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Research paper

Recovery performance in xylem hydraulic conductivity is correlated with cavitation resistance for temperate deciduous tree species

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Woody species hydraulically vulnerable to xylem cavitation may experience daily xylem embolism. How such species cope with the possibility of accumulated embolism is unclear. In this study, we examined seven temperate woody species to assess the hypothesis that low cavitation resistance (high vulnerability to cavitation) is compensated by high recovery performance via vessel refilling. We also evaluated leaf functional and xylem structural traits. The xylem recovery index (XRI), defined as the ratio of xylem hydraulic conductivity in plants rewatered after soil drought to that in plants under moist conditions, varied among species. The xylem water potential causing 50% loss of hydraulic conductivity (Ψ_{50}) varied among the species studied, whereas only a slight difference was detected with respect to midday xylem water potential (Ψ_{\min}), indicating smaller hydraulic safety margins ($\Psi_{\min} - \Psi_{50}$) for species more vulnerable to cavitation. Cavitation resistance ($|\Psi_{50}|$) was negatively correlated with XRI across species, with cavitation-vulnerable species showing a higher performance in xylem recovery. Wood density was positively correlated with cavitation resistance and was negatively correlated with XRI. These novel results reveal that coordination exists between cavitation resistance and xylem recovery performance, in association with wood functional traits such as denser wood for cavitation-resistant xylem and less-dense but water-storable wood for refillable xylem. These findings provide insights into long-term maintenance of water transport in tree species growing under variable environmental conditions.

Keywords: gas exchange, vessel refilling, water stress, water transport, xylem embolism, xylem structure.

Introduction

Maintenance of xylem water transport is critical for plant survival, growth and development. Transpiration generates a tension or negative pressure in the xylem that drives water transport over long distances from the roots to the leaves. When xylem pressure becomes more negative with increasing transpiration rate or dehydration and exceeds a certain threshold, an air bubble is aspirated from air-filled conduit to water-filled conduit via leaky pores in the inter-conduit pit membranes and expands to block water flow in the conduit as embolism (Sperry and Tyree 1988, Jansen et al. 2009). Such cavitation-induced embolism reduces

xylem hydraulic conductivity and restricts leaf gas exchange (Nardini and Salleo 2000, Brodribb and Holbrook 2003). Consequently, the xylem dysfunction caused by embolism is a serious problem for plant productivity and development.

Stomatal control is a primary plant response for prevention of drought-induced xylem cavitation (Jones and Sutherland 1991, Brodribb et al. 2003, Franks and Brodribb 2005). Stomatal regulation of the leaf transpiration rate determines leaf water potential, which constrains xylem water potential. The difference between minimum xylem water potential (Ψ_{\min}) and xylem water potential causing 50% loss in hydraulic conductivity (Ψ_{50}) is the hydraulic safety margin, which is

employed to evaluate xylem safety against hydraulic dysfunction caused by cavitation (Meinzer et al. 2009, Johnson et al. 2012). According to earlier studies, species that are vulnerable to cavitation (higher Ψ_{50}) tend to exhibit small safety margins (Pockman and Sperry 2000, Meinzer et al. 2008a, 2008b, Markesteijn et al. 2011), and are likely to experience some embolism daily even under non-extreme drought conditions (Meinzer et al. 2009). How such species cope with the possibility of accumulated embolism in xylem is unclear.

In plants vulnerable to cavitation, rapid repair of xylem embolism caused by prolonged drought or high evaporative demand is essential for long-term maintenance of water transport. Over the past two decades, studies assessing recovery of xylem hydraulic conductivity (i.e., vessel refilling) under negative xylem pressure have been conducted on various woody species (Salleo et al. 1996, Zwieniecki and Holbrook 1998, Hacke and Sperry 2003, Ogasa et al. 2010). As proposed by various authors, vessel refilling involves the flow of water into droplets on the cavitated vessel lumen along an osmotic gradient generated by solutes (e.g., sugars hydrolyzed from starch; Bucci et al. 2003) released from the contact cells (Tyree et al. 1999, Hacke and Sperry 2003, Salleo et al. 2004). Based on dynamics of starch content in living wood cells, phloem unloading is also thought to contribute to embolism repair (Salleo et al. 2009). Although there have been many studies exploring the mechanisms of xylem repair (Johnson et al. 2012), most have been narrowly focused on a single species, primarily garden and cultivated woody species such as *Laurus nobilis* L. (Salleo et al. 1996, 2009, Tyree et al. 1999), grapevine (Holbrook et al. 2001, Brodersen et al. 2010) and poplar (Secchi and Zwieniecki 2010, 2011). Little attention has been paid to the significance of xylem recovery across different species with respect to species-specific vulnerability to cavitation.

Because vessel refilling is a physiological process entailing energy (osmoticum) costs (Salleo et al. 2004, Secchi and Zwieniecki 2011), the extent of xylem recovery performance should be coordinated with other functional traits. Recovery performance may be associated with cavitation resistance to ensure integrity of water transport on a long-term basis under variable environmental conditions. The question thus arises: does xylem recovery performance compensate for low cavitation resistance in cavitation-vulnerable species?

