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ORIGINAL PAPER

Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P_{50}) under severe drought

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Abstract The objectives of the study were to assess the phenotypic variation in the vulnerability to water stress-induced cavitation (estimated by P_{50}, or the xylem water potential which causes a 50% loss of conductivity) and the trade-offs between P_{50} and related hydraulic traits, i.e., stem specific conductivity (K_s), slope of the vulnerability curve (*slope*), wood density and branch size. Variability was examined for six *Pinus pinaster* populations covering the latitudinal range of the species and plasticity was tested through two provenance-progeny trial sites (xeric/mesic). As expected, the overall values of P_{50}, K_s and branch size decreased in the xeric site. Variation in P_{50} and K_s among populations was mainly the result of phenotypic plasticity, while wood density was genetically controlled and not affected by the environment. Stress conditions in the xeric site promoted a convergence in P_{50} and K_s as a result of the high phenotypic plasticity of the populations from mesic origins. In the mesic site, the ranking of populations for cavitation resistance and hydraulic capacity was consistent with the geographic location of the seed source. Higher resistance to cavitation was related to lower K_s, branch size and *slope*, mainly at the population level, but also as a general trend across individuals. In a warmer and drier climate, there could be a potential selection of *Pinus pinaster* populations from mesic origins, which showed a great responsiveness and adjustment to drought conditions (similar or higher P_{50} than the populations from dry origins), in addition to a high wood density and growth.

Keywords Xylem embolism · Phenotypic plasticity · Genetic variation · Environmental interaction · Drought · *Pinus pinaster*

Introduction

Vulnerability to xylem embolism induced by water stress has been recognized as one major trait in plant response to drought (Vilagrosa et al. 2003). An increase in cavitation resistance with decreasing mean annual precipitation has been documented in several woody species (Maherali et al. 2004; Pita et al. 2003), suggesting that the maintenance of functional xylem conduits is necessary to survive severe droughts. Xylem structure plays a major role in cavitation resistance (Hacke et al. 2001). Higher resistance to embolism is associated with thicker vessel walls and smaller lumen diameters and, hence, to a higher construction cost, as wood density is a function of the proportion of cell wall in the wood volume (Pittermann et al. 2006). According to the Hagen-Poiseuille law (Zimmermann 1983), smaller vessel diameter and sapwood cross section lead to a lower hydraulic efficiency. On the contrary, the lower the water transport resistance, the higher is the water flux to the canopy under a given pressure gradient, and the greater the capacity for carbon uptake. However, the trade-off between cavitation resistance and xylem water transport is controversial (Hacke and Sperry 2001; Maherali et al. 2004) and depends on the scale of study: species,

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populations and individuals (Maherali et al. 2006; Martinez-Vilalta et al. 2004). In this respect, the compromise between safety and efficiency has been confirmed and refuted at the species (Froux et al. 2002; Martinez-Vilalta et al. 2002; Piñol and Sala 2000) and population level (Choat et al. 2007; Kavanagh et al. 1999; Sparks and Black 1999). At a clonal level, Cochard et al. (2007) found that genetic variation in vulnerability to cavitation was poorly correlated with wood anatomy, but highly related to biomass production, suggesting a trade-off between xylem safety and growth potential.

Along a major environmental gradient, populations within a species may adapt to xeric environments by a greater resistance to cavitation (Kavanagh et al. 1999). Several works have assessed the range of genotypic variation in P_{50} and other relevant hydraulic parameters, and their relationship with climate differences, in conifers such as *Pinus sylvestris* (Martinez-Vilalta et al. 2009), *Pinus halepensis* (Tognetti et al. 1997), *Pseudotsuga menziesii* (Anekonda et al. 2002; Dalla-Salda et al. 2009; Kavanagh et al. 1999), *Pinus ponderosa* (Bouffier et al. 2003; Maherali and DeLucia 2000; Maherali et al. 2002; Wang et al. 2003), *Cedrus libani* (Ladjal et al. 2005) and *Austrocedrus chilensis* (Gyenge et al. 2005). We chose *Pinus pinaster* Ait. due to its importance in the Mediterranean basin. It spreads from Morocco to the French Landes and grows in a large variety of soil types and precipitation amounts, coping with diverse water availabilities. The species has shown a high level of population and/or family differentiation and plasticity throughout its natural geographical distribution in morphological and physiological parameters related to drought responses like carbon isotopic composition (Aranda et al. 2010; Corcuera et al. 2010; Correia et al. 2008; Nguyen-Queyrens et al. 1998; Tognetti et al. 2000), gas exchange (Fernandez et al. 2006), osmotic adjustment (Nguyen and Lamant 1989) and water relations (Fernandez et al. 1999).

