



## Assessing inter- and intraspecific variability of xylem vulnerability to embolism in oaks



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### ABSTRACT

The genus *Quercus* comprises important species in forestry not only for their productive value but also for their ability to withstand drought. Hence an evaluation of inter- and intraspecific variation in drought tolerance is important for selecting the best adapted species and provenances for future afforestation. However, the presence of long vessels makes it difficult to assess xylem vulnerability to embolism in these species. Thanks to the development of a flow centrifuge equipped with a large rotor, we quantified (i) the between species variability of embolism resistance in four native and two exotic species of oaks in Europe and (ii) the within species variability in *Quercus petraea*. Embolism resistance varied significantly between species, with the pressure inducing 50% loss of hydraulic conductivity ( $P_{50}$ ) ranging between  $-7.0$  and  $-4.2$  MPa. Species native to the Mediterranean region were more resistant than pan-European species. In contrast, intraspecific variability in embolism resistance in *Q. petraea* was low within provenances and null between provenances. A positive correlation between  $P_{50}$  and vessel diameter among the six oak species indicates that the more embolism resistant species had narrower xylem vessels and a higher amount of hydraulic bridges between vessels. However, this tradeoff between hydraulic efficiency and safety was not observed between *Q. petraea* provenances.

### 1. Introduction

Climate change projections predict a significant effect on the growth and survival of forests (Reyer et al., 2014) but the impact on growth rate is species specific. While species in northern latitudes are expected to benefit from a warmer climate (Talhelm et al., 2014), species growing under temperate or more southern latitudes will be negatively affected (Reyer et al., 2014). With the predicted climate change scenario (IPCC, 2014), mean annual temperatures are expected to increase and patterns and frequency of rainfall may change considerably. This will probably result in more frequent summer drought events in most parts of Europe. Such drought events will have important implications for vegetation distribution and dynamics as seen from the evidence of drought-induced forest dieback in various parts of the world (Allen et al., 2010; Cailleret et al., 2017) including Europe (Bréda et al., 2006; Anderegg et al., 2016). Understanding the mechanisms leading to such mortality events as well as the capacity of the trees to cope with

drought is therefore crucial in predicting the ecological consequences of ongoing climate change.

For trees, drought survival relies on their ability to control the loss of water during an extreme event (McDowell et al., 2008). Drought induced dieback in forest trees is more likely due to xylem hydraulic failure (Anderegg et al., 2013, 2016) caused by the formation of air bubbles (embolism) in the xylem conduits rather than carbon starvation (Adams et al., 2017) even if we cannot totally exclude this hypothesis (Hartmann 2015). Xylem embolism disrupts the water transport from the roots to the leaves (Tyree & Zimmermann, 2002) and lead to organ desiccation and plant death (Urli et al. 2013). Vulnerability to embolism ( $P_{50}$ , water potential at which 50% of hydraulic conductivity is lost) and the hydraulic safety margin (difference between  $P_{50}$  and minimum xylem water potential under natural conditions) are key physiological traits linked to tree mortality under severe drought (Brodribb and Cochard, 2009; Brodribb et al., 2010; Barigah et al., 2013; Meinzer et al., 2009; Torres-Ruiz et al., 2017a; Urli et al., 2013).

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A recent survey on 226 tree species across the world found that more than 30% of the species had narrow hydraulic safety margins, making them susceptible to drought (Choat et al., 2012). Vulnerability curves evaluate the loss of xylem conductance as the xylem pressure decreases thereby providing a valuable method to assess the drought resistance (Cochard et al., 2013). This allows the estimation of hydraulic traits such as  $P_{50}$  which is commonly used to characterize drought tolerance at local and global scales as well as across species (Maherali et al., 2004; Trueba et al., 2017).

Between-species variation in resistance to embolism was earlier reported in conifers (Bouche et al., 2014; Delzon et al., 2010) and angiosperms (Torres-Ruiz et al., 2017b). It is also reported that within-species variation can be considerably less than the between-species variation (David-Schwartz et al., 2016; Lamy et al., 2014). Intraspecific variability in resistance to embolism in conifers can be of lower magnitude than intraspecific variability in angiosperms (Anderegg, 2015) although such studies are very limited in number. At the intraspecific level, variation in resistance to embolism is largely attributed to environmental factors rather than genetics as observed in several species such as *Fagus sylvatica* (Schuldt et al., 2016; Aranda et al., 2015) and *Pinus pinaster* (Lamy et al. 2014). The issue is complicated in the long-vesselled angiosperm species, because the commonly used methods to construct vulnerability curves can lead to biased results (Torres-Ruiz et al. 2014, 2017a; Cochard et al. 2013; Ennajeh et al. 2011). This is the case for ring porous species such as oak for which maximum vessel length above 70 cm has been typically reported (Cochard and Tyree 1990; Jacobsen et al. 2007). The vulnerability to embolism may have been overestimated in previous studies due to both the so called “open-vessel” (Cochard et al. 2013; Torres-Ruiz et al. 2014, 2017a) and “cutting” (Wheeler et al., 2013; Torres-Ruiz et al., 2015) artefacts. The overestimations are especially evident by using centrifuge techniques as the flow centrifuge (Cavitron) in which the samples are spun to gradually expose them to decreasing xylem pressures while monitoring the loss of hydraulic conductivity (Cochard et al., 2005). These artefacts have therefore questioned the reliability of previous results reported on resistance to embolism of long-vesselled species, complicating the proper evaluation of their function.

