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## Research paper

# Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees

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Hydraulic failure is one of the main causes of tree mortality in conditions of severe drought. Resistance to cavitation is known to be strongly related to drought tolerance and species survival in conifers, but the threshold of water-stress-induced embolism leading to catastrophic xylem dysfunction in angiosperms has been little studied. We investigated the link between drought tolerance, survival and xylem cavitation resistance in five angiosperm tree species known to have contrasting desiccation resistance thresholds. We exposed seedlings in a greenhouse to severe drought to generate extreme water stress. We monitored leaf water potential, total plant water loss rate, leaf transpiration, stomatal conductance and CO<sub>2</sub> assimilation rate during drought exposure and after rewatering (recovery phase). The time required for the recovery of 50% of the maximum value of a given ecophysiological variable after rewatering was used to determine the critical water potential corresponding to the threshold beyond which the plant failed to recover. We also investigated the relationship between this potential and stem xylem cavitation resistance, as assessed from vulnerability curves. This minimum recoverable water potential was consistent between ecophysiological variables and varied considerably between species, from −3.4 to −6.0 MPa. This minimum recoverable water potential was strongly correlated with  $P_{50}$  and  $P_{88}$ , the pressures inducing 50 and 88% losses of stem hydraulic conductance, respectively. Moreover, the embolism threshold leading to irreversible drought damage was found to be close to 88%, rather than the 50% previously reported for conifers. Hydraulic failure leading to irreversible drought-induced global dysfunction in angiosperm tree species occurred at a very high level of xylem embolism, possibly reflecting the physiological characteristics of their stem water-transport system.

**Keywords:** cavitation resistance, drought resistance, hydraulic failure, mortality, recovery, water stress.

## Introduction

Extreme drought decreases both tree growth (Waring 1987, Ciais et al. 2005, Bigler et al. 2006, Hogg et al. 2008, Michaelian et al. 2011, Eilmann and Rigling 2012, Sanchez-Salguero et al. 2012) and survival (Pockman and Sperry 2000, Breshears et al. 2005, Bigler et al. 2006, Gitlin et al. 2006, van Mantgem and Stephenson 2007, Hogg et al. 2008, Galiano et al. 2010, Michaelian et al. 2011). Many cases of drought-related forest dieback have already been reported (see the review by Allen et al. 2010) and such diebacks are likely to

become more frequent in the future, as models have predicted more frequent and intense extreme climatic events, such as long, severe droughts (Meehl and Tebaldi 2004, Schar et al. 2004, Burke et al. 2006, Jentsch et al. 2007).

Two main hypotheses have been proposed concerning the mechanisms underlying drought-induced mortality in trees: hydraulic failure and carbon starvation (McDowell et al. 2008, Adams et al. 2009, Sala 2009, Galiano et al. 2011, McDowell 2011, Anderegg et al. 2012, Plaut et al. 2012). Hydraulic failure occurs when water transport is disrupted in a large number of

embolized vessels (cavitation), resulting in the desiccation of plant tissues. Stomatal closure allows plants to reduce water loss, but also decreases carbon uptake by photosynthesis. Plant metabolism requires a constant supply of carbohydrates, so stomatal closure may lead to carbon starvation. Support for both these hypotheses has been obtained in recent studies (Galiano et al. 2011, Anderegg et al. 2012), but the precise physiological mechanisms underlying plant mortality are poorly understood. This stresses the need for a clearer understanding of drought-induced mortality in trees, in particular regarding the threshold leading to hydraulic failure, which may be the primary cause of mortality during severe drought (Rice et al. 2004, McDowell et al. 2008). Species vulnerable to cavitation should be more prone to hydraulic failure because their xylem cannot sustain very negative pressure. Cavitation resistance varied widely across species and biomes (Delzon et al. 2010, Choat et al. 2012) and might therefore explain species distribution and survival (Maherali et al. 2004).

Many studies have shown that hydraulic failure plays a key role in the drought-induced mortality of leaves, peripheral shoots and roots (Rood et al. 2000, Davis et al. 2002, Rice et al. 2004, Hoffmann et al. 2011) and have reported the existence of relationships between hydraulic failure and either minimum water potential (Pockman and Sperry 2000, Choat et al. 2012) or climate (Tyree and Ewers 1991, Tyree and Cochard 1996, Davis et al. 1999, Pockman and Sperry 2000, Maherali et al. 2004, Choat et al. 2012). However, few studies have investigated the direct relationship between drought-induced tree mortality and hydraulic failure (Tyree et al. 2002, 2003, Brodribb and Cochard 2009, Kursar et al. 2009, Brodribb et al. 2010). Tyree et al. (2002, 2003) and Kursar et al. (2009) measured the native embolism of severely wilted tropical angiosperm tree seedlings from forests in Panama. Their results suggested that death occurred at a threshold corresponding to ~80% loss of xylem conductance per leaf area (but they did not measure cavitation resistance). Brodribb and Cochard (2009) and Brodribb et al. (2010) assessed the link between cavitation and plant death for seven southern hemisphere conifer species with different levels of water stress tolerance. They found a strong linear relationship between the water potential inducing a 50% loss of stem conductivity ( $P_{50}$ ) and the lethal water potential. No such direct evidence is available for angiosperm trees, despite the large number of economically important species belonging to these taxa.

We studied the physiological significance of xylem embolism in angiosperm tree species, by quantifying the relationship between drought-induced mortality and hydraulic failure. We selected five angiosperm tree species on the basis of the large range of water stress tolerance they covered: *Populus tremula* L., *Quercus robur* L., *Fagus sylvatica* L., *Quercus petraea* (Matt.) and *Quercus ilex* L. Seedlings of each species were exposed to water stress until they were severely wilted and close to death.

We assessed the minimum recoverable potential, by determining the leaf potential inducing a failure of various ecophysiological variables to recover (predawn leaf water potential, total plant water loss rate, leaf transpiration, stomatal conductance and  $\text{CO}_2$  assimilation rate). We specifically addressed the following questions: Is there a relationship between irreversible drought damage and xylem embolism in angiosperm tree species? Is the threshold of embolism leading to drought damage similar between angiosperm species and close to the one previously reported for conifer species?

## Materials and methods

### Plant materials

We selected five angiosperm tree species widespread in Europe and presenting a broad range of water stress tolerance. *Populus tremula* L. is a deciduous Eurasian species generally found in open areas, such as waste ground and glades on mesic soils (Rameau et al. 1989). *Quercus robur* L. is a Eurasian deciduous species common throughout Europe other than the Mediterranean region, as it is susceptible to summer droughts (Rameau et al. 1989). *Fagus sylvatica* L. and *Quercus petraea* (Matt.) Lielb. are deciduous species inhabiting plains and mountains throughout Europe; they tolerate drier soils than *P. tremula* and *Q. robur* (Rameau et al. 1989). *Quercus ilex* L. is a typical evergreen Mediterranean species found around the entire Mediterranean Basin (Alia et al. 2009).

