

## An ecophysiological approach of hydraulic performance for nine Mediterranean species

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**Summary** The existence of an efficient but also safe hydraulic system seems to be essential for plant survival under water limiting conditions. To investigate any common pattern in this safety–efficiency trade-off, static (xylem anatomy and vulnerability to xylem cavitation) and dynamic ( $K_{\text{plant}}$ , soil to leaf hydraulic conductance and  $\Psi_{\text{leaf}}$ , leaf water potential) hydraulic properties of nine Mediterranean species belonging to four functional groups (semi-deciduous malacophyllous, sclerophylls, deciduous and herbaceous) were studied across two altitude sites. Static parameters did not show any pattern, but a strong exponential relationship between  $K_{\text{plant}}$  and  $\Psi_{\text{leaf}}$  was evident for all the studied species. Furthermore, each species is represented by a different part and/or range of the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship, indicative of its adaptive mechanisms and capacity for survival under water stress conditions. The use of  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship as a tool for understanding the mechanisms of plant responses to water stress is discussed.

*Keywords:* embolism, soil to leaf hydraulic conductance, vulnerability curves, water potential, water stress, xylem anatomy.

### Introduction

Water availability may be considered as the major limiting environmental factor for terrestrial plant life. However, plants have evolved special adaptations, being able to survive even under the harshest conditions. Mediterranean ecosystems, with almost complete lack of precipitation during the summer period, coinciding with high temperatures and high light intensities, are ranked among the most demanding environments for plant survival.

Survival in a given environment largely depends on the ability of a plant to create an efficient (highly conductive) but also safe (able to maintain intact water column under negative pressures) hydraulic system. To achieve this, plants seem to control some features that influence this safety–efficiency trade-off. When plants are under water stress it

is essential to maintain water potential and transpiration within the limits defined by the cavitation threshold, the point upon which expansion of cavitation events will lead the hydraulic system to fail (Sperry and Pockman 1993, Kolb and Sperry 1999, Salleo et al. 2000).

Accordingly, the design of the plant's hydraulic architecture is considered to play a major role in the control of water movement from the roots to the leaves. A lot of research has been devoted to understanding how plants' hydraulic systems have evolved to accommodate survival under different environments. Anatomical characteristics of the xylem, hydraulic conductance and vulnerability to xylem cavitation are some of the parameters that have been studied to achieve this goal. Comparatively long and wide vessel elements are of adaptive value in mesic habitats, while shorter and narrower but more numerous vessel elements per unit area have evolved under drier conditions (Dickison 2000, Carlquist 2001). Hydraulic conductivities tend to be larger in mesic sites than in xeric sites (Willigen and Pammenter 1998) or on well-watered than drought-stressed plants (Tognetti et al. 1997). In a study with 167 species, Maherali et al. (2004) found that cavitation resistance increases with decreasing mean annual precipitation.

Despite the above trends, species growing under the same environment may exhibit totally different hydraulic properties (Kolb and Davis 1994, Martinez-Vilalta et al. 2002, 2003). This interspecific variation sometimes may be ascribed to differentiations among functional types. For example, conifers are more resistant to freeze–thaw-induced embolism compared with vessel-bearing species (Sperry and Sullivan 1992); deciduals display higher hydraulic conductivities (Wang 2005) and mean diameter of their widest xylem conduits (Castro-Diez et al. 1998) compared with evergreens. Nevertheless, there are studies where variation of hydraulic traits cannot be explained by categorization of species in functional types (Nardini and Salleo 2000, Nardini 2001, Martinez-Vilalta et al. 2002, 2003).

Studies dealing with intraspecific variation of hydraulic properties along an ecological gradient indicate that plants from arid environments are more resistant to cavitation and

exhibit lower conductivity (Kavanagh et al. 1999, Sparks and Black 1999). However, these studies are usually based on greenhouse experiments and they do not consider the plasticity of hydraulic traits that occurs within species due to the different growing environments. Studies dealing with field data present ambiguous results on the correlation between environmental factors and vulnerability to cavitation (Matzner et al. 2001) or xylem anatomy (Noshiro and Suzuki 1995, Villar-Salvador et al. 1997). The wide distribution of some species indicates the adaptation of their hydraulic system to different environments, but the contribution of some hydraulic properties to this adaptation is not well understood yet.

