lateral roots, main roots, stem, and leaf mid-rib veins of three poplar species*

Values are estimate ±SE. Different letters (a, b, c) in a species line indicate significant difference between organs within this species. Different letters (A, B, C) in an organ column (below the Ψ_{PLC50} value) indicate significant difference between species for this organ.

	Lateral root	Main root	Stem	Mid-rib vein
P. alba cv. 2AS11	−1.45±0.09 b A	−1.49±0.03 b A	−1.53±0.03 b C	−1.12±0.08 a B
P. euphratica	−1.32±0.08 b A	−1.51±0.03 b A	−0.70±0.02 a A	−0.72±0.04 a A
P. trichocarpa×koreana	−1.52±0.10 bc A	−1.64±0.02 c B	−1.41±0.03 b B	−1.04±0.08 a B

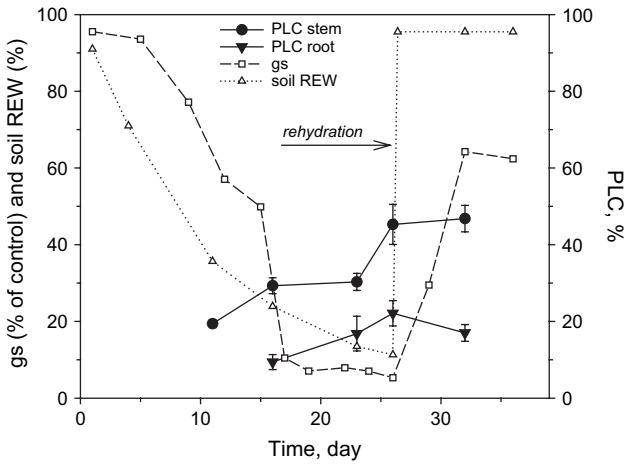


Fig. 5. Time-course of relative extractable soil water (*REW*), stomatal conductance to water vapour (g_s, expressed as a percentage of controls), and percentage loss of hydraulic conductivity (*PLC*) in stems and main roots of drought-stressed *P. euphratica* plants during a gradually imposed drought and after rewatering. Vertical bars correspond to standard errors (n=5).

approximately −0.7 MPa. This extends the earlier results of Hacke and Sauter (1996), Tognetti *et al.* (1999), and Tyree *et al.* (1992) showing that poplars are among the most vulnerable trees tested so far. Surprisingly, the most vulnerable of the three poplars tested was *P. euphratica* with 50% loss of conductivity occurring in the stem at a water potential around −0.70 MPa. This high susceptibility estimated through pressurization, was confirmed by the time-course of embolism development *in vivo* during a gradually imposed soil water depletion: a severe loss of stem conductivity and a smaller but significant one in roots were recorded in drought-stressed plants that displayed stomata that were still open. Moreover, the water-stressed *P. euphratica* were able to recover functionality shortly after re-irrigation (as photosynthesis, stomatal conductance; stem growth, and root growth returned to 80%, 60%, 60%, and 80% of controls, respectively, data not shown), but the embolism in stems did not display any reversibility and remained stable at about 45%. Stomatal closure is frequently described as being a key process able to protect trees from the occurrence of massive embolism (Cochard *et al.*, 1996*a*; Tyree and Sperry, 1988; Nardini and Salleo, 2000). A safety margin usually exists between the water potential at which stomata close and the cavitation induction threshold. This margin can be rather small in temperate tree species (Cochard *et al.*, 1996*a*), but may reach large values in mediterranean species like *Cedrus atlantica* (Froux *et al.*, 2005). In the case of *P. euphratica*, no such safety margin was recorded and stomatal closure was apparently unable to protect stems against cavitation, similar to what had been observed in *P. trichocarpa×koreana* cv. Peace by Cochard *et al.* (1996*b*). It is therefore possible to conclude that drought-induced embolism in stems and roots of *P. euphratica* was: (i) irreversible, at least in the short term, (ii) of limited consequence once the plants were rewatered, and (iii) not avoided through stomatal control of transpiration. *P. euphratica* is therefore by no means intrinsically tolerant to drought. Its distribution in rather arid deserts of Central Asia can only be due to its ability to access deep water tables, as shown *in situ* by Gries *et al.* (2003), which makes it very vulnerable when water tables deepen as a result of river management and dams. In this respect, populations of *P. euphratica* behave similarly to other riparian poplars endangered by heavy river management (Scott *et al.*, 1999).