In this study, we tested the hypothesis that resistance to xylem cavitation is coordinated with xylem recovery performance across species. We chose seven temperate woody species that were expected to show a range of xylem vulnerability to cavitation. Four of the seven species were closely related members of the genus *Salix*, chosen to allow comparison of xylem recovery trends in both related and contrasting species. Vulnerability to cavitation was evaluated in plants subjected to dehydration. In addition to monitoring the extent of recovery in

hydraulic conductivity after rewatering, we also measured functional and structural traits such as maximum xylem hydraulic conductivity, gas exchange rate and xylem structure. These physiological and structural traits were examined because of their possible roles in the hypothetical coordination between cavitation resistance and xylem recovery performance (Ishida et al. 2008).

Materials and methods

Plant material and growth conditions

Seven temperate deciduous diffuse-porous species were selected for study: *Salix integra* Thunb., *Salix babylonica* L., *Salix cheilophila* C.K. Schneid., *Salix psammophila* C. Wang & Chang Y. Yang, *Betula platyphylla* var. *japonica* (Miq.) Hara, *Carpinus tschonoskii* Maxim. and *Cerasus jamasakura* (Siebold ex Koidz.) Ohba. All nomenclature follows The Plant List (2010); *S. babylonica* is also listed as *Salix matsudana* Koidz. in Flora of China (1999). We chose four species from the genus *Salix* to allow comparison of the variation in xylem recovery performance among both related and contrasting species. Potted saplings of the four *Salix* species were produced from 20-cm-long freshly rooted cuttings. The other species were 2–3-year-old potted seedlings. Potted plants were grown in an experimental field at Okayama University, Okayama, Japan, and were irrigated daily and fertilized periodically until experiments were initiated (plant size information is given in Table 1). All measurements were collected during 2009–2011, when climatic conditions such as the mean air temperature and hours of daylight were similar among years (Japan Meteorological Agency).

Water potential and hydraulic conductivity measurements

Xylem water potential (Ψ_{xylem}) was measured in shoot tips of *Salix* species and *Ca. tschonoskii*—which have very short petioles—and in leaves of the other two species using a pressure chamber (models 600 and 1000; PMS Instrument Company, Corvallis, OR, USA). A few hours before Ψ_{xylem} measurements, shoots (including shoot tips or leaves to be

Table 1. The plant materials used in this study. Values are the means (with SD in parentheses) for all plants used in the experiments ($n = 21\text{--}41$). Values for *Salix* species are the lengths of shoots sprouted from rooted cuttings. For the other three species, the heights of seedlings are listed.

	Length or height (m)		Basal diameter (mm)	
<i>Salix integra</i>	0.93	(0.32)	8.3	(1.2)
<i>S. babylonica</i>	1.12	(0.24)	7.6	(1.2)
<i>S. cheilophila</i>	1.17	(0.23)	7.9	(1.5)
<i>S. psammophila</i>	1.09	(0.29)	6.9	(1.3)
<i>Betula platyphylla</i>	1.39	(0.41)	14.3	(4.36)
<i>Carpinus tschonoskii</i>	1.15	(0.24)	10.8	(1.54)
<i>Cerasus jamasakura</i>	1.21	(0.21)	11.0	(2.28)

measured) were enclosed and covered with black plastic bags and aluminum foil to equilibrate water potential between shoot tips/leaves and stem xylem.

Following the Ψ_{xylem} measurements, the hydraulic conductivity (K_h) of stems was measured, with one set of Ψ_{xylem} and K_h measurements obtained per plant. The segments used to measure K_h were cut under water from the middle of a 4–10-cm length of the current stem for the four *Salix* species and from the middle of the main stems for the other species. Segment length varied among individual plants because the portion convenient for conductivity measurements was restricted, especially for *Salix* species, because of small internodes and leafy shoots. Each segment was connected to a tubing apparatus based on Torres-Ruiz et al. (2012), and the initial K_h ($K_{h \text{ init}}$) was measured gravimetrically with 0.1- μm -filtered 20-mM KCl solution. K_h was calculated as the flow rate of the measuring fluid (kg s^{-1}) through the stem segment divided by the pressure gradient along the segment (MPa m^{-1}). The K_h measurements were taken under each hydraulic head: 80 cm in the *Salix* species and 60 cm in the other species. We had previously confirmed that artificial embolism reversal (i.e., a flushing effect) did not occur under the species-specific hydraulic heads. To improve the accuracy of conductivity values obtained, the background flow rate of the segment under no hydraulic head was also measured before and after measuring the pressure-induced flow rate. It was then averaged and subtracted from the pressure-induced flow rate. This procedure allowed us to obtain the net flow from the segments during perfusion of solution (Davis et al. 1999, Torres-Ruiz et al. 2012). During measurements of K_h for the construction of vulnerability curves (see the next section) in *Ca. tschonoskii* and *Ce. jamasakura*, background flow was not measured. We verified, however, that the effect of background flow on net flow was negligible for these species, as the maximum background flow during these measurements was <3% of the gross flow, based on a similar dataset from rehydration experiments (see the section 'Dehydration–rehydration treatments'). After measuring $K_{h \text{ init}}$, each segment was flushed at 100 kPa for 10–30 min to remove any xylem embolism, and its maximum K_h ($K_{h \text{ max}}$) was measured.