The genus *Quercus* comprises approx. 415 reported species across America, Asia, North Africa and Europe (Oh and Manos, 2008; Hubert et al., 2014). In Europe, *Quercus petraea* and *Q. robur* are the two widely distributed species extending from Spain to Scandinavia (Ducousso and Bordacs, 2004). Their pan European distributions suggest their high ability to adapt to very diverse growing conditions and, therefore their suitability for future climates (Epron & Dreyer, 1993; Arend et al., 2011; Eaton et al., 2016). The evergreen oak species, *Q. ilex* and *Q. suber* are two of the most common species of the Mediterranean forests and they have demonstrated capacities to withstand drought (David et al., 2007). While drought-induced mortality events are reported for *Q. robur* (Urli et al., 2014) and *Q. petraea* (Cochard et al., 1992), other *Quercus* species have shown a high tolerance to drought (Baquedano & Castillo, 2007) including capacity to recover their canopy after a drought event (Lloret et al., 2004). Such different species responses to drought could be due to inherent differences in morphological and physiological traits of adaptive significance and/or phenotypic plasticity. Although there are many studies within the genus *Quercus* reporting difference in functional traits between oak species under drought (Chiatante et al. 2015; Peguero-Pina et al. 2014), an accurate quantification of the interspecific variation in resistance to embolism within the genus is still lacking. This scientific gap is mainly due to the difficulty in measuring long vesselled species while avoiding the open vessel artefact described above. In a recent review, Robert et al. (2017) reported that the average  $P_{50}$  value of oaks highly differ whether all vulnerability curves or s-shaped curves only are considered. This result confirms the urgent need to better characterize hydraulic traits in oaks. Moreover, experimental evidence of the adaptive significance of the resistance to embolism is also absent in oaks. Therefore, an evaluation

of the genetic variation in resistance to embolism is crucial in order to anticipate the response of major European *Quercus* species to climate change.

The aim of the present study was to assess inter- and intraspecific variation in xylem vulnerability to embolism between six oak species (*Quercus* spp.) widely distributed in Europe. Vulnerability to embolism was assessed by using a newly developed prototype of Cavitron equipped with a 1 m-diameter rotor (Charrier et al. 2018). This reduces the amount of cut-open vessel in the xylem samples and, therefore, prevents artefactual losses in hydraulic conductance by avoiding the ‘open-vessel’ artefact. Secondly we investigated the amount of genetic differentiation among provenances in resistance to embolism, by sampling four *Q. petraea* provenances growing in a common garden and originating from temperate and Mediterranean latitudes in Europe. Results are expected to provide relevant information not only about the ability of the different *Quercus* species to withstand the adverse effects of drought events, but also show its capacity to adapt to the new climate conditions imposed by the human-induced climate change.

## 2. Materials and methods

### 2.1. Interspecific variation in xylem vulnerability to embolism for six oak species

Vulnerability to embolism was evaluated in six oak species: four were native European species (*Quercus petraea*, *Q. ilex*, *Q. robur* and *Q. suber*) and two were exotic species introduced from North America (*Q. palustris* and *Q. rubra*). Their native distribution range is given in Fig. S1 in supplementary information. For each species, two branches were collected from 5 to 16 healthy mature trees growing in the same climatic conditions in Southern France (INRA and University of Bordeaux campus). All branches were 3–5 years old and were a minimum of two meters long ranging between 18 and 20 mm in diameter. They were collected in the early morning using a pole pruner from the sunny side of the crown. Sampling was made within a period of six weeks in summer 2015. Once collected, transpiration losses from the branches were prevented by removing the leaves immediately after sampling and wrapping them in moist paper to keep them wet during their transportation to the lab. Once in the lab, branches were stored at 3 °C until resistance to embolism was assessed (within three weeks of sampling).

### 2.2. Intraspecific variation in xylem vulnerability to embolism for *Quercus petraea*

For evaluating the genetic differentiation in vulnerability to embolism between *Quercus petraea* (sessile oak) provenances, we used a common garden experiment planted in 1986 and 1987 in the Forêt Domaniale de Sillégné (France) which contains in total 107 sessile oak provenances. The initial density of plantation was 1904 individuals per hectare (spacing 3 m × 1.75 m) with each provenance replicated from ten to fifteen times having 24 trees per replicate. Four provenances selected for the present study; Grésigne (Southeastern France), Killarney (Southern Ireland), Vachères (Southwestern France) and Göhrde (Northern Germany) (Fig. 1, Table 1) represent different climatic regions, ranging from dry Mediterranean region in France to continental temperate climate in Germany. For each provenance, the aridity index (AI) was calculated as;

$$AI = MAP/MAE$$

where MAP = Mean Annual Precipitation and MAE = Mean Annual Potential Evapotranspiration.

We collected 12–15 trees per provenance in the common garden experiment. Trees of each provenance were sampled in five replications. Trees of the common garden were on average 10 m tall, and 2 m long branches were cut from upper part of the tree. Branches were wrapped in wet paper before being immediately transported to the lab