### Vulnerability curves

Xylem cavitation was assessed with the CAVITRON technique, a centrifugation-based technique, as described by Cochard et al. (2005), on 11 and 30 mature trees for *P. tremula* and *F. sylvatica*, respectively. Centrifugal force was used to establish negative pressure in the xylem and to provoke water-stress-induced cavitation, using a custom-built honeycomb rotor (Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5, Asheville, NC, USA). The xylem pressure ( $P_i$ ), hydraulic conductance ( $k_i$ ) and percentage loss of conductance (PLC) were determined at various speeds, to obtain a vulnerability curve (the VC is the percentage loss of xylem conductance plotted against xylem pressure; see Delzon et al. 2010 for details). For *Q. robur*, *Q. petraea* and *Q. ilex*, VCs were replotted from previous studies (Cochard et al. 1992, Tyree and Cochard 1996). Vulnerability curves were measured on branches of mature trees by the air-dehydration method (Sperry et al. 1988), with samples brought to the laboratory and dehydrated on a bench over various periods of time to obtain branches with various degrees of stress (water potentials ranging from  $-2$  to  $-5$  MPa; see Cochard et al. 1992 for details of the procedure). Vulnerability to cavitation has also been measured in two different organs (root and stem) of two *Q. petraea* seedlings following the same protocol.

For each species and sample, a sigmoid function (Pammenter and Vander Willigen 1998) was fitted (proc NLIN, SAS version 9.2, SAS Institute, Cary NC, USA) to the VC, according to the following equation:

$$PLC = \frac{100}{(1 + \exp(S / 25(P_i - P_{50})))} \quad (1)$$

where  $P_{50}$  (MPa) is the xylem pressure inducing a 50% loss of conductance and  $S$  (% MPa<sup>-1</sup>) is the slope of the VC at the inflexion point. The xylem pressure inducing 88% of PLC ( $P_{88}$ , MPa) was calculated from the equation of Domec and Gartner (2001):

$$P_{88} = -\frac{50}{S} + P_{50} \quad (2)$$

### Experimental design

In April 2010, we transplanted 30–50 cm high seedlings into 8 l cylindrical plastic containers filled with loam. All the seedlings used were 2–3 years old, with the exception of *Q. ilex* seedlings, which were 4–5 years old. We arranged 275 plants (55 per species) into a randomized complete block design (5 blocks) in a greenhouse at the INRA Forest Research Station (0°45'58.60, 44°44'18.6N, Cestas, France). The leaves of each plant had fully expanded before the beginning of the experiment. All plants were watered two or three times per week, to keep soil water content at field capacity until the start of the experimental drought period. Air temperature and relative humidity were monitored hourly with sensors (HOBO, H08-032-08, Onset, Cape Cod, MA, USA). Mean temperature and relative humidity during the experiment (from May to August) were  $23 \pm 6$  °C and  $65 \pm 24\%$ , respectively. Pesticides were applied to prevent the development of diseases caused by insects (aphids) or fungi (mildew, rust). The soil surface was covered with plastic film to prevent evaporation.

After 2 weeks of acclimation in the greenhouse, two different water regimes were applied. Five plants per species were watered to keep the soil at field capacity (control plants), whereas drought was induced by withholding irrigation for 50 plants per species (water-stressed plants). The drought phase lasted until predawn leaf water potential could no longer be measured due to leaf desiccation (no more leaves, complete necrosis or withered leaves). The duration of this phase was thus species-dependent, ranging from 43 days for *P. tremula* to about 80 days for more water-stress-resistant species. The percentage of dead leaves was visually assessed on five plants per species at each level of water stress. At the end of the experiment, the three remaining water-stressed seedlings per species were rewatered to check for mortality 1 year later.

Predawn water potential was monitored during the drought phase, for the selection of five levels of increasing water stress

per species. Ecophysiological variables were measured on five water-stressed and one control plant per stress level and species. Three of the five water-stressed plants were rewatered until the soil was fully saturated. These plants (recovered plants) were then watered daily to keep the soil at field capacity during the recovery phase. During this period, the same ecophysiological measurements were performed on the plants, at intervals of 1–4 days, depending on the recovery rate of the plants.

### Ecophysiological measurements

For each species, plant water status, gas exchanges and total plant water loss rate were assessed throughout the experiment on water-stressed, control and recovered plants. Plants were placed overnight in a dark room within the greenhouse. The following morning, predawn leaf water potential ( $\Psi_p$ , MPa) measurements were performed on one to three healthy leaves per individual, with a Scholander pressure chamber (Precis 2000, Gradignan, France). The same seedlings were then exposed to a standard controlled-light environment (created with four high-energy gas-discharge lamps) for 90 min. Photosynthetically active radiation (PAR), measured at mid-plant level, reached  $566 \pm 281$   $\mu\text{mol m}^{-2} \text{s}^{-1}$  on average.

For each plant, total plant water loss rate ( $W$ , mmol m<sup>-2</sup> s<sup>-1</sup>) was determined by gravimetric measurement, as follows:

$$W = \frac{1}{A_L} \frac{\Delta w}{\Delta t} \quad (3)$$

where  $\Delta w/\Delta t$  (mmol s<sup>-1</sup>) is the loss of water over a given time interval (30–90 min) under controlled-light conditions, as measured with an electronic balance (PM34K, Mettler Toledo, Columbus, OH, USA) and  $A_L$  (m<sup>2</sup>) is the total plant leaf area. Leaf size was measured on at least 15 seedlings per species, with a leaf area meter (WinFolia 2007b, Regent Inst., Quebec, Canada). For each species, we then determined  $A_L$  by multiplying the mean leaf size for the species by the number of leaves.

Stomatal conductance ( $g_s$ , mmol m<sup>-2</sup> s<sup>-1</sup>), leaf transpiration ( $E_l$ , mmol m<sup>-2</sup> s<sup>-1</sup>) and CO<sub>2</sub> assimilation rate ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were measured with a portable open system including an infrared gas analyser (CIRAS 2, PPSsystems, Amesbury, MA, USA). Mean PAR was  $1493 \pm 5$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ , CO<sub>2</sub> concentration was  $391 \pm 15$  ppm and vapor pressure deficit in the leaf chamber was  $1460 \pm 480$  hPa during the experiment. Immediately after gas exchange measurements, midday leaf water potential ( $\Psi_m$ , MPa) was measured on one to three leaves per plant, during the drought phase only.

### Determination of the minimum recoverable water potential

The minimum recoverable potential ( $\Psi_{\text{reco}}$ ) was determined as described by Brodribb and Cochard (2009) and Brodribb

et al. (2010), for all five species, based on five ecophysiological variables (predawn leaf water potential, total plant water loss rate, leaf transpiration, stomatal conductance and  $\text{CO}_2$  assimilation rate). During the recovery phase, the time required for each variable ( $\Psi_p$ ,  $W$ ,  $E$ ,  $g_s$  and  $A$ ) to reach 50% of its maximum value ( $t_{1/2}$ ) was calculated per water stress level and species. The 50% maximum value of each variable was calculated by dividing the 99th quantile of the distribution of each variable by 2 (on control, water-stressed and recovered plants). For each species and variable, linear regressions were fitted to the inverse of  $t_{1/2}$  versus midday water potential (proc GLM, SAS version 9.2, SAS Institute, Cary, NC, USA). The minimum recoverable potential, corresponding to the water potential inducing a failure of the studied variable to recover, was assessed by determining the x-intercept of the linear regression. One-way analyses of covariance were carried out to test the difference in slope of linear regressions between minimum recoverable potentials and  $P_{88}$  or  $P_{50}$  (proc GLM, SAS version 9.2, SAS Institute).