Vulnerability curves

Vulnerability curves (VCs) were obtained using the dehydration method (Sperry and Tyree 1990) and plotted as $K_{h \text{ init}}/K_{h \text{ max}}$ vs. Ψ_{xylem} . Irrigation was withheld from potted plants, which were dehydrated naturally under field conditions and then transported to the laboratory. Ψ_{xylem} and the corresponding K_h values were then measured in accordance with the procedures described earlier. For the construction of VCs, 13–20 plants of each species were used. Vulnerability curves were obtained during the summer of 2009 for *Ca. tschonoskii* and *Ce. jamasakura*, in the summer of 2010 for *S. cheilophila*, and in the

summer of 2011 for the remaining *Salix* species. A similar dataset was derived for *B. platyphylla* from Ogasa et al. (2010). As an indicator of cavitation resistance (Tyree and Zimmermann 2002), xylem water potential inducing 50% loss of hydraulic conductivity (Ψ_{50}) was obtained from fitted vulnerability curves (see the section 'Statistical analysis').

Dehydration – rehydration treatments

To assess the extent of recovery in hydraulic conductivity, plants were subjected to dehydration–rehydration treatments in accordance with Ogasa et al. (2010). Plants were dried by stopping irrigation to reach a xylem water potential near Ψ_{50} (Table S1 available as Supplementary Data at *Tree Physiology* Online). Plant water status was monitored occasionally by measuring the water potential of randomly chosen leaves using a pressure chamber. When a plant's water potential approximated the value of the species-specific Ψ_{50} , it was rewatered to field capacity and held for 12 h overnight for rehydration (Ogasa et al. 2010). After the rehydration period, Ψ_{xylem} and K_h (both $K_{h \text{ init}}$ and $K_{h \text{ max}}$) were measured immediately (termed 'rewatered' in Figure 3). We also measured K_h under moist conditions (as 'controls' in Figure 3) and under drought conditions at a level of Ψ_{50} (as 'stressed to Ψ_{50} ') using other sets of the plants. Ψ_{xylem} measured in drought-stressed plants and Ψ_{xylem} immediately before rewatering for rewatered plants were not significantly different within species (P values were 0.11–0.82 according to a t -test), providing evidence that rewatered plants had experienced Ψ_{50} -level water stress. $K_{h \text{ max}}$ per xylem cross-sectional area (K_s , see below) was similar within species throughout the experiments, indicating that no irreversible embolism was induced by the experimental drought stress. In the experiment, 3–6 plants were used per treatment (controls, stressed to Ψ_{50} , or rewatered). The relative value of K_h (i.e., $K_{h \text{ init}}/K_{h \text{ max}}$) obtained from each plant was arcsine-transformed and averaged per species and per treatment. Experiments were conducted during the summer of 2010 for *S. cheilophila* and during the summer of 2011 for the other species. For *B. platyphylla*, a similar dataset was derived from Ogasa et al. (2010) with the same experimental design used in this study.

Calculation of xylem recovery index

The extent of xylem recovery (xylem recovery index; XRI) was defined as the ratio of $K_{h \text{ init}}/K_{h \text{ max}}$ measured 12 h after rewatering to that obtained under moist conditions. Because of the destructive nature of K_h measurements, the average $K_{h \text{ init}}/K_{h \text{ max}}$ per treatment ('rewatered' or 'controls') was used for the calculation. The use of XRI assumes that the values of $K_{h \text{ init}}/K_{h \text{ max}}$ measured at the Ψ_{50} level and the values measured immediately before rewatering are equivalent (see the previous section), because rewatered plants were subjected to dehydration at the Ψ_{50} level. For example, values of XRI will approach 1.0 when xylem hydraulic conductivity is largely restored,

whereas a lack of recovery or progress of cavitation despite rewatering leads to XRI values of 0.5 or lower. Because the $K_{h \text{ init}}/K_{h \text{ max}}$ obtained under moist conditions was used in the denominator for XRI calculations, the effect of any emboli induced prior to experimental drought could be ignored when evaluating xylem recovery.

Xylem-specific hydraulic conductivity

To obtain xylem area-specific hydraulic conductivity (K_s) as a measure of transport efficiency, xylem area was determined from thin cross sections excised from all segments used for K_h measurements in the dehydration–rehydration experiments. Xylem area was determined using a microscope (SZX-ILLB100; Olympus, Tokyo, Japan), image processing software (GIMP 2.6; GIMP Development Team) and an image analyzer (ImageJ 1.44p; National Institutes of Health, Bethesda, MD, USA). K_s was calculated by dividing $K_{h \text{ max}}$ by the xylem cross-sectional area for each segment and was averaged for each species ($n = 9\text{--}13$).