Extreme weather events, such as the drought and heat wave that occurred in Europe in 2003, are expected to increase in frequency and intensity (IPCC 2007). Population genetic differentiation and/or phenotypic plasticity in hydraulic traits could be key factors in the adaptation to the new climatic conditions. There is documented variation in vulnerability to cavitation at different genetic levels, mainly interspecific. Less is known about the intraspecific variation at the population, family or clone levels (Martinez-Vilalta et al. 2009). Nowadays, the new cavitron technique (Cochard et al. 2005) has meant a great advance in the rapid generation of vulnerability curves and allows its application to a large number of genotypes. The aim of the study was to assess *P. pinaster* variation in the vulnerability to xylem embolism (P_{50}) and related hydraulic parameters like xylem-specific conductivity (K_s), slope of the vulnerability curve (*slope*) and wood density at the population, family and individual level. We chose six populations from two provenance-progeny trials located in "contrasting climatic locations" and a subsample of 13 families from one of the populations in a single trial site. We hypothesized that vulnerability to cavitation could vary in response to the climate of the site of origin due to local adaptations, as well as in response to the experimental growing conditions (phenotypic plasticity). We also evaluated possible relationships between (1) P_{50} and wood density, (2) P_{50} and the slope of the vulnerability curve, and the trade-offs between (3) safety (estimated from P_{50}) and efficiency (estimated from K_s) and (4) safety and growth (estimated from branch size).

Materials and methods

Study sites and plant material

Data were taken from a nested structure of families within populations. The term "family" will refer to a group of individuals who have one or both parents in common (half-sib or full-sib families, respectively). "Population" denotes the group of individuals within which there is gene exchange, and "provenance" the geographic place of origin of the population. Natural populations have been subjected to selection from their particular set of local environmental conditions and may differ in performance when grown at a common site. The progeny trial is the best way to evaluate the genetic worth of the selected parents to determine the best provenances of the species. The plantings were established using seedlings grown in nurseries from open-pollinated seeds (individuals with one parent common and the other parent unknown), collected in natural stands of maritime pine. In the mesic site, 2-year old seedlings were planted in 2005 at a spacing of 3 × 2 m in a randomized complete block design with four replications of 71 blocks, 225 families and 4 plants per experimental unit (a total of 16 plants per family). In the xeric site, 1-year old seedlings were planted in 2004 at a spacing of 2 × 3 m in an α-lattice incomplete block design with three replications of 65 blocks, eight families by block and four plants by experimental unit. Both trials were designed in a nested structure of families within populations. In November 2008, we selected six populations covering the latitudinal range of *Pinus pinaster* (Table 1: *Pleucadec* and *Mimizan* (French Landes), *San Cipriano* (northwest Spain), *Arenas* (central Spain), *Oria* (south Spain) and *Tamrabta* (Morocco)). Populations were grown in two provenance-progeny trials located in (a) Parderrubias, NW Spain, at a low elevation, near the Atlantic ocean, exposed to wet and mild winters (hereinafter, mesic site) and (b) Calcena, NE

Spain, at an interior mid-high elevation, with a continental climate and colder and drier winters (hereinafter, xeric site). Climate and soil properties for the progeny trials and seed sources are presented in Table 1.

In the xeric site, where survival rates ranged between 23 and 36%, depending on the population, due to drought and poor soil conditions, we analyzed 9–11 individuals per population. From each individual we cut one branch. Therefore, we analyzed 9–11 branches per population. In the mesic site, survival rates were >95% and plant mortality was due to human errors. Higher water availability promoted growth of tall weeds and mechanical removing was necessary twice a year, which caused some deaths of the tested material. Higher availability of genetic material at the mesic site allowed us to consider the family structure within populations. In the mesic site, we tested three families per population and four individuals per family. Like in the xeric site, we took a branch per individual and, therefore, analyzed 4 branches per family and a total of 12 branches per population. In addition, we obtained the variation in P_{50} within individuals in the *Arenas* population from the mesic site and sampled 4 branches per individual (48 branches for this population). Measured trees were selected at random.

Both sites undergo summer drought. However, the De Martonne's aridity index, or the ratio between the mean annual values of precipitation (P) and temperature (T) plus 10°C, that is P/(T+10), is 20 (semiarid–Mediterranean) for the xeric site and 30 (sub-humid) for the mesic site. Moreover, in the mesic site, cumulated precipitation in winter and spring, higher air relative humidity and water holding capacity of the soil probably reduced summer drought stress. In the "sampling year", the xeric site was drier (395.4 vs. 461.2 mm) and warmer (12.9 vs. 12.3°C) than an average year. On the contrary, the mesic site experienced higher precipitation (854.4 vs. 722.0 mm) and lower temperatures (11.9 vs. 14.4°C) than the average.