For each species and variable, predicted native embolism ( $\text{PLC}_p$ ) was derived from the  $\Psi_{\text{reco}}$  and VCs. Differences in  $\Psi_{\text{reco}}$ ,  $\Psi_m$  at the most severe level of water stress and  $\text{PLC}_p$  between species were tested using one-way analyses of variance and Tukey tests (proc GLM, SAS version 9.2, SAS Institute).

## Results

### Cavitation resistance

All VCs for each of the species followed a sigmoid shape (Figure 1). We found substantial differences in cavitation resistance between species, with  $P_{50}$  values ranging from  $-2.36$  MPa for *P. tremula* to  $-5.52$  MPa for *Q. ilex*. Cavitation-resistant species (with the lowest  $P_{50}$  values) had the VCs with the shallowest slopes (Figure 1), increasing the between-species differences for  $P_{88}$ , which ranged from  $-3.09$  MPa for *P. tremula* to  $-7.08$  MPa for *Q. ilex*. For *Q. petraea*, VCs measured on the stem and root of two seedlings showed no difference with those obtained on mature trees (Figure 1c; no difference of cavitation resistance between developmental stages and organs).

### Ecophysiological response to drought

Leaf death increased more rapidly for *P. tremula* and *Q. robur* (<60 days) than for other species (>60 days) and reached 50% (*F. sylvatica*) to 100% (*P. tremula* and *Q. robur*) at the most severe level of water stress (Figure 2). Predawn and midday water potentials decreased in a similar manner during drought for all species. Midday water potential (i.e.,  $\Psi_m$ ) differed significantly between species, ranging from  $-3.1$  MPa for *P. tremula* to  $-5.8$  MPa for *Q. ilex* for the most severe level of

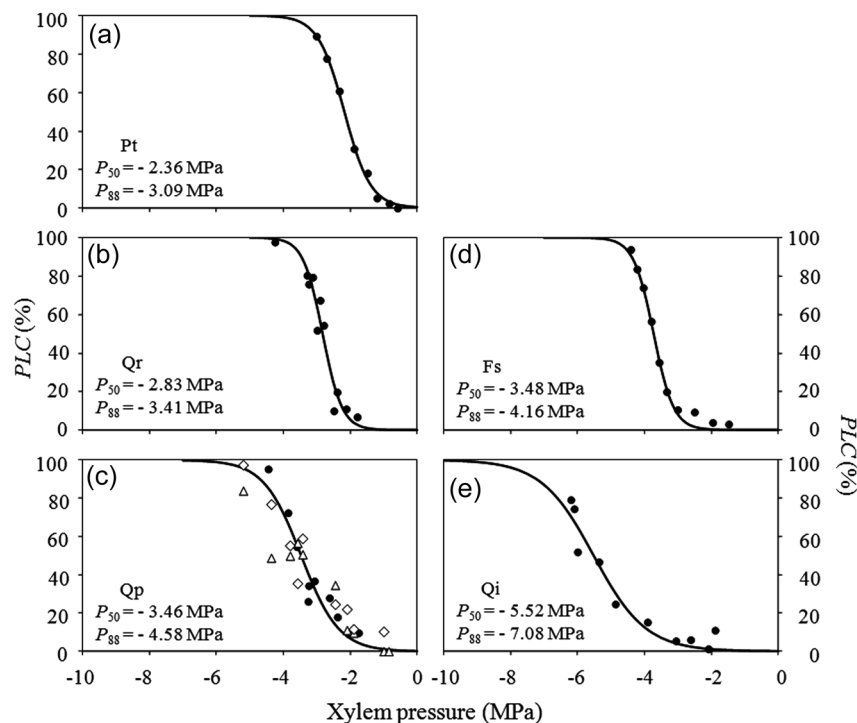


Figure 1. Percentage loss of hydraulic conductance (PLC) versus xylem pressure for each of the species studied. Vulnerability curves were obtained with the CAVITRON technique on mature trees for *P. tremula* and *F. sylvatica* and correspond to one individual per species. The air-dehydration method was used for the long vessel oak species (mature tree, closed circles; stem seedling, triangles; root seedling, diamonds). (a) Pt, *P. tremula*; (b) Qr, *Q. robur*; (c) Qp, *Q. petraea*; (d) Qi, *Q. ilex*.



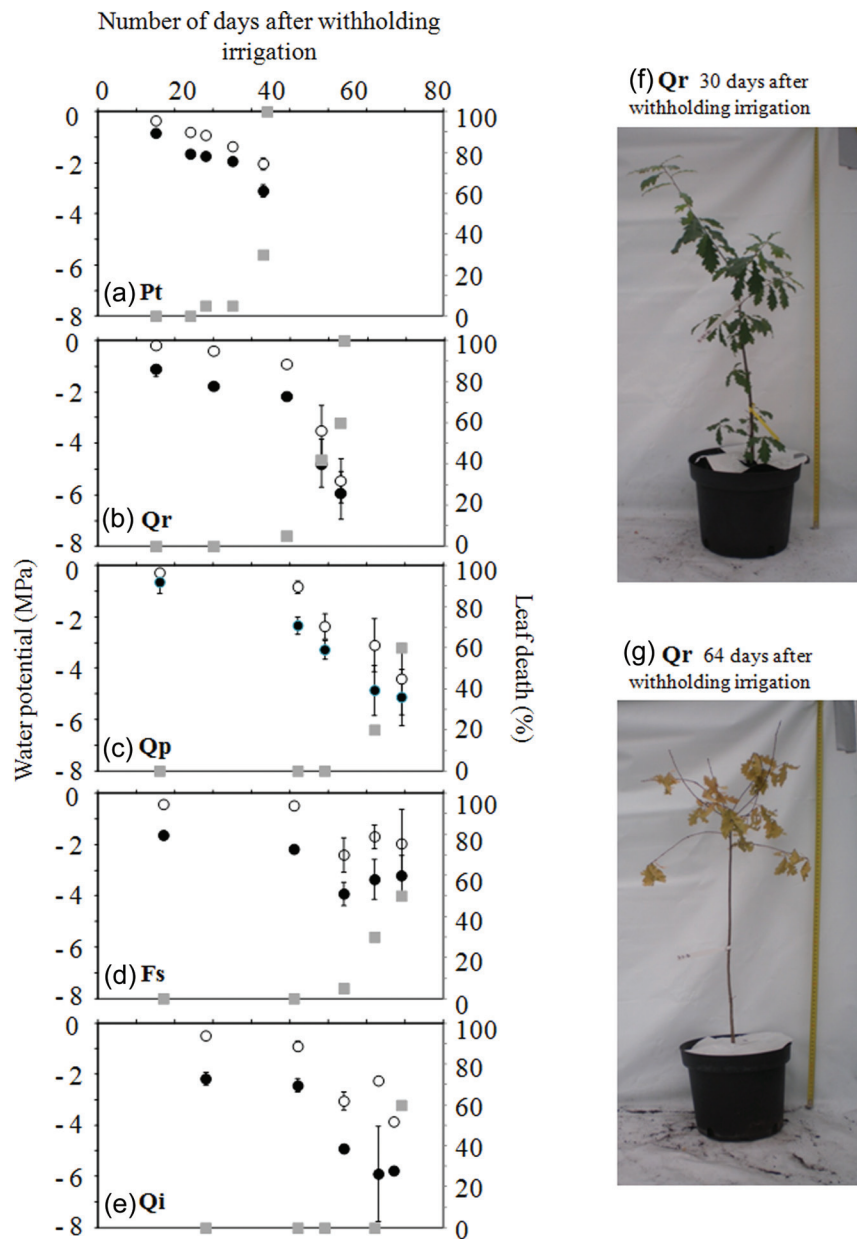


Figure 2. Changes in predawn (open circles) ( $\Psi_p$ ) and midday (closed circles) ( $\Psi_m$ ) water potential, and percentage of leaf death (grey squares) over time and with increasing drought duration: (a) *Pt*, *P. tremula*; (b) *Qr*, *Q. robur*; (c) *Fs*, *F. sylvatica*; (d) *Qp*, *Q. petraea*; (e) *Qi*, *Q. ilex*. Values of predawn and midday water potentials are the mean values for at least five individuals per species and per level of water stress; the bars indicate the standard error on the mean value. Photos illustrate leaf desiccation for two individuals of *Q. robur* at (f) 30 and (g) 64 days after withholding irrigation.