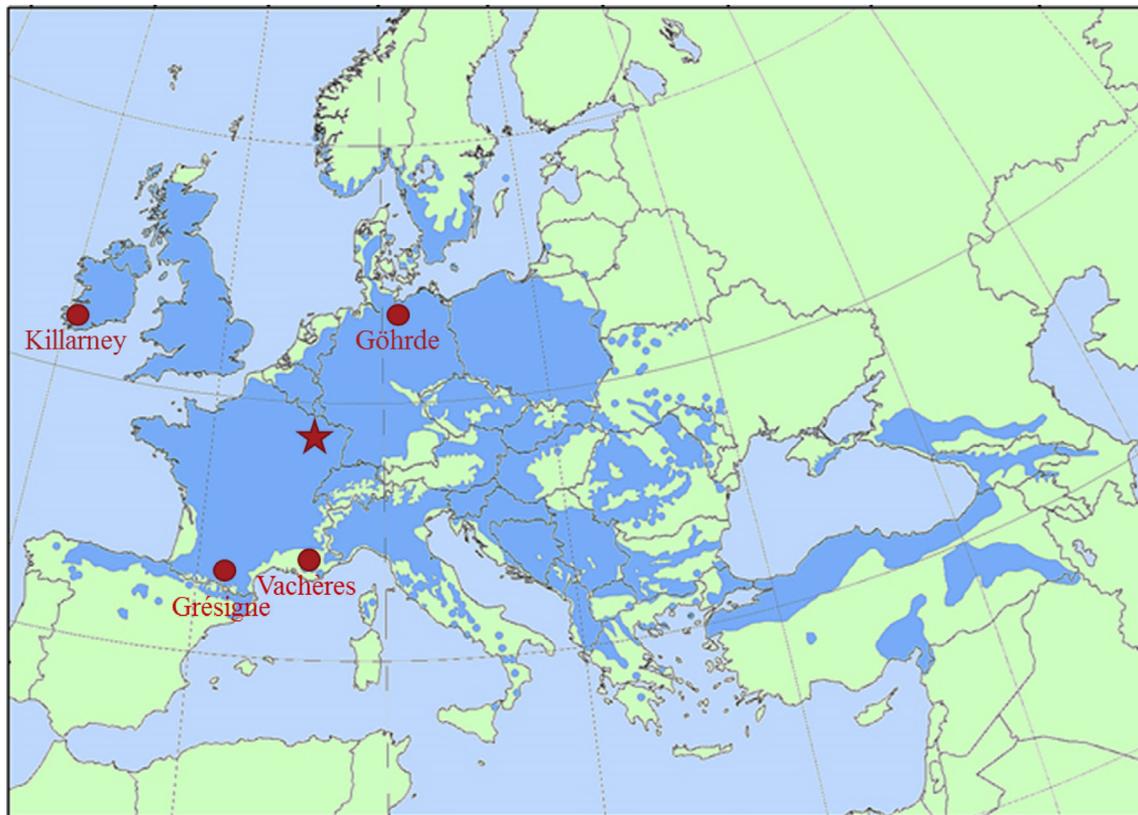


Fig. 1. *Q. petraea* provenances (full circles) planted in the common garden experiment located in the core of the species distribution range (full star) (Map source: EUFORGEN).

and then processed as branches collected for the species comparison.

### 2.3. Vulnerability curves

Vulnerability to drought-induced embolism was determined at the Caviplace (University of Bordeaux, Talence, France; <http://sylvain-delzon.com/caviplace>) with the Cavitrone technique (Cochard 2002; Cochard et al., 2005). To prevent artefactual losses in hydraulic conductance due to the presence of open vessels in the samples (Pivovarov et al., 2016; Torres-Ruiz et al. 2017a), the 2-m long branches were recut under water to a 1 m-long length after pressure relaxation, debarked at both ends and then installed in a large cavitrone equipped with a 1 m-diameter custom-built honeycomb rotor (DGMeca, Gradignan, France). Several samples per species were used to test the presence of open vessels by air injection at 2 bars and none of the studied species presented open vessels in 1 m long branches. Samples were not flushed in order to generate vulnerability curves from functional vessels only and to avoid possible effects of cavitation fatigue due to a stretching or degradation of the pit membranes during previous embolism events. Samples were spun for two minutes at a given speed to decrease the xylem pressure progressively at its center from  $-0.8$  MPa to  $-10.5$  MPa (those pressures correspond to centrifugation rotation from 764 rpm to 2768 rpm respectively) while measuring the sample

variation in hydraulic conductance using an ionic solution of 10 mM KCl and 1 mM CaCl<sub>2</sub>. Samples were three to five years old with a diameter varying from 10.3 to 12.2 mm. As this technique enables measuring the hydraulic conductance of the samples under negative pressure, the vulnerability curves were generated by plotting the percentage loss of hydraulic conductivity (PLC) against the different target pressures applied. This protocol has been validated on long vesselled species belonging to the *Vitis* genus as described in Charrier et al. (2018). For each branch, the relationship between PLC and the xylem pressure induced by centrifugation was fitted with the following sigmoidal equation (e.g., Pammenter and Vander Willigen 1998):

$$PLC = \frac{100}{1 + \exp\left(\frac{S}{25} * (P - P_{50})\right)}$$

where  $P_{50}$  (MPa) is the xylem pressure inducing a 50% loss of conductivity and  $S$  (% MPa<sup>-1</sup>) is the slope of the vulnerability curve at the inflexion point. Mean values of embolism vulnerability parameters ( $P_{50}$  and  $S$ ) correspond to the average values of 5–16 samples per species. The  $P_{50}$  value shows the ability of the branch to maintain its conductance at negative pressures and is commonly used as a proxy for the tree drought resistance. The lower the  $P_{50}$  value, the more drought tolerant the species or the provenance (Choat et al., 2012; Delzon

Table 1  
Climatic data, location and elevation of the studied *Q. petraea* provenances.