Vulnerability curves

The percent loss of hydraulic conductivity (*PLC*) was measured on lateral branches with the cavitron technique (Cochard et al. 2005). In September 2008, branches were cut from the plant, wrapped in wet filter paper, enclosed in bags and sent to the laboratory in Clermont-Ferrand, France. From each sample, lateral branches and bark were removed and sample ends were cut under water with a razor blade so that each sample was 28-cm long. The sample was then inserted in two polycarbonate tubes filled with distilled water and 10 mmol KCl. The xylem segment and the tubes were placed on the rotor of the centrifuge.

Xylem pressure (P) was decreased in 0.5-MPa steps and maintained constant for several minutes till a steady-state flux was obtained. The hydraulic conductance (K) was computed in every step with a specific designed software. Due to the high resin content of the species, sap flux took a long time to stabilize in every pressure step. P higher than −2.0 MPa gave 0% *PLC*. Therefore, at the beginning of the curve, P was set at −2.0 MPa and the initial xylem conductance (K_o) was obtained. The procedure was completed on reaching a *PLC* of 90–99%. Logistic functions were fitted to the vulnerability curves (Pammenter and Van der Willigen 1998), and both, the pressure inducing a 50% loss of xylem conductance (P_{50}) and the slope of the linear part of the vulnerability curve (*slope*) were obtained. $PLC\,(\%) = 100/(1 + e\,(slope/25*(P - P_{50})))$.

We calculated P_{12}, an estimate of the xylem water potential at which embolism begins, and P_{88}, an approximation of the xylem water potential at full embolism (Sperry and Tyree 1988). The pressures at 12% (P_{12}) and 88% (P_{88}) loss of

Table 1 Location and description of the seed sources and progeny trials

Locality	*Arenas*	*Oria*	*Mimizan*	*San Cipriano*	*Pleucadec*	*Tamrabta*	Calcena (xeric site)	Parderrubias (mesic site)
Latitude (N)	40°02′	37°52′	44°08′	42°08′	47°47′	34°0′	41°37′	42°14′
Longitude (W)	5°08′	2°37′	1°18′	8°42′	2°20′	5°0′	1°44′	7°56′
Elevation (m)	1,359	1,232	37	310	80	1,600	1,017	460
P (mm)	1,257	348	995	661	855	763	461	722
T (°C)	13.8	12.5	13.8	15.1	11.6	17.2	12.3	14.4
Tmax (°C)	34.2	30	25	25.9	24.2	29.1	28.6	27.8
Tmin (°C)	0.28	3.0	3	1.9	2.2	1.2	1.1	1.5
Soil	Leached on sandstone	Sandstone	Sandy	Sandy loam	Sandy	Limestone	Siliceous, over slate	Sandy siliceous

P mean annual precipitation (mm); *T* mean annual temperature (°C); *Tmax* mean of maximal temperatures of the warmest month (°C); *Tmin* mean of minimum temperatures of the coldest month (°C) conductivity were obtained from $P_{12} = P_{50} + 50/slope$ and $P_{88} = P_{50} - 50/slope$, respectively.

Stem specific hydraulic conductance (K_s), or the hydraulic efficiency of the xylem in relation to wood's transverse section, was calculated as:

K_s = (cross-sectional area of the cuvette—cross-sectional area of the sample) × K × 0.28/18 (mmol m s^{-1} MPa^{-1}).

Wood density

Samples were dried at 60° in the oven for 3 days to obtain the dry weight. Afterward, they were saturated with water in a vacuum chamber and the saturated and submerged weights were measured.

Wood density at 23°C could then be calculated following the Archimedes' principle with a density determination kit coupled to a Sartorius balance, according to the following formula:

Wood density = dry weight/volume

Volume = (saturated weight−submerged weight)/water density at 23°C (0.9978 g cm^{-3}).

Statistical analysis

According to the experimental design and on the basis of the subsampling carried out, a set of mixed models was used for all variables. Normality and homocedasticity of data were checked successfully. Due to the influence of the branch size on the hydraulic parameters, the diameter of the twigs (d) was included as a covariate.