water stress (Figure 2, Table 1, analysis of variance (ANOVA),  $F = 4.07$ ,  $P = 0.0171$ ). All the ecophysiological variables studied responded strongly to drought, with a sharp decline, from 0 to  $-2$  MPa of predawn water potential. However, different patterns were observed for the different species, with, for example, an even more abrupt decline in  $W$ ,  $g_s$ ,  $E_i$  and  $A$  for *Q. robur* compared with *Q. ilex* (Figure 3).

### Recovery from drought

The time required for each variable ( $\Psi_p$ ,  $W$ ,  $E_i$ ,  $g_s$  and  $A$ ) to reach 50% of its maximum value ( $t_{1/2}$ ) depended strongly on the level

of water stress experienced by the plant, regardless of the species considered. Individuals subjected to moderate water stress (open circles in Figure 4) generally recovered overnight. Recovery time after rewetting was no longer than 2 days in any case. Plants that had experienced severe water stress (closed circles in Figure 4) generally did not recover, but in all cases in which recovery was observed,  $t_{1/2}$  exceeded 2 days.

### Minimum recoverable potential and cavitation resistance

The minimum recoverable potential—the potential associated with a failure to recover—differed significantly between species

Table 1. Values of minimum recoverable potential associated with predawn leaf water potential ( $\Psi_{\text{reco-}\Psi_p}$ , MPa), total plant water loss rate ( $\Psi_{\text{reco-}W}$ , MPa), leaf transpiration ( $\Psi_{\text{reco-}E_l}$ , MPa), stomatal conductance ( $\Psi_{\text{reco-}g_s}$ , MPa) and CO<sub>2</sub> assimilation rate ( $\Psi_{\text{reco-}A}$ , MPa), minimum recoverable potential averaged for the five ecophysiological variables studied ( $\Psi_{\text{reco}}$ , MPa), midday potential at the most severe level of water stress ( $\Psi_m$ , MPa) and predicted (PLC<sub>p</sub>, %) native embolism, determined for each species. Mean values with the same letter between species are not significantly different ( $\alpha = 0.05$ ).

	<i>P. tremula</i>	<i>Q. robur</i>	<i>Q. petraea</i>	<i>F. sylvatica</i>	<i>Q. ilex</i>
$\Psi_{\text{reco-}\Psi_p}$	-3.38	-3.74	-4.24	-4.67	-5.3
$\Psi_{\text{reco-}W}$	-2.77	-3.9	-4.79	-3.49	-5.34
$\Psi_{\text{reco-}E_l}$	-3.74	-4.21	-4.59	-4.52	-6.78
$\Psi_{\text{reco-}g_s}$	-3.48	-3.04	-4.62	-4.90	-5.82
$\Psi_{\text{reco-}A}$	-3.77	-2.88	-4.25	-4.48	-6.97
$\Psi_{\text{reco}}$	-3.43 ± 0.18 a	-3.55 ± 0.26 ab	-4.50 ± 0.11 b	-4.41 ± 0.24 b	-6.04 ± 0.35 c
$\Psi_m$	-3.10 ± 0.24 a	-5.45 ± 1.01 b	-5.12 ± 1.10 ab	-3.21 ± 0.78 ab	-5.76 ± 0.00 ab
PLC <sub>p</sub>	92.78 ± 4.22 a	82.97 ± 9.18 a	85.77 ± 2.34 a	87.40 ± 2.34 a	63.39 ± 9.27 a

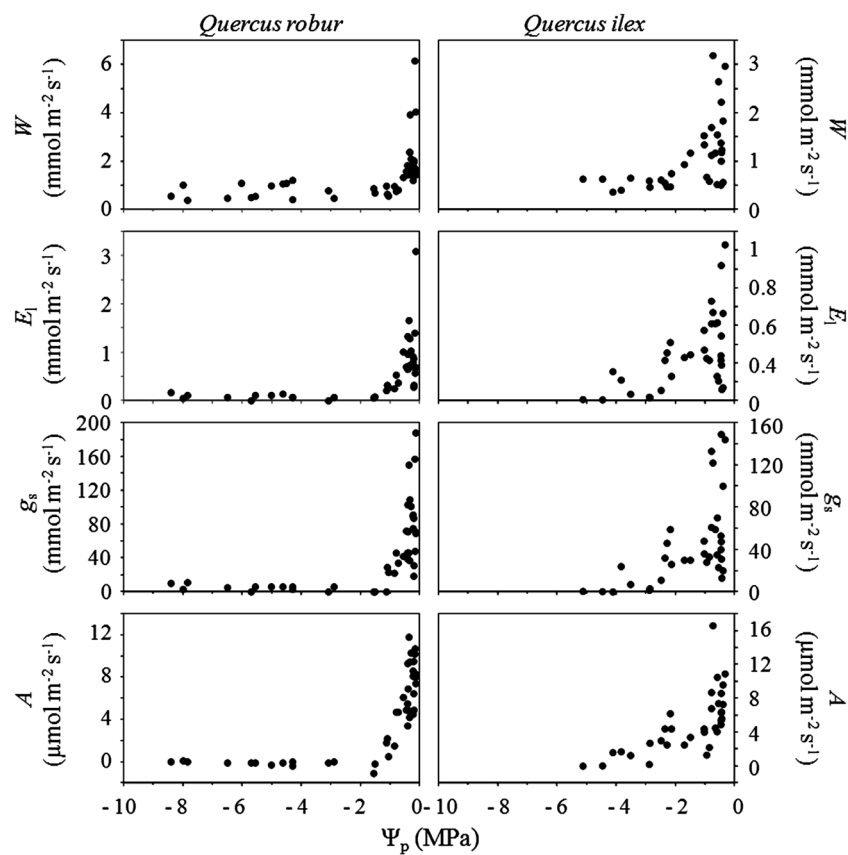


Figure 3. Changes in total plant water loss rate ( $W$ ), leaf transpiration ( $E_l$ ), stomatal conductance ( $g_s$ ) and CO<sub>2</sub> assimilation rate ( $A$ ) with decreasing predawn water potential during drought for two of the five species studied: we present one of the least drought-tolerant species, *Q. robur* (left panel), and the most drought-tolerant species, *Q. ilex* (right panel). Each value corresponds to a single individual.