Provenance	Country	Latitude	Longitude	Altitude (m)	MAT	MAP	MAE	AI
Grésigne	France	44.04	1.75	310	11.9	806	995	0.81
Killarney	Ireland	52.01	-9.50	50	10.1	1374	657	2.09
Vachères	France	43.98	5.63	650	10.7	779	847	0.92
Gohrde	Germany	53.10	10.85	85	8.4	629	699	0.90

MAT = mean annual temperature (°C), MAP = Mean Annual Precipitation (mm), MAE = Mean Annual Potential Evapotranspiration, AI = aridity index.

2015).

### 2.4. Anatomical traits

For each oak species and *Q. petraea* provenance, 30 μm–50 μm sections were cut from the center of 5–10 of the branches used for vulnerability curve measurements using a sliding microtome (GLS1-microtome, Schenkung Dapples, Switzerland). After sectioning, they were bleached, rinsed with milliQ water and stained with a 1:2 mixture of safranin (0.5% in 50% ethanol, Safranin - Fisher scientific - General purpose grade) and alcian blue (1% in water, Alcian Blue 8GX - Alfa Aesar). After this, sections were washed with milliQ water and five times with 50, 70 and 96% ethanol. Slides of the sections were mounted with Euparal (Euparal 100 g - ROTH) and left to fix at room temperature. After one or two days, slides were placed in the oven at 60 °C and the best ones were selected to be scanned. The entire transverse section of each sample was digitized at 20X magnification using a desktop single slide scanner (Nanozoomer 2.0 HT, Hamamatsu; see Fig. S6 of supplementary material). The pictures from the scanner were later processed and analyzed ring by ring to quantify several anatomical traits such as vessel diameter in μm (*d*), vessel density in n mm<sup>-2</sup> (*VD*), hydraulically weighted vessel diameter in μm (*d<sub>h</sub>*, that is the sum of all conduit diameters (Σ *d*<sup>3</sup>) divided by the total number of conduits (Σ *d*<sup>2</sup>; Sperry et al., 1994) and relative theoretical conductivity in kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup> (*K<sub>s</sub>*). *K<sub>s</sub>* (sensu Tyree and Ewers 1991) was calculated by adding the conductivities of the conduits found in the cross-section, using the Hagen–Poiseuille equation to calculate the conductivity of every single conduit:

$$K_s = \pi r^4 / 8\eta$$

where *r* is the internal radius of the conduit and  $\eta$  the dynamic viscosity of water taken as 10<sup>-9</sup> MPa s at 20 °C. The average of each trait was calculated for the whole cross section (*d*, *d<sub>h</sub>*, *VD*, *K<sub>s</sub>*) and for the last ring (*d<sub>ring</sub>*, *d<sub>h,ring</sub>*, *VD<sub>ring</sub>*, *K<sub>s,ring</sub>*).

Finally, the mean amount of cells between two vessels were estimated (Hydraulic bridge; Cai et al., 2014). This was done by randomly selecting vessel couples in the last year ring and counting the number of cells in between two vessels.

### 2.5. Statistical analysis

Before the analyses, we confirmed the normal distribution of values for all variables measured (Shapiro-Wilk test;  $\alpha = 0.05$ ). To compare vulnerability to embolism between oak species and provenances of *Q. petraea*, we used one-way analyses of variance with post hoc Tukey's honest significant difference using 95% confidence intervals to compare hydraulic traits values (*P*<sub>50</sub>, *P*<sub>12</sub>, *P*<sub>88</sub>, *S*) across species and provenances. Coefficients of variations (CV<sub>intra</sub>) were estimated within each species and provenance. For *Q. petraea*, the between-provenance variability (CV<sub>inter</sub>) was calculated from the between-provenance standard deviation and the overall mean value. Pearson's correlation analysis was used to test for relationships between hydraulic and anatomical traits between and within species at the individual level. All analyses were performed in SAS 9.4 (SAS Institute, Cary NC).

## 3. Results

### 3.1. Interspecific variation among oak species

For each species, vulnerability curves showed similar shapes (Figs. S2, 2) with the air-entry (*P*<sub>12</sub>) ranging from -4.93 in *Q. ilex* to -2.14 in *Q. rubra* (Table 2). Values of *P*<sub>50</sub> significantly varied between species (F = 39.12; P < 0.0001), ranging from -7.13 MPa in *Q. ilex* to about -4.5 MPa as recorded in *Q. petraea*, *Q. robur* and *Q. rubra* (Table 2). Similar significant variation in *P*<sub>12</sub> (F = 7.94; P < 0.0001) was observed across species with *Q. ilex* and *Q. rubra* at the extremes of the

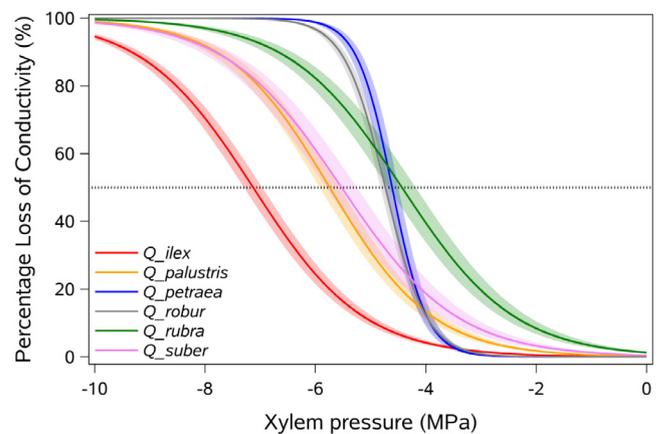


Fig. 2. Mean vulnerability curves for each of the six studied oak species showing percentage loss of hydraulic conductance in xylem (%) as a function of xylem pressure (MPa). Shaded bands represent standard errors, and 50% loss in conductance is indicated by a horizontal dotted line.