The general model established was:

$$y_{ijn} = \mu + d_n + s_i + p_j + s_i \times p_j + \varepsilon_{ijn}.$$

In the mesic site the following genetic hierarchical model was used

$$y_{jkn} = \mu + d_n + p_j + f_{k(j)} + \varepsilon_{jkn}$$

and in *Arenas* population for evaluating the variability at intrapopulation level:

$$y_{knl} = \mu + d_l + f_k + i_{n(k)} + \varepsilon_{knl}$$

where, y_{ijknl} is the value of the variable for the *lth* branch of the *nth* seedling from the *jth* population within the *kth* family and measured at the *ith* site; d is the value of the branch diameter; μ is the overall mean of the variable; p_j is the effect of the *jth* population (i = 1–6); f_k is the effect of the *kth* family (k = 1–3) within the *ith* population; s_i is the effect of the *ith* site (i = 1–2); i_n is the effect of the *kth* individual (n = 1–11); ε_{ijknl} is the residual (n = 1–11 or l = 1–3 when more than 1 branch is sampled).

The models were analyzed as mixed with fixed (population, site, family and individual when applicable) and random (individual and residual) effects, where the component of the variance was obtained by restricted likelihood (REML). The best linear unbiased estimators and predictors (BLUE and BLUP) for fixed and random factors, respectively, were obtained (SAS 1999).

Results

Values of xylem vulnerability to cavitation were in the range of values previously reported for *P. pinaster* (Martinez-Vilalta and Piñol 2002; Fig. 2a) and the vulnerability curves showed a typical sigmoid fit ($R^2 > 0.99$; Fig. 1).

We found significant genetic differences in the hydraulic traits among populations. In the xeric site, the *San Cipriano* and *Pleucadec* populations displayed higher wood density (data not shown) and branch size (Fig. 3b) and the *Arenas* population the highest resistance to cavitation (Fig. 2a). In the mesic site, the *Tamrabta* population presented the

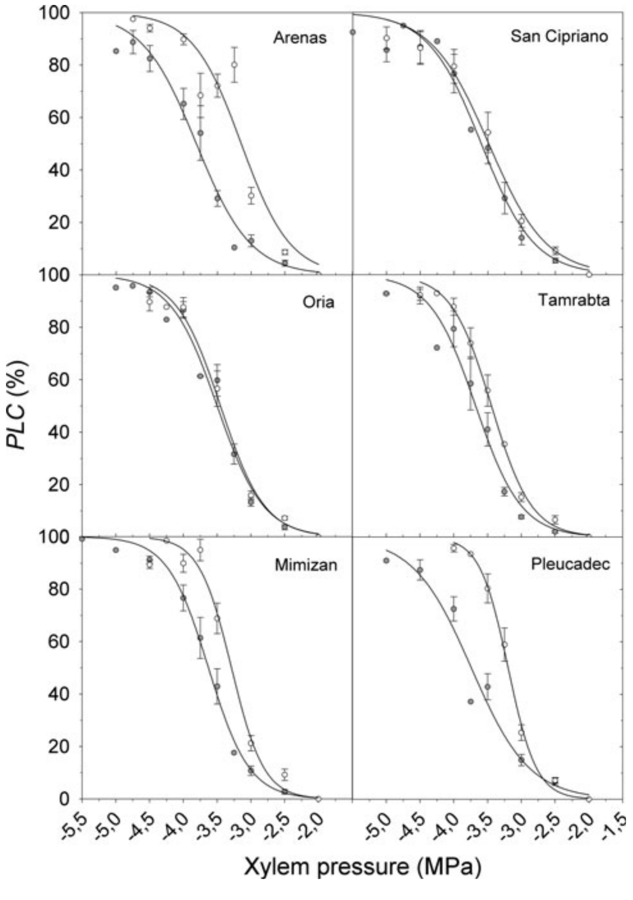

Fig. 1 Vulnerability curves expressed as percentage loss of conductivity (*PLC %*) versus xylem pressure for the six *Pinus pinaster* populations from the mesic (*open circles*) and xeric (*gray filled circles*) trial sites. *Vertical bars* are standard errors (mesic site: n = 12, except for Arenas where n = 48; xeric site: n = 11). *Lines* are sigmoid fits through the means

highest branch size (Fig. 3b), the *San Cipriano*, *Oria* and *Tamrabta* populations a higher cavitation resistance (Fig. 2a), and the *Mimizan* and *Pleucadec* populations (from mesic origins) the highest hydraulic efficiency (Fig. 2b).

The *site* (mesic/xeric) had a significant effect on P_{50} and K_s (Table 2). P_{50} and K_s yielded significantly lower values in the xeric site (Fig. 2b). Environmental conditions (*site*) and $G \times E$ interactions (*site* × *Pop*) absorbed the highest proportion of variance for P_{50} and K_s (22–24 and 12–13%, respectively, Table 4). The contrary was observed for wood density, with higher variation due to population (17%) than to environment (5%) or genotype by environment interaction ($G \times E$, 2%).