(Table 1, ANOVA,  $P < 0.0001$ ), particularly for *Q. ilex*, which had the most negative values for all the variables studied. During the experiment, all species except *F. sylvatica* experienced leaf water potentials close to their minimum recoverable potential, as demonstrated by the values of midday water potential measured at the most severe level of water stress (Table 1).

Significant linear regressions between minimum recoverable water potential and cavitation resistance ( $P_{50}$  or  $P_{88}$ ) were found for all variables ( $\Psi_{\text{reco-}\Psi_p}$ ,  $\Psi_{\text{reco-}W}$ ,  $\Psi_{\text{reco-}E_l}$ ,  $\Psi_{\text{reco-}g_s}$  and  $\Psi_{\text{reco-}A}$ ; Figure 5,  $P \leq 0.0003$ ). The slopes of the regression with  $P_{88}$  were closer to 1 (deviation from 1 of 0.03 to 0.11, Table 2), whereas those obtained with  $P_{50}$  deviated to a greater

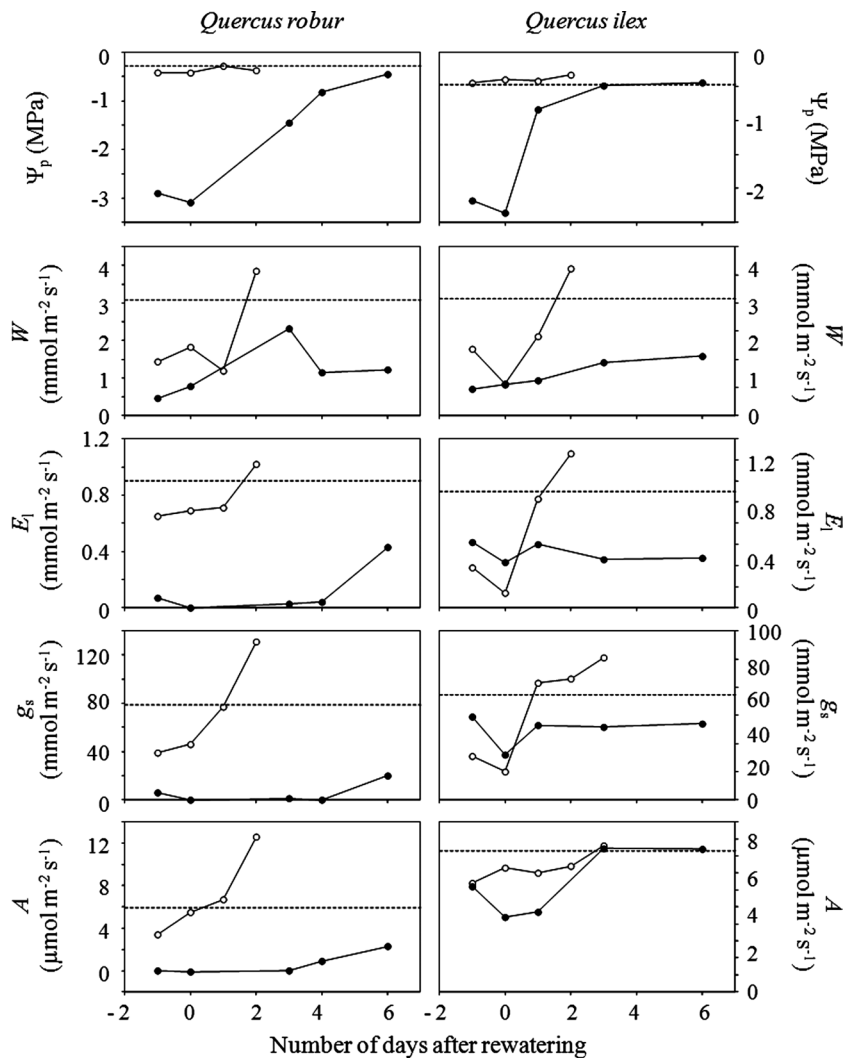


Figure 4. An example of the recovery over time of predawn water potential ( $\Psi_p$ ), total plant water loss rate ( $W$ ), leaf transpiration ( $E_l$ ), stomatal conductance ( $g_s$ ) and  $\text{CO}_2$  assimilation rate ( $A$ ) for moderate (open circles) and severe (closed circles) stresses, in rewatered plants of *Q. robur* (left panel) and *Q. ilex* (right panel). Measurements of ecophysiological variables before and on the day of rewetting indicated the level of water stress experienced by the plants. Dashed lines indicate 50% of the maximal value for ecophysiological variables.

extent from 1 (deviation from 1 of 0.13 to 0.25, Table 2). Moreover, the estimated embolism rates reached at  $\Psi_{\text{reco-}\Psi_p}$ ,  $\Psi_{\text{reco-}W}$ ,  $\Psi_{\text{reco-}E_l}$ ,  $\Psi_{\text{reco-}g_s}$ , or  $\Psi_{\text{reco-}A}$ , averaged for all species, were systematically  $>50\%$  ( $82 \pm 10$ ,  $72 \pm 11$ ,  $93 \pm 3$ ,  $82 \pm 8$  and  $83 \pm 8\%$ , respectively) and close to 88%. Small differences in minimum recoverable potential were found between ecophysiological variables (standard error between 0.11 for *Q. petraea* and 0.35 for *Q. ilex*; Table 1). Moreover, we found no difference in slope of linear regression according to the variable used to estimate the minimum recovery potential for  $P_{88}$  (Figure 5, analysis of covariance (ANCOVA),  $F = 1.51$ ,  $P = 0.2347$ ) or for  $P_{50}$  (ANCOVA,  $F = 1.32$ ,  $P = 0.2969$ ).

The 1 : 1 relationship between  $P_{88}$  and the overall minimum recovery potential corresponding to recovery failure (estimated from the mean of the five ecophysiological variables studied per species) was highly significant (Table 2). The predicted

native embolism ( $\text{PLC}_p$ ) at the minimum recoverable potential was  $>80\%$  for all species other than *Q. ilex* ( $\text{PLC}_p = 63\%$ ; Table 1).

## Discussion

Our findings demonstrate that xylem cavitation clearly limits the desiccation resistance of angiosperm trees and that the minimum recoverable potential corresponds to the water stress threshold associated with hydraulic failure at the plant level. The five species studied here were exposed to severe drought conditions and were unable to recover if their water potential dropped below the pressure inducing 88% xylem embolism in the stem. The use of several post-drought recovery variables to estimate the severity of water stress renders our findings both valid and reliable for estimating the global limit of desiccation