Table 2

Xylem embolism vulnerability parameters of the six studied oak species. Different letters indicate significant differences between species, (p-value in parenthesis).

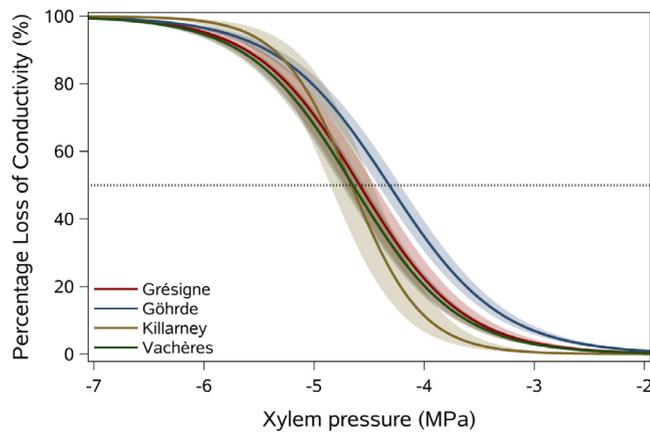
Species	n	<i>P</i> <sub>50</sub>	<i>P</i> <sub>12</sub>	<i>P</i> <sub>88</sub>	<i>S</i>	CV
<i>Quercus ilex</i>	10	-7.13 (0.16) c	-4.93 (0.29) c	-9.33 (0.23) d	24.98(2.24) a	7.27
<i>Quercus palustris</i>	5	-5.74 (0.18) b	-3.53 (0.47) b	-7.95 (0.33) c	26.87(6.41) a	6.97
<i>Quercus petraea</i>	16	-4.61 (0.11) a	-3.55 (0.19) b	-5.67 (0.17) a	79.54(23.31) a	9.78
<i>Quercus robur</i>	8	-4.74 (0.09) a	-3.81 (0.20) b	-5.66 (0.09) a	67.07(14.09) a	5.17
<i>Quercus rubra</i>	6	-4.43 (0.25) a	-2.14 (0.48) a	-6.72 (0.29) b	24.52(4.08) a	13.72
<i>Quercus suber</i>	7	-5.52 (0.28) b	-3.12 (0.42) b	-7.93 (0.44) c	24.00(4.07) a	13.25

n = number of samples; *P*<sub>50</sub>, *P*<sub>12</sub> and *P*<sub>88</sub> are water potential values at 50%, 12% and 88% loss of conductivity respectively; *S* = slope of vulnerability curve; CV = coefficient of variation in *P*<sub>50</sub> (%).

range. For *P*<sub>88</sub> (F = 40.50; P < 0.0001), the extreme values were found for *Q. ilex* and *Q. robur*. The vulnerability curve slope, *S* (F = 2.15; P < 0.07) was not significantly different among the species. The species with lowest *P*<sub>50</sub> and hence more resistant to xylem embolism were the two Mediterranean species *Q. ilex* and *Q. suber* and the North American species *Q. palustris* whereas the more vulnerable species were *Q. rubra*, *Q. petraea* and *Q. robur* (Fig. 2). The coefficient of variation in *P*<sub>50</sub> (CV) is low and varied from 5.17% in *Q. robur* to 13.71% in *Q. rubra* (Table 2).

### 3.2. Intraspecific variation in *Q. Petraea*

In *Q. petraea*, all provenances exhibited sigmoidal vulnerability curves (Fig. 3) and no significant differences were found for any embolism resistance trait between the four provenances (Fig. 3; Table 3, Fig. S3). The maximum difference in *P*<sub>50</sub> was only 0.34 MPa (Table 3) and was not significantly different between the provenances (F = 1.53; P = 0.22). Similarly, the maximum differences between provenances were only 0.62 and 0.26 MPa, for *P*<sub>12</sub> and *P*<sub>88</sub>, respectively (Table 3) and no significant differences were found for those traits between provenances. The vulnerability curve slope, *S* (F = 1.90; P = 0.14) was also not significantly different between the provenances (Table 3). The *P*<sub>50</sub> values measured for a given provenance showed a relatively moderate variability with average CV<sub>intra</sub> for provenances of 10.5% (Table 3). However this within-provenance variability was much larger



**Fig. 3.** Mean vulnerability curves for each of the four studied sessile oak provenances showing percentage loss of hydraulic conductance in xylem (%) as a function of xylem pressure (MPa). Shaded bands represent standard errors, and 50% loss in conductance is indicated by a horizontal dotted line.

than the inter provenance variability ( $CV_{inter} = 3.5\%$ ).

### 3.3. Correlation between $P_{50}$ and xylem anatomical traits

Significant differences were found between species for  $d_{ring}$  ( $F = 3.84$ ;  $P = 0.009$ ),  $d_h$  ( $F = 4.52$ ;  $P = 0.004$ ),  $d_{h,ring}$  ( $F = 9.61$ ;  $P < 0.0001$ ),  $K_s$  ( $F = 2.57$ ;  $P = 0.05$ ) and  $K_{s,ring}$  ( $F = 4.36$ ;  $P = 0.004$ ) indicating that Mediterranean species (*Q. ilex* and *Q. suber*) have smaller vessels than the others (Table S4 in supplementary information). The other analyzed anatomical traits did not show any significant differences between species. Among the six oak species,  $P_{50}$  was positively correlated with  $d_{ring}$ ,  $d_{h,ring}$ ,  $d_h$  and  $K_{s,ring}$  (Table 4, Fig. 4). These correlations were calculated at the individual tree level over all species, but the distribution of the species mean values on the regression diagram (Fig. 4) indicates that the correlation is mostly driven by the species effect: the more resistant to embolism the species, the narrower the vessels. There were no significant correlations between hydraulic traits and vessel density ( $VD$  and  $VD_{ring}$ ).