Minimum xylem water potential and leaf gas exchange

Using a pressure chamber, minimum xylem water potential (Ψ_{min}) was measured on a clear summer day in 2010 in *B. platyphylla*, *Ca. tschonoskii* and *Ce. jamasakura*, and in 2011 in the four *Salix* species. A day before Ψ_{min} measurements, shoots were enclosed in black plastic bags and covered with aluminum foil to equilibrate water potential between shoot tips/leaves and stem xylem when measuring Ψ_{xylem} . Measurements were conducted from 11:00 to 15:00, when Ψ_{xylem} was at a minimum. Ψ_{min} was measured from one shoot tip (for *Salix* species and *Ca. tschonoskii*) or matured leaf (for *Ce. jamasakura* and *B. platyphylla*) per plant, using three plants per species. Because all plants were well watered on the day before measuring, we can assume that Ψ_{min} was the result of stomatal control under non-extreme moderate conditions.

During the same year and season that Ψ_{min} was measured, the maximum photosynthetic rate (A) and stomatal conductance (g_s) were measured from 6:00 to 15:00 using a portable photosynthesis system (LI-6400; Li-Cor, Lincoln, NE, USA). Fixed measurement conditions in the apparatus leaf chamber were 370 $\mu\text{mol mol}^{-1}$ CO_2 concentration, 25 °C air temperature and <2 kPa vapor pressure deficit. A and g_s were measured at saturating photosynthetic photon flux densities of 1600 $\mu\text{mol m}^{-2} \text{s}^{-2}$ for *Ca. tschonoskii*, *Ce. jamasakura* and *B. platyphylla*, 1500 $\mu\text{mol m}^{-2} \text{s}^{-2}$ for *S. integra* and *S. babylonica*, and 2000 $\mu\text{mol m}^{-2} \text{s}^{-2}$ for *S. psammophila* and *S. cheilophila*. Measurements were conducted on a fully expanded and matured leaf of each plant for 3–5 plants.

Xylem structural traits

Xylem anatomical traits were measured on the three to six stem segments used for measuring hydraulic conductivity.

From a digital image of thin cross sections excised from each segment, the arithmetic mean vessel diameter (D_v , μm), mean hydraulic vessel diameter ($D_h = \Sigma D^5 / \Sigma D^4$, μm), vessel density (VD, # mm^{-2}) and vessel lumen fraction (F_v , dimensionless) in a given xylem cross-sectional area were determined as wood porosity indicators. F_v was calculated as the ratio of the sum of the vessel lumen area to xylem area (Preston et al. 2006, Zanne et al. 2010). We measured all vessels in any observed area that included >100 vessels. All image analyses were conducted using GIMP 2.6 and ImageJ software.

Wood density (g cm^{-3}) was determined for another set of four to six plants of similar size and age as the saplings used for K_h measurements. Stem samples were excised from the same position at which K_h was measured. After removal of bark, cambium and, if possible, pith, samples were immersed in water and the weight of water displacement was converted into volume. Sample dry weight was obtained after oven-drying at 80 °C for 48 h. Wood density was calculated as wood dry weight per unit of fresh volume.

The leaf area to xylem area ratio

The ratio of leaf area (A_L) to xylem area (A_S) was determined for four or five of the plants used for K_h measurements. All leaves distal to segments used for K_h measurements were harvested and oven-dried at 80 °C for 48 h, and their dry weight was then determined using an electric balance. The original leaf area was estimated based on the proportional relationship between leaf dry weight and leaf area, which had already been obtained for each species. A_S had already been determined for use in K_s calculations.

Statistical analysis

Vulnerability curve data expressed as $K_{h \text{ init}}/K_{h \text{ max}}$ vs. Ψ_{xylem} were fitted to a Weibull function (Neufeld et al. 1992, Hacke et al. 2006):

$$\frac{K_{h \text{ init}}}{K_{h \text{ max}}} = \exp\left(-\left(\frac{-\Psi_{\text{xylem}}}{b}\right)^c\right),$$

where b and c were the fitted parameters. Parameter fitting was conducted using KaleidaGraph graphics software (ver. 4.0; Synergy Software, Reading, PA, USA). The Ψ_{xylem} at which $K_{h \text{ init}}/K_{h \text{ max}}$ was identical to 0.5 was Ψ_{50} .

The means of xylem water potential under moist, drought-stressed and rewatered conditions were compared for each species using one-way analysis of variance at significance levels of $\alpha = 0.05$ to confirm recovery in xylem water status after rewatering. When a significant difference was found, a multiple comparison of the means (Dunnett's test) was conducted. These statistical analyses were conducted using Statistica software (StatSoft, Tulsa, OK, USA).

Bivariate relationships between Ψ_{50} , XRI and other traits were analyzed using standardized major axis (SMA) regression. The SMA method is more appropriate than ordinary linear regression because, as type II regression, the relationship between two variables can be ascertained in the presence of error in both variables. In addition, the SMA method provides the best slope estimate by minimizing the sum of the product of x and y deviations rather than the sum of vertical deviations alone (Smith 2009). SMATR 2.0 statistical software (Warton et al. 2006) was used for SMA regression analysis.

Results

The xylem water potential causing 50% loss of hydraulic conductivity (Ψ_{50}) ranged from -1.0 to -3.9 MPa among the

species studied (Figure 1; Table S1 available as Supplementary Data at *Tree Physiology Online*). *Salix babylonica* was the least cavitation resistant of the four *Salix* species. *Betula platyphylla* showed higher cavitation resistance than *Salix* species, and *Ca. tschonoskii* exhibited the most negative Ψ_{50} (-3.9 MPa).