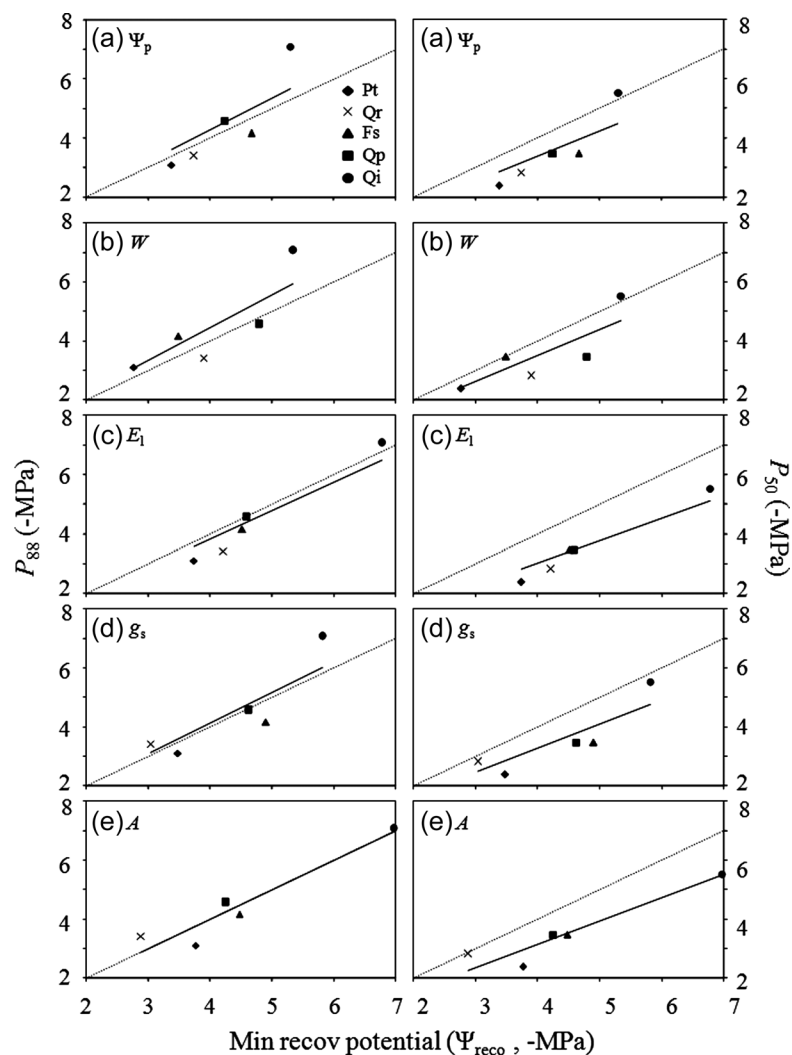


Figure 5. Relationships between  $P_{88}$  (left panel) or  $P_{50}$  (right panel) and minimum recoverable water potential associated with predawn leaf water potential ( $\Psi_{\text{reco-}\Psi_p}$ ) (a), total plant water loss rate ( $\Psi_{\text{reco-}W}$ ) (b), leaf transpiration ( $\Psi_{\text{reco-}E_1}$ ) (c), stomatal conductance ( $\Psi_{\text{reco-}g_s}$ ) (d) and  $\text{CO}_2$  assimilation rate ( $\Psi_{\text{reco-}A}$ ) (e), for the five species studied. Estimated slopes for linear regression forced through the origin are shown in Table 2.

Table 2. Slopes of the linear regression between cavitation traits ( $P_{50}$  and  $P_{88}$ ) and the minimum recoverable potential associated with predawn leaf water potential ( $\Psi_{\text{reco-}\Psi_p}$ ), total plant water loss rate ( $\Psi_{\text{reco-}W}$ ), leaf transpiration ( $\Psi_{\text{reco-}E_1}$ , MPa), stomatal conductance ( $\Psi_{\text{reco-}g_s}$ ) and  $\text{CO}_2$  assimilation rate ( $\Psi_{\text{reco-}A}$ ), and minimum recoverable potential averaged for the five ecophysiological variables studied ( $\Psi_{\text{reco}}$ , MPa) and the  $P$ -values ( $P$ ) for the slope estimates. The values given are for regression lines forced through the origin.

	Slope ( $P_{50}$ )	$P$	Slope ( $P_{88}$ )	$P$
$\Psi_{\text{reco-}\Psi_p}$	0.84	0.0002	1.07	0.0003
$\Psi_{\text{reco-}W}$	0.87	0.0003	1.11	0.0003
$\Psi_{\text{reco-}E_1}$	0.75	<0.0001	0.95	<0.0001
$\Psi_{\text{reco-}g_s}$	0.82	0.0001	1.03	0.0002
$\Psi_{\text{reco-}A}$	0.82	<0.0001	1.04	<0.0001
$\Psi_{\text{reco}}$	0.82	<0.0001	1.04	<0.0001

resistance. Furthermore, this threshold clearly differs from that reported for conifer species ( $P_{50}$ ).

**Estimation of the minimum recoverable potential with various post-drought recovery variables**

Previous studies determined the potential associated with recovery failure in trees from total plant water loss (Blackman et al. 2009, Brodribb and Cochard 2009, Brodribb et al. 2010) or leaf photosynthesis (Resco et al. 2009), but no previous study has ever used more than one variable to characterize a global limit for desiccation resistance. We used several ecophysiological variables (predawn leaf water potential, plant water loss, leaf transpiration, stomatal conductance and  $\text{CO}_2$  assimilation rate) to estimate the threshold of water stress leading to recovery failure. The minimum recoverable potential obtained differed only slightly between the five ecophysiological variables studied, indicating a



failure of water potential, transpiration and photosynthesis to recover at similar water stress thresholds. However, the overall minimum recoverable water potential, corresponding to a global dysfunction at the plant level, differed considerably between species and values were ranked in the same order as for cavitation resistance (*P. tremula* and *Q. robur* being the least resistant species and *Q. ilex* the most resistant species; see Table 1).

Such global dysfunction always leads to plant death in conifer species (Brodribb and Cochard 2009) but does not always result in death in angiosperm species (Blackman et al. 2009). Indeed, in our experiment, two out of the three remaining individuals of *P. tremula* and *Q. robur* that experienced water potentials close to their species minimum recoverable potential and displayed total leaf mortality (100% of leaf death at the most severe level of water stress) did not die and resprouted the following year. Some angiosperm species can resprout or refill an embolism after partial or complete leaf desiccation. This is typically the case in the genus *Populus*, for instance, in which leaves are shed to avoid water stress, with resprouting occurring after drought (Lu et al. 2010).

### Relationship between drought resistance and cavitation resistance

The overall minimum potential associated with the failure of ecophysiological processes to recover was highly correlated with stem cavitation resistance. Moreover, we found that the minimum recoverable potential was always much lower (more negative) than  $P_{50}$ , providing evidence that hydraulic failure occurred at a threshold well beyond 50% stem embolism ( $P_{50}$ ). Indeed, the minimum potential leading to hydraulic failure was much closer to the pressure inducing 88% embolism in the stem ( $P_{88}$ ), for all the ecophysiological variables ( $\Psi_p$ ,  $W$ ,  $E_i$ ,  $g_s$  and  $A$ ). The values of measured and estimated native embolism ( $PLC_p$ ) related to recovery failure were well beyond 50% and close to 88% in four of the five species studied. The high stem embolism values found here for angiosperm species are consistent with results from previous studies showing a progressive loss of leaves (fallen, necrotic or wilted leaves) for very high values of stem embolism (50–90% for *Quercus coccifera* L. and 90–100% for *Pistacia lentiscus* L. (Vilagrosa et al. 2003)). In tropical seedlings native to Panama, severely wilted stages were reached when ~80% of stem conductivity was lost (Tyree et al. 2002, 2003, Kursar et al. 2009). Complete leaf mortality occurred at ~90% stem embolism for *Populus tremuloides* Michx. (Lu et al. 2010) and 85% PLC for *Tamarix ramosissima* Ledeb. (Ayup et al. 2012). Thus, our results indicate that the water potential leading to irrevocable drought damage is associated with massive xylem dysfunction in the stem, whereas near-complete leaf embolism does not lead to death (Blackman et al. 2009). Therefore, as in conifers (Brodribb et al. 2010), stem vulnerability seems to be more informative about the survival limits of angiosperms than leaf hydraulics.