No significant differences of anatomical traits were observed between provenances within *Q. petraea* except for  $VD$  ( $F = 4.20$ ;  $P = 0.01$ ), (Table S5 in supplementary information). However,  $P_{50}$  was negatively correlated with  $d$ ,  $d_h$ ,  $d_{h,ring}$  as well as  $K_s$  (Table 4, Fig. 4): the more resistant to embolism the individual, the larger the vessels. As there were no significant differences between provenances, we computed correlation among traits at the within provenance level (individual tree level) by merging the data of all provenances (Fig. 4). Here the correlations are mostly driven by the individual tree effect, rather than by the provenance effect (Fig. 4). There were no significant correlations between hydraulic traits and vessel density ( $VD$  and  $VD_{ring}$ ). Quite strikingly regression coefficients between anatomical and hydraulic traits were of opposite signs at the species and provenance level (Table 4).

The mean number of fiber cells between two adjacent vessels was 13

**Table 3**

Xylem embolism vulnerability parameters of the four sessile oak provenances. Different letters indicate significant differences between species, (p-value in parenthesis).

Species	n	$P_{50}$	$P_{12}$	$P_{88}$	S	$CV_{intra}$
Grésigne	12	-4.58(0.14)a	-3.42(0.19)a	-5.74(0.20)a	53.05(7.96)a	10.28
Killarney	13	-4.65(0.19)a	-3.72(0.14)a	-5.59(0.31)a	77.70(15.91)a	14.78
Vachères	14	-4.64(0.10)a	-3.51(0.20)a	-5.78(0.14)a	52.95(5.80)a	8.15
Göhrde	15	-4.31(0.10)a	-3.10(0.20)a	-5.52(0.14)a	49.44(5.18)a	9.00

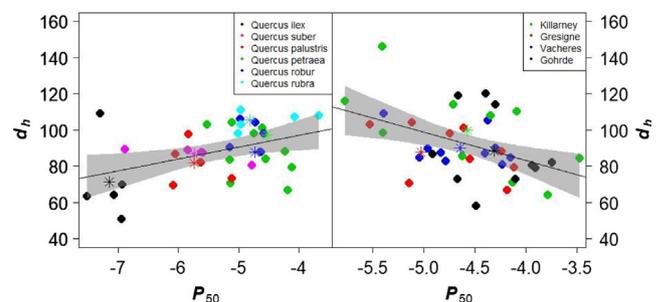
n = number of samples;  $P_{50}$ ,  $P_{12}$  and  $P_{88}$  are water potential at 50%, 12% and 88% loss of conductivity respectively; S = slope of vulnerability curve;  $CV_{intra}$  = coefficient of variation in  $P_{50}$  within provenance (%).

**Table 4**

Pearson correlation coefficient (r) between  $P_{50}$  and various anatomical traits.

Trait	Anatomical traits	r	P-value
<i>Among the 6 oak species</i>			
$P_{50}$	$VD_{ring}$	0.03	0.88
	VD	0.17	0.33
	$d_{ring}$	0.47	0.004
	d	0.31	0.07
	$d_{h,ring}$	0.63	< 0.0001
	$d_h$	0.42	0.01
	$K_{s,ring}$	0.42	0.01
<i>Within Q. petraea</i>	$VD_{ring}$	-0.05	0.78
	VD	-0.01	0.95
	$d_{ring}$	-0.30	0.06
	d	-0.41	0.01
	$d_{h,ring}$	-0.35	0.03
	$d_h$	-0.45	0.004
	$K_{s,ring}$	-0.29	0.08
$K_s$	-0.46	0.004	

$VD_{ring}$  = vessel density of the last ring ( $n\ mm^{-2}$ ),  $VD$  = vessel density ( $n\ mm^{-2}$ ),  $d_{ring}$  = vessel diameter of the last ring ( $\mu m$ ),  $d$  = vessel diameter ( $\mu m$ ),  $d_h$  = hydraulically weighted vessel diameter ( $\mu m$ ),  $d_{h,ring}$  = hydraulically weighted vessel diameter of the last ring ( $\mu m$ ),  $K_s$  = relative theoretical conductivity ( $kg\ m^{-1}\ MPa^{-1}\ s^{-1}$ ),  $K_{s,ring}$  = relative theoretical conductivity of the last ring ( $kg\ m^{-1}\ MPa^{-1}\ s^{-1}$ ),  $P_{50}$  = water potential at 50% loss of conductivity (MPa).



**Fig. 4.** Weighted regression between  $P_{50}$  and  $d_h$  (hydraulically weighted vessel diameter in  $\mu m$ ) between the 6 oak species (left) and between the four provenances within *Q. petraea* (right), mean values per species/provenance is indicated by stars,  $d_h$  = hydraulically weighted vessel diameter ( $\mu m$ );  $P_{50}$  = water potential at 50% loss of conductivity (MPa).

for *Q. ilex*, 11 each for *Q. suber* and *Q. palustris*, 7 for *Q. rubra* and 6 each for *Q. petraea* and *Q. robur*. A significant correlation was found between  $P_{50}$  and the mean number of cells between adjacent vessels between the six studied species (Fig. 5) i.e. those species with lower  $P_{50}$  values also possessed on average more number of cells between two xylem vessels.