A daily minimum xylem water potential (Ψ_{\min}) corresponding to $<10\%$ loss of hydraulic conductivity was measured for *S. integra* (Figure 1a, vertical lines). Similar results were observed for *S. cheilophila*, *B. platyphylla* and *Ca. tschonoskii* (Figure 1). In contrast, for *S. psammophila*, $\sim 35\%$ loss of hydraulic conductivity was estimated at Ψ_{\min} (Figure 1d).

The more cavitation-resistant species tended to be hydraulically safer, as estimated from the difference between their Ψ_{\min} and Ψ_{50} (Figure 2). The *Salix* species exhibited a relationship between Ψ_{50} and Ψ_{\min} close to the 1 : 1 line, implying

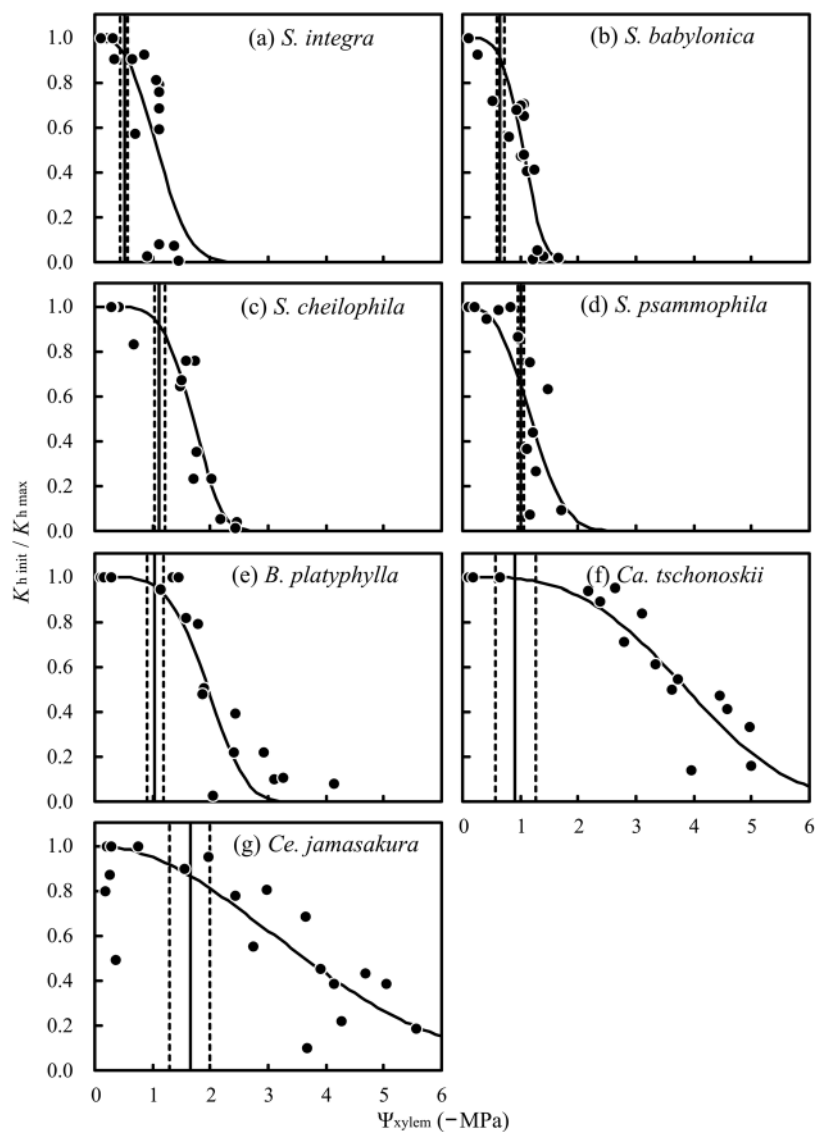


Figure 1. Vulnerability curves expressed as $K_{h\text{ init}}/K_{h\text{ max}}$ vs. xylem water potential ($\Psi_{\text{ xylem}}$). Each data point corresponds to a different plant. Curves are fitted to a Weibull function. Solid and dashed vertical lines are the means and SE of minimum xylem water potential measured on a clear summer day ($n = 3$).

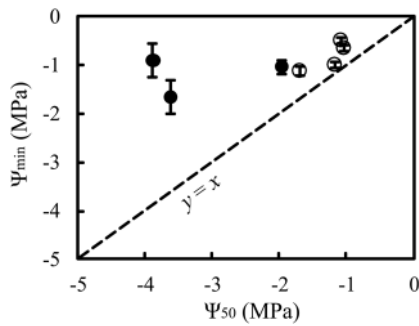


Figure 2. Minimum xylem water potential (Ψ_{\min}) on a clear day shown against Ψ_{50} (xylem water potential yielding 50% loss of hydraulic conductivity). Ψ_{50} was obtained from the vulnerability curves shown in Figure 1. Open symbols, *Salix* species; closed symbols, the other three species. Means \pm SE are shown for Ψ_{\min} ($n = 3$). The dashed line represents $y = x$.

a narrow safety margin (Figure 2, open circles and dashed line).