Differences among populations in wood density were significant, independently of the site, and displayed no interaction with the site (Table 2), which shows that differences in vulnerability to cavitation were not driven by changes in wood density. P_{50}, K_s and *slope* discriminated between populations only in the mesic site (Table 3), which was confirmed by the higher population variation in P_{50} (14%, Table 5), K_s (31%) and *slope* (10%) in the mesic than in the xeric site (8 and 7%, respectively, Table 5).

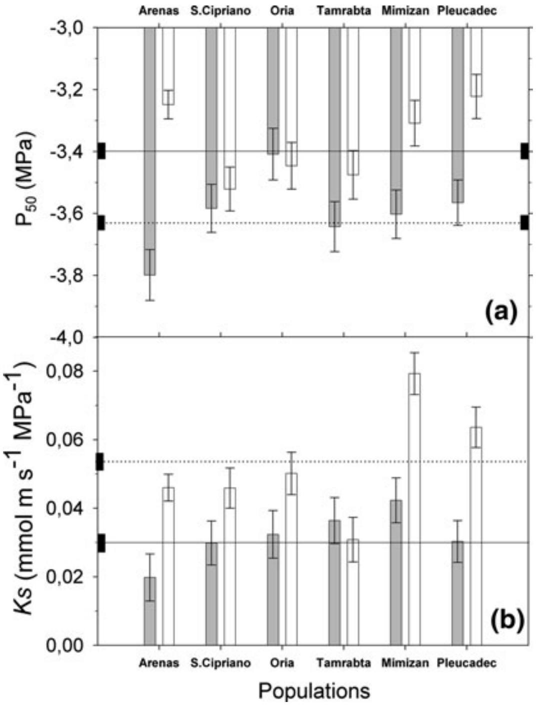

Fig. 2 Representation of the means and standard errors of P_{50} (**a**) and stem specific conductivity (K_s, mmol m s^{-1} MPa^{-1}, **b**) for the six *Pinus pinaster* populations in the mesic (*white bars*) and xeric (*gray bars*) trial sites (*n* like in Fig. 1). *Dash* and *dotted lines*: mean of the populations in the xeric and mesic sites, respectively, with standard errors represented as *boxes*

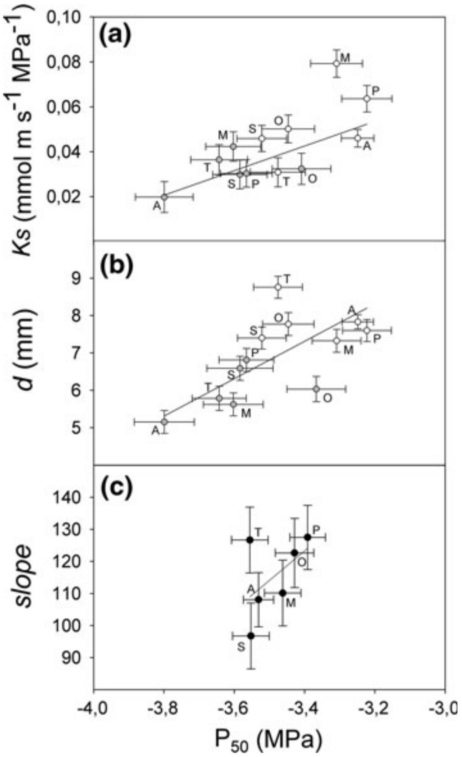

Fig. 3 Population means in the xeric (*gray filled circles*) and mesic (*open circles*) sites, population means from both sites (*black filled circles*) and standard errors. **a** Relationship between K_s and P_{50}. Line: linear regression fit through population means in both sites ($R^2 = 0.59$, $P = 0.0036$). **b** Relationship between P_{50} versus branch size. Line: sigmoid fit through population means in both sites ($R^2 = 0.55$, $P = 0.0057$). **c** Relationship between P_{50} and *slope*. Line: linear regression fit through population means ($R^2 = 0.27$, $P < 0.0081$)

Arenas, *Mimizan* and *Pleucadec* populations, from mesic origins, had higher resistance to cavitation (lower P_{50} values) in the xeric site (Fig. 2a), which was related to a lower K_s in the three populations (Fig. 2b) and to lower slopes in the *Arenas* and *Pleucadec* populations (data not shown). *San Cipriano*, *Oria* and *Tamrabta* populations showed no differences between sites in P_{50} and K_s. At the family level, differences in P_{50} and K_s were also significant (Table 3). Differences in P_{50} at the individual level were also presented (Table 3) and the 93% of the population variation in P_{50} was partitioned among individuals (Table 5). The high percentage of variation found among individuals, but not among the branches within individuals (5%, Table 5), indicates that this trait is stable for the whole plant.