Although the VCs have been obtained on mature trees, the  $P_{50}$  and  $P_{88}$  values estimated here seem very representative for a given species. Indeed, we did not find any differences of cavitation resistance between developmental stages in *Q. petraea* and previous studies reported similar results between seedlings and mature trees for three oak species (see Cochard et al. 1992, Higgs and Wood 1995) and for *F. sylvatica* (Cochard et al. 1999). Rice et al. (2004) also found similar vulnerability to cavitation between seedlings and adult trees for *Corymbia erythrophloia* (Blakely) K.D. Hill and L.A.S. Johnson but slight differences for a *Eucalyptus* species. This apparent lack of variation as trees get older might likely be due to strong genetic constraints (canalization) as evidenced for cavitation resistance (Lamy et al. 2011).

The threshold ( $P_{88}$ ) for recovery failure in angiosperms is more negative than that reported for conifers: Brodribb and Cochard (2009) and Brodribb et al. (2010) showed a significant 1 : 1 relationship between lethal water potential, defined as the potential associated with recovery failure, and  $P_{50}$ , in seven conifer species native to Australia. The observed differences of threshold between angiosperm and conifer trees may reflect the greater ability of vessel-bearing species to repair embolism, as reported in *F. sylvatica* (Holbrook and Zwieniecki 1999, Cochard et al. 2001, Bucci et al. 2003, Salleo et al. 2004), and, therefore, to cope with higher levels of embolism. In conifers, refilling of stem embolism has also been observed (Edwards et al. 1994, Sobrado et al. 1992, McCulloh et al. 2011) but embolism repair mostly occurred in distal organs (leaves and roots) for this taxa (Johnson et al. 2012). Moreover, the dynamics of gas exchange recovery in these species matches the rate of hydraulic repair due to xylem regrowth (Brodribb et al. 2010). The replacement of xylem tissues in conifer trees can lead to the recovery of 15–20% of lost hydraulic function per month, whereas, in angiosperm trees such as ring-porous oak species, the building of a new tree ring, which becomes the sole functioning tree ring, allows the recovery of 100% hydraulic conductance. For these species, the cost of the loss of a functional tree ring, due to drought, at the end of the summer is small, because that ring would have become non-functional in the following winter anyway.

Choat et al. (2012) recently showed that 42% of angiosperms traversed the  $P_{50}$  safety margin (difference between the minimum xylem pressure a stem experiences during a season and the pressure inducing 50% loss of hydraulic conductance,  $[\Psi_m - P_{50}]$ ), confirming that  $P_{50}$  was not the point of hydraulic failure in angiosperms. Where a measure of the threshold of mortality is the desired metric, we here demonstrated that a more conservative safety margin calculated as  $[\Psi_m - P_{88}]$  would seem much more meaningful. The  $P_{88}$  safety margin of angiosperms showed a similar convergence of vulnerability across forest biomes, but angiosperms have a lower safety margin than conifer species (Choat et al. 2012). The fact that conifers

maintain much higher safety margins than angiosperms might be simply due to the lower stem embolism threshold associated with recovery failure. However, Johnson et al. (2012) suggested that the lower safety margin of angiosperms compared with that of conifers could be related to higher levels of xylem parenchyma and non-structural carbohydrate concentrations in their stems, both of which may be required for embolism repair (Zwieniecki and Holbrook 2009, Nardini et al. 2011).

## Conclusion

Knowing the thresholds at which tree mortality occurs is of critical importance to making predictions of mortality across biomes. In contrast to what has been reported for conifers, a 50% loss of stem conductivity does not lead to plant death in angiosperm trees. Indeed, the stem embolism threshold associated with irrevocable drought damage in angiosperms is well beyond this limit and close to an 88% loss of stem conductivity. These findings are essential to understand how community structure will change in response to increased aridity and should be integrated in dynamic global vegetation models to predict with more accuracy shifts in species distribution. Nevertheless, nanotomography observations in planta are required to check this threshold of global hydraulic dysfunction and the potential occurrence of refilling under tension (Brodersen et al. 2010, Pittermann et al. 2012).

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## Conflict of interest

None declared.

## References

- Adams H, Guardiola-Claramonte M, Barron-Gafford G, Villegas J, Breshears D, Zou C, Troch P, Huxman T (2009) Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proc Natl Acad Sci USA* 106:7063–7066.
- Alia R, García del Barrio JM, Iglesias S, Mancha JA, De Miguel J, Nicolas JL, Pérez F, Sanchez de Ron D (eds) (2009) *Regiones de procedencia de especies forestal en España*. Ministerio de Medio Ambiente y Medio Rural y Marino, Madrid, Spain.
- Allen CD, Macalady AK, Chenchouni H et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For Ecol Manag* 259:660–684.
- Anderegg WR, Berry JA, Smith DD, Sperry JS, Anderegg LD, Field CB (2012) The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proc Natl Acad Sci USA* 109:233–237.
- Ayup M, Hao X, Chen Y, Li W, Su R (2012) Changes of xylem hydraulic efficiency and native embolism of *Tamarix ramosissima* Ledeb. seedlings under different drought stress conditions and after rewetting. *S Afr J Bot* 78:75–82.
- Bigler C, Bräker OU, Bugmann H, Dobbertin M, Rigling A (2006) Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* 9:330–343.
- Blackman CJ, Brodribb TJ, Jordan GJ (2009) Leaf hydraulics and drought stress: response, recovery and survivorship in four woody temperate plant species. *Plant Cell Environ* 32:1584–1595.
- Breshears DD, Cobb NS, Rich PM et al. (2005) Regional vegetation die-off in response to global-change-type drought. *Proc Natl Acad Sci USA* 102:15144–15148.
- Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA (2010) The dynamics of embolism repair in xylem: *in vivo* visualizations using high-resolution computed tomography. *Plant Physiol* 154:1088–1095.
- Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiol* 149:575–584.
- Brodribb TJ, Bowman DJ, Nichols S, Delzon S, Burlett R (2010) Xylem function and growth rate interact to determine recovery rates after exposure to extreme water deficit. *New Phytol* 188:533–542.
- Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Sternberg LDL (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. *Plant Cell Environ* 26:1633–1645.
- Burke EJ, Brown SJ, Christidis N (2006) Modeling the recent evolution of global drought and projections for the twenty-first century with the Hadley centre climate model. *J Hydrometeorol* 7:1113–1125.
- Choat B, Jansen S, Brodribb TJ et al. (2012) Global convergence in the vulnerability of forests to drought. *Nature* 491:752–755.
- Ciais P, Reichstein M, Viovy N et al. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437:529–533.
- Cochard H, Breda N, Granier A, Aussenac G (1992) Vulnerability to air-embolism of 3 European oak species (*Quercus petraea* (Matt) Liebl, *Quercus pubescens* Willd, *Quercus robur* L). *Ann Sci For* 49:225–233.
- Cochard H, Lemoine D, Dreyer E (1999) The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. *Plant Cell Environ* 22:101–108.
- Cochard H, Lemoine D, Améglio T, Granier A (2001) Mechanisms of xylem recovery from winter embolism in *Fagus sylvatica*. *Tree Physiol* 21:27–33.
- Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiol Plant* 124:410–418.
- Davis SD, Ewers FW, Wood J, Reeves JJ, Kolb KJ (1999) Differential susceptibility to xylem cavitation among three pairs of *Ceanothus*