In all the species studied, native hydraulic conductivity was only slightly reduced from its maximum value before experimental drought was imposed (Figure 3a). When Ψ_{xylem} reached a value corresponding to Ψ_{50} during the drought treatment, the measured loss of hydraulic conductivity was close to the predicted value of 50% for all species except *Ca. tschonoskii*. *Carpinus tschonoskii* exhibited somewhat lower values in $K_{h \text{ init}}/K_{h \text{ max}}$, probably because of individual differences in plants used for Ψ_{50} calculations and for drought treatments. In the same situation, *Salix* species had large error bars with respect to the mean of $K_{h \text{ init}}/K_{h \text{ max}}$, notwithstanding the very small variations observed in Ψ_{xylem} ; this is presumably because of the large variation in $K_{h \text{ init}}$, as inferred from the steep slope of the vulnerability curve at Ψ_{50} (Figure 1). In all species studied, xylem water potential recovered to its pre-dehydration levels 12 h after rewatering ($P = 0.09\text{--}0.84$, Dunnett's test); however, recovery of hydraulic conductivity after rewatering varied among species (Figure 3). Xylem recovery index (XRI), which indicates the extent of recovery of hydraulic conductivity in stem xylem, was high (0.8–1.0) in the four *Salix* species (Table S1 available as Supplementary Data at *Tree Physiology* Online). Lower XRI values were observed in *Ca. tschonoskii* (XRI = 0.4) and *Ce. jamasakura* (XRI = 0.5).

No correlation was found between Ψ_{50} and XRI across the four *Salix* species ($P = 0.62$, Figure 4, open symbols). When all of the species studied were considered, however, a significant correlation was observed (Figure 4). Photosynthetic capacity and Ψ_{\min} were correlated with Ψ_{50} and XRI, respectively (Table 2), while both Ψ_{50} and XRI were significantly correlated with wood density across all species (Table 2, Figure 5). Significant correlations were not observed when only *Salix* species were analyzed (Figure 5, see r^2 with its significance in parentheses).

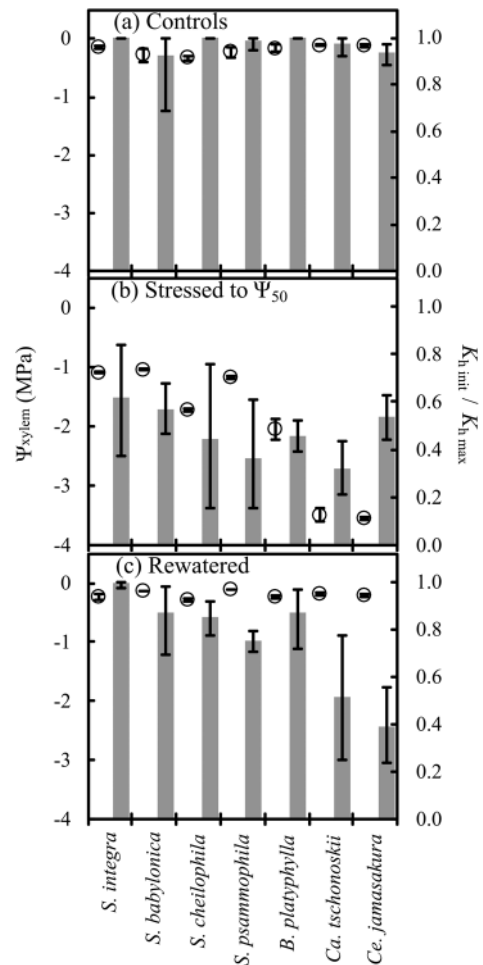


Figure 3. Xylem water potential (Ψ_{xylem} , open circles) and $K_{h \text{ init}}/K_{h \text{ max}}$ (gray columns) in stems under contrasting water status in the dehydration–rehydration experiment. (a) ‘Controls’, moist conditions in the absence of water stress. (b) ‘Stressed to Ψ_{50} ’, drought conditions yielding 50% loss of conductivity (Ψ_{50}). (c) ‘Rewatered’, 12 h after rewatering following dehydration at the species-specific Ψ_{50} level. Values are the means \pm SE for xylem water potential data and the means \pm 95% of confidence intervals for $K_{h \text{ init}}/K_{h \text{ max}}$ data ($n = 3\text{--}6$ per treatment).

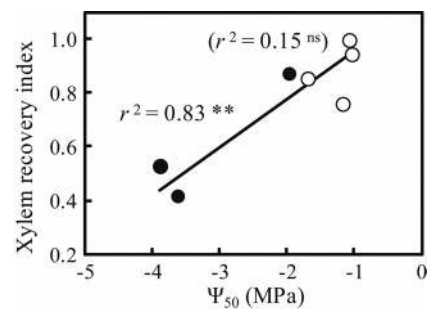


Figure 4. Relationship between Ψ_{50} and xylem recovery index for *Salix* (open circles) and the other three species (closed circles). The solid line represents standardized major axis regression across the seven species studied. The coefficient of determination, r^2 , is also shown with its level of significance (** $P < 0.01$; ns, $P > 0.05$); r^2 of the regression (for the genus *Salix* only) is in parentheses.