In general, relationships among the variables (considering all values) were weaker than at the population level (data not shown). A higher resistance to cavitation was related to a lower slope of the vulnerability curve ($R^2 = 0.34$, $P < 0.0001$), lower stem specific conductivity ($R^2 = 0.19$, $P < 0.0001$) and lower branch size ($R^2 = 0.26$,

Table 2 Summary of ANOVA significances for the populations in the two sites (xeric/mesic)

Source of variation	Num DF	Den DF	P_{50}		K_s		Wood density		Slope	
			F value	Pr > *F*	*F* value	Pr > *F*	*F* value	Pr > *F*	*F* value	Pr > *F*
Branch size	1	137	0.39	0.5323	0.47	0.4958	3.86	**0.0516**	2.61	0.1082
Site	1	137	18.14	**<0.0001**	20.66	**<0.0001**	1.02	0.3146	0.19	0.6595
Pop	5	137	1.82	0.1125	6.31	**<0.0001**	6.99	**<0.0001**	1.21	0.3094
Pop x *site*	5	137	4.73	**0.0005**	3.10	**0.0110**	1.52	0.1864	3.33	**0.0072**

Bold numbers denote significant values, Pr > 0.05

P_{50} xylem pressure required to reduce hydraulic conductance by 50%; *slope* slope of the curve at the inflexion point; *Num DF* number of degrees of freedom; *Den DF* Denominator of degrees of freedom; *Pr* Probability; *F* F values; *d* branch diameter; *Pop* population

Table 3 Summary of ANOVA significances for the populations and families in the mesic site, families and individuals of *Arenas* in the mesic site and populations in the xeric site

Source of variation	Num DF	Den DF	P_{50}		K_s		*Slope*	
			F Value	Pr > *F*	*F* value	Pr > *F*	*F* value	Pr > *F*
Mesic site								
Branch size	1	69	0.27	0.6039	0.17	0.6774	3.64	0.0604
Pop	5	69	4.47	**0.0014**	6.90	**<0.0001**	2.57	**0.0345**
Fam (Pop)	12	69	1.85	**0.0565**	2.17	**0.0226**	1.00	0.4593
Arenas								
Branch size	1	17	12.85	**0.0023**	6.01	**0.0253**	0.48	0.4993
Fam	2	17	28.73	**<0.0001**	8.60	**0.0026**	3.47	**0.0544**
Ind (Fam)	6	17	60.31	**<0.0001**	1.52	0.2311	4.38	**0.0075**
Xeric site								
Branch size	1	55	1.44	0.2354	0.15	0.6979	0.11	0.7457
Pop	5	55	2.05	0.0862	1.77	0.1338	2.03	0.0891

Rest of abbreviations as in Table 2

Fam family, *Ind* individual

Table 4 Percentages of variance in the two sites (xeric/mesic)

Source of variation	P_{50} (%)	K_s (%)	Wood density (%)	Slope (%)
Branch size	1		1	2
Site (xeric/mesic)	24	22	5	
Pop		7	17	
Pop x *site*	12	13	2	9
Residual	63	58	76	89

Abbreviations as in Table 2. Empty boxes denote zero values

$P < 0.0001$). The among-population differences in vulnerability to embolism were associated with differences in xylem conductivity ($R^2 = 0.59$, $P = 0.0036$; Fig. 3a), branch size ($R^2 = 0.55$, $P = 0.0057$; Fig. 3b) and *slope* ($R^2 = 0.27$, $P < 0.0081$; Fig. 3c). Populations showing smaller size, lower specific hydraulic conductance and slope of the vulnerability curve had a greater resistance to xylem embolism. A higher wood density was related to a higher branch size at the population level ($R^2 = 0.40$, $P < 0.0001$; data not shown).

Discussion

Inter- and intra-population variation in hydraulic traits

Genetic variation appeared within sites. The most important source of variation in cavitation resistance and specific stem conductivity was “the site”, but significant differences appeared at the genetic level as well (*population* and *family*). In the mesic site, we found significant population and family variations, consistent and related to their

Table 5 Percentages of variance in the mesic and xeric site and for *Arenas* population in the mesic site

Source of variation	P_{50} (%)	K_s (%)	Wood density (%)	Slope (%)
Mesic site				
Branch size			15	5
Pop	14	31	3	10
Fam (Pop)	13	14		1
Residual	73	55	82	84
Xeric site				
Branch size	2			
Pop	8	7	27	7
Residual	90	93	73	93
Arenas				
Branch size	2	9		1
Fam		36		
Individual	93	8	23	54
Residual	5	47	77	45

Abbreviations as in Tables 2 and 3. Empty boxes denote zero values

original climate. Three populations from sub-humid climates (*Pleucadec, Mimizan, Arenas*) were the most vulnerable to xylem embolism and displayed higher K_s (excluding the *Arenas* population). On the contrary, no differences between populations appeared in P_{50} or K_s in the xeric site. The fact that population differences were only expressed in the mesic site indicate that P_{50} and K_s are complex traits influenced by the genotype and environment. Sometimes the environment allows the genetic expression of the character, while others phenotypic plasticity blurs ecotypic divergence in response to water stress (Baquedano et al. 2008).