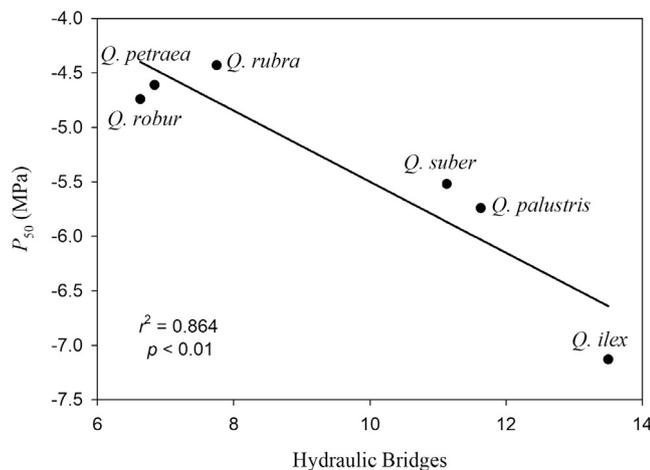


Fig. 5. Correlation between  $P_{50}$  (pressure inducing 50% loss of hydraulic conductivity) and hydraulic bridges (the mean number of cells between two adjacent xylem vessels) among the six studied oak species.

## 4. Discussion

### 4.1. Oak species show large interspecific variation in embolism resistance

Large variation in resistance to embolism was found between the six oak species studied, with a higher resistance in those occurring in the Mediterranean biome, such as *Q. ilex*, than those occurring in temperate cooler climates. Previous studies have shown that resistance to xylem embolism is a key trait allowing trees to survive in an extreme water scarcity environment (Urli et al., 2013; Brodrribb and Cochard 2009; Anderegg, 2015). Such variation between species was earlier observed among angiosperms where embolism resistance was negatively correlated with mean annual precipitation of the species distribution range (Maherali et al., 2004; Choat et al., 2012). In addition, Mediterranean species are well-known for being more resistant to embolism than temperate species (Urli et al., 2014). Indeed, we found the temperate species *Q. rubra* as being the most vulnerable to embolism, even more vulnerable than its European counterparts as previously reported by Cochard et al. (1992). A similar study by Cochard et al. (1992) compared three native European oak species. According to their results, *Q. robur* was more vulnerable to embolism ( $P_{50}$  of  $-2.5$  MPa) than *Q. petraea* and *Q. pubescens* ( $P_{50}$  of  $-3.3$  MPa for both species). Those values are substantially less negative from those obtained in this study but they still show a similar trend among the species. Indeed large discrepancies in  $P_{50}$  values for a given species have been previously reported mainly due to use of different methods for determining their vulnerability to embolism (see Cochard et al. 2013). For instance, Martin-StPaul et al. (2014) showed that the  $P_{50}$  values in *Q. ilex* varied anywhere between  $-2$  and  $-8.2$  MPa due to the “open-vessel artefact” involved in the traditional methods for obtaining vulnerability curves to embolism. Also, Venturas et al. 2016 showed very different results in *Q. robur* with a  $P_{50}$  of  $-1.4$  MPa when vulnerability curves were measured using single vessel air injection technique. To avoid any artefactual results due to presence of open vessel in the samples, a large rotor (1 m in diameter) was used in this study to determine the resistance to embolism for the six *Quercus* species. Thus, we obtained S-shaped curves for each species, and *Quercus robur* showed a similar  $P_{50}$  value ( $-4.7$  MPa) as reported by Choat et al. (2015) using the reference microCT technique ( $-4.2$  MPa). This suggests that, when using large diameter rotor (1m), the centrifuge technique reports accurate  $P_{50}$  values for long-vesseled species as *Q. robur* while standard rotors (0.27 m in diameter or less) overestimate their vulnerability to embolism thus supporting open vessel artefact hypothesis (Choat et al., 2015; Cochard et al., 2010; Martin-StPaul et al., 2014; McElrone et al., 2012; Torres-

Ruiz et al., 2014, 2017). Caution is still advised when applying this technique to species with vessel lengths that exceed the diameter of the rotor used in centrifuge methods.

### 4.2. Lack of genetic differentiation between provenances in resistance to xylem embolism

Within *Q. petraea*, we found no significant genetic differentiation in resistance to embolism between provenances originating from different climatic regimes. Exploring the genetics behind plant hydraulics is still a developing area of research (Venturas et al., 2017) and very few studies have investigated the genetic variability in resistance to embolism in angiosperms. Lack of genetic differentiation in embolism resistance between provenances for  $P_{50}$  has been reported for conifer species (Corcuera et al. 2011; Lamy et al. 2011; Sáenz-Romero et al. 2013). Lamy et al. (2011) found a very narrow range in embolism resistance (0.2 MPa) for six *Pinus pinaster* populations grown in a provenance trial. Narrow ranges of xylem embolism resistance variability between populations of the same tree species suggest a highly canalized response driven by uniform selection of this trait (Lamy et al. 2011, 2014). Contrastingly, a recent study reported weak but significant genetic differentiation between *Pinus halepensis* provenances (David-Schwartz et al., 2016). It is worth noticing that, in conifers, the phenotypic plasticity is much larger than the genetic variation but remains low (Lamy et al. 2014). In angiosperms, previous studies on *Fagus sylvatica* provenance trials have also reported weak or no genetic differentiation between populations both in hydraulic as well as anatomical traits (Wortemann et al., 2011; Hajek et al., 2016). Likewise our *Q. petraea* provenances from different climates and with different evolutionary histories (Petit et al. 2002) shared similar embolism resistance value, suggesting an evolutionary process behind the lack of variability of this trait.