Table 2. Coefficients of determination (r^2) and their P values, with their positive (+) or negative (–) relationships, for comparison of functional and structural traits with Ψ_{50} (xylem water potential causing 50% loss of conductivity) and XRI (xylem recovery index) for the seven species studied. Correlations were analyzed using standardized major axis regression. Asterisks indicate significant correlation ($*P < 0.05$).

	Ψ_{50}		XRI	
	r^2	P	r^2	P
Physiological traits				
Ψ_{\min}	0.38	0.142 (+)	0.58*	0.045 (+)
K_s	0.06	0.593 (–)	0.00	0.929 (–)
g_s	0.51	0.070 (+)	0.17	0.351 (+)
A	0.61*	0.039 (+)	0.26	0.241 (+)
Xylem structural traits				
WD	0.57*	0.049 (–)	0.59*	0.043 (–)
D_v	0.19	0.336 (–)	0.06	0.594 (–)
D_h	0.29	0.209 (–)	0.11	0.466 (–)
VD	0.15	0.386 (+)	0.08	0.553 (+)
F_v	0.04	0.674 (–)	0.04	0.680 (–)
Morphological traits				
$A_L:A_S$	0.47	0.089 (–)	0.21	0.306 (–)

Symbols/abbreviations and units are: Ψ_{\min} (MPa), daily minimum xylem water potential; K_s ($\text{kg m}^{-1} \text{MPa}^{-1} \text{s}^{-1}$), xylem specific hydraulic conductivity; g_s ($\text{mol m}^{-2} \text{s}^{-1}$), maximum stomatal conductance; A ($\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum net photosynthetic rate; WD (g cm^{-3}), wood density; D_v (μm), mean vessel diameter; D_h (μm), mean hydraulic vessel diameter; VD ($\# \text{mm}^{-2}$), vessel density; F_v ($\text{mm}^2 \text{mm}^{-2}$), the ratio of the sum of vessel lumen area to xylem area; $A_L:A_S$ ($10^3 \text{m}^2 \text{m}^{-2}$), leaf area per xylem area.

Discussion

To ascertain the functional coordination involved in maintenance of water transport, the relationship between cavitation resistance and xylem recovery performance was studied using temperate woody species. The cavitation resistance values obtained in this study coincide with or approximate those reported previously for *Salix* (Cochard et al. 2007, Savage and Cavender-Bares 2011), *Betula* (Sperry and Saliendra 1994, Barigah et al. 2006), *Carpinus* (Cochard et al. 2005) and *Prunus* (closely related to *Cerasus*; Cochard et al. 2008).

Although xylem hydraulic conductivity in *S. psammophila* appeared to decrease by >35% as Ψ_{xylem} declined at midday (Figure 1d), native embolism was not observed (Figure 3a). These results suggest that xylem cavitation in *S. psammophila* may have been reversed on a daily basis by refilling of cavitated vessels. In the other species, xylem dysfunction was restricted to <10% of maximum hydraulic conductivity at midday, possibly because of stomatal regulation moderating the leaf-to-xylem water potential gradient and preventing cavitation (Salleo et al. 2000, Meinzer et al. 2009). Considering that little hydraulic conductivity loss was observed in the absence of drought stress (Figure 3a), vessel refilling to avoid accumulated

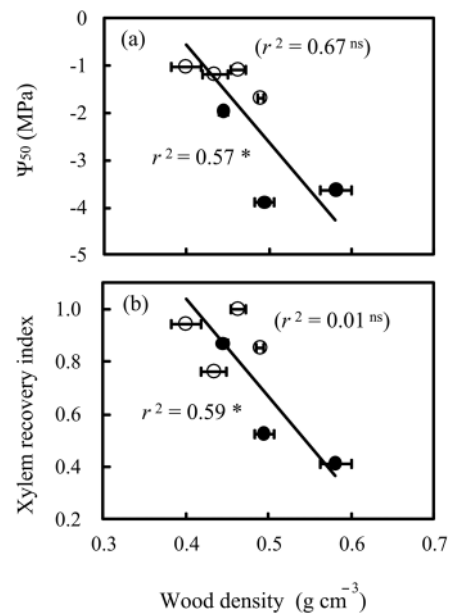


Figure 5. (a) Ψ_{50} and (b) XRI as a function of wood density in *Salix* (open circles) and the other three species (closed circles). Wood density values are the means \pm SE ($n = 4-6$). The solid line shows standardized major axis regression across the seven species. The coefficient of determination, r^2 , is shown with its significance ($*P < 0.05$; ns, $P > 0.05$) in each graph; r^2 values of the regression (for *Salix* only) are in parentheses.

emboli during growth periods might have occurred even in these species.

Across all the species studied, cavitation resistance was significantly correlated with xylem recovery performance (Figure 4). This correlation is the first evidence that upon release of drought stress, higher xylem recovery performance can compensate for lower resistance to cavitation. While the cavitation mechanism is simply a physical process related to xylem structural characteristics (Tyree and Zimmermann 2002), short-term vessel refilling is a physiological process associated with living wood cells (Bucci et al. 2003, Salleo et al. 2004, 2009) and phloem (Salleo et al. 2006). The relationship between cavitation resistance and xylem recovery performance observed in our study can therefore be assumed to be a functional coordination.