A significant variability in cavitation vulnerability was detected among organs in the three poplar species. The apical (younger) region of the stem was slightly less vulnerable to cavitation than the basal (older) ones. A different result recorded in *P. trichocarpa×koreana* was probably due to high pith content and so was artefactual. This gradient of cavitation vulnerability along the growing stem is probably due to the dimensions and the physical properties of the xylem conduits. In basal regions of the stem, mature vessels would be expected to be larger and longer than those in apical regions and so, as a consequence, would be more vulnerable to cavitation and embolism. Earlier studies have focused on cavitation vulnerability in stems and branches, and few data are available about petioles and mid-leaf ribs. *P. alba* and *P. trichocarpa×koreana* cv. Peace displayed comparatively high cavitation vulnerability in the leaf mid-rib veins with respect to other organs. Therefore, as water availability becomes limiting and xylem water potential decreases *in planta*, it would be expected that large rates of embolism would be recorded in

the leaves at an early stage of drought stress. Such an intensive cavitation in petioles and mid-rib veins could ultimately lead to a large leaf shedding, as described in walnut by Tyree *et al.* (1993), leading to the protection of perennial organs (twigs and branches). This process has been called 'hydraulic segmentation through cavitation' during which the survival of the plant during drought may come at the expense of the renewable leaf biomass, the loss of which would help safeguard against drought stress in other plant tissues (Tyree and Ewers, 1991). Nevertheless, in *P. euphratica*, a real vulnerability segmentation was not evident as leaf mid-ribs displayed a similar level of vulnerability as stems. Moreover, only limited leaf shedding occurred during drought, while stems had already begun to cavitate.

Similarly, roots attracted less attention in this respect, despite the fact that their embolization could be very detrimental for tree survival. In *P. euphratica*, roots were found to be less vulnerable than stems and leaf mid-ribs. This pattern had already been found in silver maple (Tsuda and Tyree, 1997), but is opposite to many others where roots were the most vulnerable organ (Sperry and Saliendra, 1994; Alder *et al.*, 1996; Hacke and Sauter, 1996; Kavanagh *et al.*, 1999; Martinez-Vilalta *et al.*, 2002; Froux *et al.*, 2005). In *P. euphratica*, where stomatal closure seems to occur lately, well after embolism significantly affected shoot conductivity, the lower vulnerability of roots may participate to a survival strategy. Indeed, *P. euphratica* has been described as producing large numbers of root suckers, especially after periods of stress or mechanical damage to the existing stem (Sharma *et al.*, 1999). The lower vulnerability of roots and their suckering capacity would enable the plant to recover from a period of drought even in the case of a catastrophic level of damage to the above-ground tissue.

From the point of view of its hydraulic architecture, *P. euphratica* displays a very poor level of drought tolerance, confirming its phreatophytic habit. This leads to the question: how can such a highly vulnerable species reproduce, as one would expect seedlings (assuming a temporary flood has allowed the establishment of seedlings) to be very prone to cavitation and therefore rapidly destroyed by water depletion from the superficial soil layers? The answer is probably that *P. euphratica* displays very little reproduction by offspring and relies on a large production of root suckers, which depend on the 'mother' tree for their water supply at least during the first growth seasons. Rood *et al.* (1994) showed that the reproduction mode (seed, root sucker, or shoot sucker) of different *Populus* species along four rivers in southern Alberta depended on the distance to the river. In the closest areas, the majority of trees originated from seedlings, while, in those at a distance, the fraction of trees issuing from suckers was much larger. Suckering would in time produce colonies of clonal and intergrafted trees able to colonize areas where water is only accessible at depth in the soil. The clonal nature of some *P. euphratica* populations has been demonstrated in a few cases (Fay *et al.*, 1999), but the occurrence of functional grafts enabling water exchange among adults remains to be demonstrated. This behaviour is probably not specific to *P. euphratica*, but may occur among species from arid zones relying on the occurrence of deep soil water, like in the case of the Sahelian acacia *Faidherbia albida* (Roupsard *et al.*, 1999).

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