In our study, the range of within-provenance differentiation is much larger (Table 3) than the range of the between provenance variation ( $CV_{inter}$  3.5%). Interestingly, this moderate genetic differentiation within provenances was maintained even with seemingly no genetic differentiation between provenances. *Q. petraea* is a wind pollinated species and has previously been found to be predominantly outcrossing with random mating (Bacilieri et al. 1996). High levels of gene flow between populations across landscapes have also been reported in the species (Gerber et al. 2014). Sessile oak usually occurs in large populations and the absence of genetic differentiation between populations as seen from our results suggest that selection can happen within these populations for adaptive traits (Scotti et al. 2016). Selected genotypes can be maintained within these populations even if there is a high gene flow between these populations (Aitken and Bemmels, 2016; Lowe et al. 2015; Kremer et al. 2012). This has indeed been observed for phenological traits in *Quercus petraea* populations (Alberto et al. 2011, Firmat et al. 2017, Vitasse et al. 2009), in which the maintenance of a large within-population genetic variation was associated with a clinal population differentiation along an elevation gradient. By working on a common garden trial with provenances coming from contrasting climatic conditions, we were able to quantify genetic differentiation but not the environmental effects. A more elaborate sampling of branches from trees growing in more diverse growing conditions is required to draw more conclusions regarding the phenotypic plasticity of embolism resistance. The need for testing provenances from extreme sites is also recommended in future in order to test whether marginal populations have evolved more embolism resistant xylem.

### 4.3. Relationship between embolism resistance and xylem anatomical traits

The positive correlation observed between  $P_{50}$  and vessel diameter indicates that the more embolism resistant species possess narrower xylem vessels (Table 4, Fig. 4). This agrees with former studies in other

angiosperms showing greater vulnerabilities to embolism for those species with wider vessels (Hajek et al., 2014; Cai and Tyree, 2010; Hacke et al., 2006). This was not the case in all genera studied since, in contrast with our results; vessel density and length, rather than vessel diameter (Lens et al., 2010) played a role in embolism resistance in *Acer* species. Jacobsen et al. (2005) found that the effect of vessel density on embolism resistance is due to its role in modifying fiber properties in wood. A reverse pattern was observed in the genus *Cistus*, where species with narrow vessels were found to be more prone to xylem embolism in the Mediterranean region (Torres-Ruiz et al., 2017b). Gleason et al. (2016) tested this trade-off across many angiosperms and gymnosperm species and found a positive correlation between hydraulic conductivity and  $P_{50}$  and concluded that there is a weak tradeoff between hydraulic efficiency and safety among species. Unlike the interspecific pattern, we found an opposite trend at the intraspecific level with no significant difference between *Q. petraea* provenances in  $P_{50}$ . Regardless of the provenance, individual trees within a provenance having lower  $P_{50}$  tend to have larger vessel sizes. These contrasting trends between  $P_{50}$  and vessel diameter suggest that vulnerability to embolism depends on anatomical traits other than vessel size. In fact, Li et al. (2016) reported recently that the thickness of the pit membranes between two adjoining vessels plays a major role in plant resistance to embolism. To explain the contrasting trends when comparing species or provenances within a given species, it would be necessary to evaluate if changes in pit membrane thickness are related to the diameter of the xylem vessels and how this possible relationship changes within and among species. Indeed, the link between vessel size and vulnerability to embolism might be indirect in angiosperms such as oak, and the ease of air-seeding could be driven by the single and thinnest pit membrane of a vessel. The trend observed at the intraspecific level (individuals with large vessels are more embolism resistant) suggests that the chances of identifying ‘elite’ genotypes combining high performance for both traits are high. Also, we have found that the amount of fiber cells between adjacent vessels is higher in species having a more resistant xylem to embolism. This finding confirms the hypothesis on hydraulic functionality of fiber bridge by Cai et al. (2014) that suggests that fiber cells in between two vessels act as a barrier against embolism spreading among vessels. As these fiber bridges confer an extra safety against embolism propagation between adjacent vessels, species with a higher number of solitary and thinner vessels would show a higher resistance to embolism despite the thickness of the pit membranes, giving an alternative explanation to the mentioned link between vessel size and vulnerability to embolism. Further research in this direction is therefore required for drawing valid conclusions regarding the protective effect of hydraulic bridges against spreading of xylem embolism.

## 5. Conclusions

Our results in oaks are in agreement with earlier data obtained in other genera showing that vulnerability to embolism exhibits tremendous variation between species (Maherali et al. 2004, Delzon et al. 2010, Bouche et al. 2014, Torres-Ruiz et al., 2017b) but little intraspecific variations variation (Lamy et al., 2011, 2014). Assuming that the within-population component of variation is partly due to genetic effects, our results suggest that there is potential for divergent evolution between populations. As no population differentiation is actually observed, we may conclude that differentiation is so narrow that it could not be detected by existing technologies. We may also conclude that selection is constrained and canalized, or that uniform selection may have contributed to the evolutionary stasis observed in vulnerability to embolism. This interpretation raises new avenues of research along technological and biological pathways. An immediate concern is to evaluate the adaptive value of the hydraulic parameters derived from the cavitron technique ( $P_{50}$ ) under controlled conditions, or in nature by assessing their selection gradients.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2018.04.031>.

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