We expected that populations from drought-prone environments had a higher cavitation resistance under drought conditions. However in the xeric site, populations that experienced high water availability in their origins had equal or even higher resistance to cavitation than the populations from arid environments. There was a convergence of lower K_s values in the xeric site as well. This is an advantage for the populations from mesic origins since a low K_s helps to reach lower P_{50} in very dry conditions. High K_s could also allow populations to maintain high leaf water potentials, but only when soil water availability is high. This is in accordance with Piñol and Sala (2000), who observed that evergreen conifers from drier habitats were not more resistant to xylem cavitation than codominant species from moister sites, when tested at the same location. Lack of intraspecific variation in this trait is frequent in common garden studies. Sometimes, the adjustment in the ratio of conducting organs to transpiring leaf area allows the maintenance of a constant water potential and a constant cavitation rate (Mencuccini and Comstock 1997). When populations were sampled at their original habitat, no differences in P_{50} were observed either. Martinez-Vilalta et al. (2009) found that vulnerability to xylem embolism of *P. sylvestris* populations across Europe was unrelated to drought of the seed source. The hydraulic adjustment to local climatic conditions occurred through modifications in leaf-to-sapwood area ratio and stomatal control.

Genetic differentiation in wood density among populations was not affected by any site effect. This was confirmed by the variance decomposition where most of the population variation in wood density was attributable to genotype rather than to environment. The stability of wood density with sites and its variability with populations show that differences in vulnerability to cavitation were not driven by changes in wood density. Wood density was not correlated with P_{50} in *Prunus* species either (Cochard et al. 2007, 2008). However, Martinez-Meier et al. (2008) observed a plastic response and sensitivity to the environmental stress by increasing mean and latewood density in *Pseudotsuga menziesii* trees surviving extreme droughts. *Pleucadec* and *San Cipriano* populations, from sub-humid climates, displayed higher wood density, which is in agreement with Bouffier et al. (2003), who found higher wood density in ponderosa pine populations from the wetter origins. However, higher wood density is usually related to drier sites (Martinez-Cabrera et al. 2009) as small tracheid diameter and/or thicker cell walls are more resistant to xylem tensions during drought. High wood density was not associated with low K_s at the population level. Hence, thicker cell walls and/or a superior latewood production could have influenced the higher wood density displayed by *Pleucadec* and *San Cipriano*.

Trade-offs

We found several trade-offs among the hydraulic traits when considering all the individuals within the species and when categorized by populations. This is in accordance with previous works where the relationships among hydraulic traits demonstrated that drought tolerance in *Pinaceae* species was the result of the combined effect of several inter-dependant traits. However, these relationships change with the scale of study or genetic level (species/populations/families; Martinez-Vilalta et al. 2004). As an example, in this study the relationship among wood density and branch size was only found at the population level and the relationships among the rest of the parameters were stronger among populations than among individuals.

We observed a trade-off between branch size and resistance to xylem cavitation. High and negative correlations between cavitation resistance and aboveground biomass production were also reported in poplar and willow clones (Cochard et al. 2007) and could be related to a higher construction cost of thicker walls and/or biomass allocation to roots. However, the trend of populations with bigger branches having higher density in our study contradicts the trade-off between carbon production costs and plant growth (Hacke et al. 2001).

As originally expected, individuals with the highest stem specific conductivity tended to be more vulnerable to cavitation, in accordance with the safety versus conducting efficiency (Martinez-Vilalta et al. 2002; Oliveras et al. 2003). This trade-off was displayed at the population level too and agrees with previous works (Sparks and Black 1999).

We found a relationship between the slope of the vulnerability curve and P_{50}. A vulnerability curve with a less steep slope should allow cavitation to occur gradually over a larger water potential range, resulting not only in less vulnerability but in a greater safety margin of cavitation. This was the case of the mesic populations in the xeric site. On the contrary, low absolute P_{50} values related to a higher *slope* were displayed by these populations in the mesic site.