A possible mediator of this correlation may be wood density (Table 2). Correlations between Ψ_{50} and wood density have been extensively observed across diverse species in arid (Hacke et al. 2000, Jacobsen et al. 2007a), tropical (Markesteijn et al. 2011) and temperate regions (Hoffmann et al. 2011). As proposed by Hacke et al. (2001) and supported by Jacobsen et al. (2005), cavitation resistance might be closely associated, with respect to withstanding xylem negative pressure, with xylem mechanical strength to resist implosion. Consequently, wood density has been used as an integrative proxy for mechanical reinforcement to resist cavitation and implosion.

Across the *Salix* species investigated, however, Ψ_{50} was not correlated with wood density (Figure 5a); this might be because

of the small sample size ($n = 4$) and restricted range of Ψ_{50} and wood density values used for the correlation analysis. Some other studies have also found wood density and Ψ_{50} to be poorly correlated across closely related species because of low trait variation and the fact that wood density is only an indirect estimate for mechanical strength as cavitation resistance (Cochard et al. 2007, Fichot et al. 2010). Xylem cavitation resistance across closely related species, as observed in our four *Salix* species, is better represented by smaller and direct scales of mechanical strength or wood microstructure: double wall thickness relative to vessel lumen diameter (Hacke et al. 2001), fiber function for mechanical support of surrounding vessels (Jacobsen et al. 2005, 2007a, Cochard et al. 2007) and pit membrane porosity (Choat et al. 2004, Jansen et al. 2009).

The negative relationship observed between XRI and wood density across the seven species in our study (Figure 5b) indicates that xylem recovery performance is associated with other functional traits. Less dense wood is indicative of cavitation-vulnerable xylem (see above), but is also associated with a higher fraction of living wood cells (Carlquist 2001, Jacobsen et al. 2007b) and greater sapwood water storage capacity (Pratt et al. 2007, Meinzer et al. 2008a). Water stored in xylem tissue is accessible to vessels, as evidenced by its probable use in buffering fluctuations in xylem tension (Goldstein et al. 1998, Meinzer et al. 2003) and maintaining hydraulic safety during drought (Meinzer et al. 2003, 2008b). Water stored in xylem contact cells and wood fibers may also contribute to vessel refilling; it may serve as a source of water discharged from contact cells to cavitated vessels (Brodersen et al. 2010), with replenishment occurring when water supply improves. This hypothesis—that wood capacitance is a key factor in xylem refilling—is supported by the proposal of Johnson et al. (2012) that differences in hydraulic safety observed between angiosperms and conifers may have stemmed from divergent xylem anatomical composition. Vessel dimension and arrangement were not related to the extent of vessel refilling in our study (Table 2). No correlation was observed between XRI and wood density across *Salix* species, perhaps because trait values were similar among closely related species, as discussed above. From a phylogenetic point of view, the similar XRI values observed among *Salix* species suggest that their xylem recovery performance is intrinsic to this genus.

Incidentally, even though apoplastic water status was standardized to xylem pressure of ' Ψ_{50} ' among the species studied, less negative Ψ_{50} values were associated with a high degree of xylem recovery. This suggests that the extent of vessel refilling depends on the vigor of living cells (Salleo et al. 1996) in addition to xylem capacitance, i.e., symplastic physiological status as well as apoplastic water status. In fact, *Ca. tschonoskii* and *Ce. jamasakura* experienced severe leaf dieback at their Ψ_{50} , as has been observed in *Acer negundo* L. (Hacke and Sperry 2003). Consequently, the poor recovery

observed in these species may be due not only to drawdown of stored water but also to decreased symplast activity at Ψ_{50} . Vilagrosa et al. (2010) reported that cavitation resistance and symplastic (e.g., photosystem II efficiency and thermal energy dissipation) resistance against plant dehydration were mutually related although not consistent among species.

In conclusion, our research uncovered the novel finding that cavitation resistance is coordinated with xylem recovery performance across the species studied. Because wood density is correlated with both cavitation resistance and xylem recovery performance, it probably mediates this coordination through contrasting functionality of wood, i.e., dense and cavitation-resistant xylem vs. less-dense but refillable xylem. These findings would explain long-term maintenance of water transport in species showing varying degrees of vulnerability to cavitation. With respect to biomass allocation, a trade-off usually exists between wood density and growth rate in many different woody species (Preston et al. 2006, Chave et al. 2009, Poorter et al. 2010). This trade-off imposes limitations on plant structure and function (Ishida et al. 2008), with constraints on wood density affecting trade-offs between cavitation resistance and xylem recovery performance, similar to relationships previously observed between wood density and gas exchange rate (Santiago et al. 2004), stomatal response (Hoffmann et al. 2011) and hydraulic properties (Meinzer et al. 2009, Gotsch et al. 2010, Fu et al. 2012). Additional experimental evidence is needed to reveal the effects of xylem structural and physiological functions on vessel refilling. In addition, more woody species should be investigated to confirm phylogenetic independence of the correlation observed in this study between cavitation resistance and xylem recovery performance (Ackerly and Reich 1999, Maherali et al. 2006). Such studies would provide additional insights into the water transport strategy, controlled by wood structure and function, employed by plants grown under variable environmental conditions.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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