- species in the Transverse Mountain Ranges of southern California. *Ecoscience* 6:180–186.
- Davis SD, Ewers FW, Sperry JS, Portwood KA, Crocker MC, Adams GC (2002) Shoot dieback during prolonged drought in *Ceanothus* (Rhamnaceae) chaparral of California: a possible case of hydraulic failure. *Am J Bot* 89:820–828.
- Delzon S, Douthe C, Sala A, Cochard H (2010) Mechanism of water-stress induced cavitation in conifers: bordered pit structure and function support the hypothesis of seal capillary-seeding. *Plant Cell Environ* 33:2101–2111.
- Domec JC, Gartner BL (2001) Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees Struct Funct* 15:204–214.
- Edwards WRN, Jarvis PG, Grace J, Moncrieff JB (1994) Reversing cavitation in tracheids of *Pinus sylvestris* L. under negative water potentials. *Plant Cell Environ* 17:389–397.
- Eilmann B, Rigling A (2012) Tree-growth analyses to estimate tree species' drought tolerance. *Tree Physiol* 32:178–187.
- Galiano L, Martinez-Vilalta J, Lloret F (2010) Drought-induced multi-factor decline of Scots pine in the Pyrenees and potential vegetation change by the expansion of co-occurring oak species. *Ecosystems* 13:978–991.
- Galiano L, Martinez-Vilalta J, Lloret F (2011) Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytol* 190:750–759.
- Gitlin AR, Sthultz CM, Bowker MA, Stumpf S, Paxton KL, Kennedy K, Munoz A, Bailey JK, Whitham TG (2006) Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. *Conserv Biol* 20:1477–1486.
- Higgs KH, Wood V (1995) Drought susceptibility and xylem dysfunction in seedlings of 4 European oak species. *Ann Sci For* 52:507–513.
- Hoffmann WA, Marchin RM, Abit P, Lau OL (2011) Hydraulic failure and tree dieback are associated with high wood density in a temperate forest under extreme drought. *Glob Chang Biol* 17:2731–2742.
- Hogg E, Brandt J, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. *Can J For Res* 38:1373–1384.
- Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension: do we need a miracle? *Plant Physiol* 120:7–10.
- Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. *Front Ecol Environ* 5:365–374.
- Johnson DM, McCulloh KA, Woodruff DR, Meinzer FC (2012) Hydraulic safety margins and embolism reversal in stems and leaves: why are conifers and angiosperms so different? *Plant Sci* 195:48–53.
- Kursar TA, Engelbrecht BM, Burke A, Tyree MT, El Omari B, Giraldo JP (2009) Tolerance to low leaf water status of tropical tree seedlings is related to drought performance and distribution. *Funct Ecol* 23:93–102.
- Lamy JB, Bouffier L, Burrett R, Plomion C, Cochard H, Delzon S (2011) Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range. *PLoS One* 6:e23476.
- Lu Y, Equiza MA, Deng X, Tyree MT (2010) Recovery of *Populus tremuloides* seedlings following severe drought causing total leaf mortality and extreme stem embolism. *Physiol Plant* 140:246–257.
- Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* 85:2184–2199.
- McCulloh KA, Johnson DM, Meinzer FC, Lachenbruch B (2011) An annual pattern of native embolism in upper branches of four tall conifer species. *Am J Bot* 98:1007–1015.
- McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol* 155:1051–1059.
- McDowell N, Pockman WT, Allen CD et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol* 178:719–739.
- Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305:994–997.
- Michaelian M, Hogg EH, Hall RJ, Arsenault E (2011) Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. *Glob Chang Biol* 17:2084–2094.
- Nardini A, Lo Gullo MA, Salleo S (2011) Refilling embolized xylem conduits: is it a matter of phloem unloading? *Plant Sci* 180:604–611.
- Pammenter NW, Vander Willigen C (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiol* 18:589–593.
- Pittermann J, Stuart SA, Dawson TE, Moreau A (2012) Cenozoic climate change shaped the evolutionary ecophysiology of the Cupressaceae conifers. *Proc Natl Acad Sci USA* 109:9647–9652.
- Plaut JA, Yezzer EA, Hill J, Pangle R, Sperry JS, Pockman WT, McDowell NG (2012) Hydraulic limits preceding mortality in a pinon-juniper woodland under experimental drought. *Plant Cell Environ* 35:1601–1617.
- Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *Am J Bot* 87:1287–1299.
- Rameau JC, Mansion D, Dumé G, Timbal J, Lecoq A, Dupont P, Keller R (eds) (1989) Flore forestière française guide écologique illustré, 1. Plaine et collines. Institut Pour le Développement Forestier, Paris, France.
- Resco V, Ewers BE, Sun W, Huxman TE, Weltzin JF, Williams DG (2009) Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C-3 woody legume, *Prosopis velutina*. *New Phytol* 181:672–682.
- Rice KJ, Matzner SL, Byer W, Brown JR (2004) Patterns of tree dieback in Queensland, Australia: the importance of drought stress and the role of resistance to cavitation. *Oecologia* 139:190–198.
- Rood SB, Patino S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. *Trees Struct Funct* 14:248–257.
- Sala A (2009) Lack of direct evidence for the carbon-starvation hypothesis to explain drought-induced mortality in trees. *Proc Natl Acad Sci USA* 106:E68–E68.
- Salleo S, Lo Gullo MA, Trifilo P, Nardini A (2004) New evidence for a role of vessel-associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. *Plant Cell Environ* 27:1065–1076.
- Sanchez-Salguero R, Navarro-Cerrillo RM, Camarero JJ, Fernandez-Cancio A (2012) Selective drought-induced decline of pine species in southeastern Spain. *Clim Chang* 113:767–785.
- Schar C, Vidale PL, Luthi D, Frei C, Haberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. *Nature* 427:332–336.
- Sobrado MA, Grace J, Jarvis PG (1992) The limits to xylem embolism recovery in *Pinus sylvestris* L. *J Exp Bot* 43:831–836.
- Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell Environ* 11:35–40.
- Tyree MT, Cochard H (1996) Summer and winter embolism in oak: impact on water relations. *Ann Sci For* 53:173–180.
- Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. *New Phytol* 119:349–360.
- Tyree MT, Vargas G, Engelbrecht BMJ, Kursar TA (2002) Drought until death do us part: a case study of the desiccation-tolerance of a

- tropical moist forest seedling-tree, *Licania platypus* (Hemsl.) Fritsch. J Exp Bot 53:2239–2247.
- Tyree MT, Engelbrecht BMJ, Vargas G, Kursar TA (2003) Desiccation tolerance of five tropical seedlings in Panama. Relationship to a field assessment of drought performance. Plant Physiol 132:1439–1447.
- van Mantgem PJ, Stephenson NL (2007) Apparent climatically induced increase of tree mortality rates in a temperate forest. Ecol Lett 10:909–916.
- Vilagrosa A, Bellot J, Vallejo VR, Gil-Pelegrin E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. J Exp Bot 54:2015–2024.
- Waring RH (1987) Characteristics of trees predisposed to die. Bioscience 37:569–574.
- Zwieniecki MA, Holbrook NM (2009) Confronting Maxwell's demon: biophysics of xylem embolism repair. Trends Plant Sci 14:530–534.