Plasticity in hydraulic traits

We found plasticity in the vulnerability to xylem embolism. The site was a significant source of variation for cavitation resistance and stem specific conductivity. As expected, *P. pinaster* responded to low water availability in the xeric site by lowering P_{50} and K_s, which indicated an acclimatization of the species to dryness. However, we should take into account that variation in a specific trait may not directly reflect a process of adjustment by itself, but be the consequence of adjustments in other related traits. For instance, a low K_s could be the consequence of smaller tracheids due to reduced growth during drought. Populations original from locations with high water availability (*Arenas*, *Mimizan* and *Pleucadec*), which showed more vulnerability to cavitation in the mesic site, presented the highest plasticity to drought (largest differences in cavitation resistance and hydraulic conductivity between the mesic and xeric sites). As a result, populations from contrasting origins, like Morocco and France, displayed similar P_{50} and K_s values in the xeric location, i.e., there was a convergence in resistance to xylem embolism and hydraulic conductivity under stressful conditions. This is an advantage, as xylem resistance to cavitation was positively related to in situ minimum xylem water potential rather than to drought tolerance by itself. Therefore, *P. pinaster* populations from mesic origins could tolerate drought as satisfactorily as the populations from dry sources in a future warmer and drier climate. On the other hand, populations from drier habitats were more resistant to xylem cavitation than populations restricted to moister origins in the mesic site. However, this was a consequence of the lack of plasticity of these populations and cannot be considered as an advantage in humid habitats. Large xylem resistance to cavitation is unnecessary if xylem tension is habitually above P_{50}, especially if increased resistance to cavitation is associated with a lower xylem conductance and growth. Higher plasticity in mesic *P. pinaster* populations in response to drought, by means of carbon isotopic discrimination, was already noted by Aranda et al. (2010). On the contrary, a low responsiveness of physiological traits to drought in maritime pine populations from dry origins was observed by Aranda et al. (2010) and Guyon and Kremer (1982).

The capacity for plastic responses in a changing environment will be advantageous for the persistence of the species. The lower values of stem specific conductivity, vulnerability to cavitation and growth obtained in the xeric site suggest a reduction in biomass production associated with a higher resistance to embolism of maritime pine, in a future drier climate. However, with population selection, we might obtain good growth and wood quality or/and drought tolerance in dry habitats, depending on forest use, production or conservation. The selection of *Pleucadec* and *San Cipriano* would provide higher growth and wood quality and a similar vulnerability to xylem embolism to that presented by the populations from xeric origins. The selection of *Arenas* would allow achieving a higher resistance to cavitation at the cost of lower growth and wood quality. On the other hand, variation within populations was much larger than variation among populations, due to the large individual variation, which leads to some caution and corroborates the necessity of future studies. We also should take into account the possible physiological and morphological changes with the ontogeny. Studies with older trees and a larger number of locations and genotypes would be needed to obtain the full plasticity and variability within the species and draw a conclusion. Drought responses, in terms of lower stem specific conductivity and higher wood density, have been previously reported at the population level in *Pinus ponderosa* (Bouffier et al. 2003). The possibility of selecting a subpopulation that combined fast growth, high wood density and reduced vulnerability to cavitation was reported by Wang et al. (2003) in *Pinus contorta*. However in a long-term perspective, the higher vulnerability of pines, in relation to other conifers (Martinez-Villalta et al. 2004), could limit their survival. Drought-induced mortality in *Pinus sylvestris* populations from Spain (Martinez-Vilalta and Piñol 2002) and the replacement of pines by more drought-resistant species after severe droughts have already been reported.

Conclusions

Variation in xylem resistance to embolism and specific conductivity across *P. pinaster* populations and families were primarily the result of phenotypic plasticity and secondly of genetic differentiation. On the other hand, wood density was genetically controlled and not influenced by the environment. Therefore, differences in vulnerability to cavitation were not driven by changes in wood density.

In the mesic site, there was a good correspondence between vulnerability to xylem embolism, hydraulic capacity and drought tolerance of the populations based on their original distribution. However, aridity led to a convergence in both traits due to the high phenotypic plasticity exhibited by populations from mesic origins.

Trade-offs among the hydraulic traits appeared where variation existed and, therefore, at different genetic levels and environmental conditions. Among populations, a higher resistance to cavitation was associated with a lower stem specific conductivity, lower slope of the vulnerability curve and smaller branch size. These are typical plant responses to dry habitats. Higher wood density was related to higher growth.

The selection of more drought-resistant *Pinus pinaster* genotypes might be possible. It seems that there could be a choice between higher resistance to cavitation but lower growth and wood quality (*Arenas*) or high wood density and growth together with a high resistance to cavitation (*Pleucadec* and *San Cipriano*).

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