Nevertheless, the common goal of a successful safety–efficiency trade-off may lead to a common pattern in hydraulic properties independently from plants' life form, leaf morphology or wood type. Trying to find such patterns, some studies correlate xylem tension at which 50% cavitation occurs ( $\Psi_{50}$ ) with specific conductivity per unit xylem area ( $K_s$ ) for a variety of species. However, Sperry et al. (1994) found weak correlation for water-stress-induced embolism, Maherali et al. (2004) found no correlation and Martinez-Vilalta et al. (2002) and McElrone et al. (2004) found a power correlation between  $\Psi_{50}$  and  $K_s$ .

The aim of this study was to investigate the existence of any pattern between efficiency and safety among different species and functional groups. Correlations between safety (embolism vulnerability and water potential) and efficiency (anatomical characteristics and soil-to-leaf hydraulic conductance,  $K_{\text{plant}}$ ) parameters were examined for nine species belonging to four functional groups. Furthermore, all species were examined seasonally in two sites with different climatic conditions to investigate any possible climate effects on anatomical parameters of the xylem and to capture the extreme responses to water stress, concerning hydraulic conductance and water potential.

## Materials and methods

### Study sites and species

This study was carried out in the Epirus region (West Greece), extending from the Ionian coast to the Pindos Mountains. This region is characterized by harsh relief (0–2637 m a.s.l.) and a variety of climatic conditions, ranging from accentuated mesomediterranean on the coast to submediterranean at the high altitudes (Tselepidakis and Theoharatos 1989). Nine species following four different growth patterns and growing in a wide range across that climatic-elevation gradient were chosen. For each species two study sites were selected meeting the following criteria: both sites bearing fully exposed mature individuals, having the same substratum and corresponding to the distribution limits of each species in the particular region (either towards the coast or towards the mountains) (Table 1).

### Meteorological data

Meteorological data for the study sites (Table 1) concern averages of 20–40 years. Data were recorded by meteorological stations in close vicinity to the study sites and were kindly provided by the Hellenic National Meteorological Service and Public Power Corporation S.A. Data from Soulis (1994) were also used.

### Field measurements

Measurements were carried out from April 2005 to September 2007, during clear sky days and were repeated about every 2 months to assess the seasonal changes. For woody species, five individuals per species and study site were chosen for the ecophysiological measurements (transpiration and water potential).

Maximum daily transpiration ( $E$ ) was measured with a steady-state porometer (PP Systems, PMR4, Massachusetts). Measurements were performed around midday (12:00–13:00 h, solar time) when transpiration reaches its maximum value. On summer days, when midday stomata closure was expected, transpiration was additionally measured from 9:30 to 10:30 h and 10:30 to 11:30 h and the maximum values were used. On woody species, five exposed leaves were measured per individual and site. Likewise, for each study site,  $E$  was measured on 30 randomly chosen exposed leaves of each herbaceous species.

Leaf water potential ( $\Psi_{\text{leaf}}$ ) was measured in the field using a Scholander-type pressure chamber (Skye, SKPM 1400/80, UK), with a lower measuring limit at  $-8.0$  MPa. Predawn and midday leaf water potential were measured on four shoots (or leaves in the case of herbaceous) per individual (two for predawn and two for midday). Shoots/leaves were wrapped in aluminium foil and sealed in plastic bags and after 10 min were cut and measured immediately. In all cases, fully exposed shoots/leaves were used. Midday measurements were performed immediately after transpiration measurements.

### Soil to leaf hydraulic conductance

Soil to leaf hydraulic conductance was estimated according to Ohm's law analogy following the formula (Koide et al. 1989)

$$K_{\text{plant}} = \frac{E}{\Psi_{\text{soil}} - \Psi_{\text{leaf}}} \quad (1)$$

It is assumed that  $\Psi_{\text{soil}}$  is in equilibrium with predawn leaf water potential and that minimum diurnal leaf water potential ( $\Psi_{\text{leaf}}$ ) is reached when transpiration reaches its maximum values. However, the first assumption may lead to a slight overestimation of  $K_{\text{plant}}$  in cases where any possible nocturnal transpiration may not allow for equilibration between  $\Psi_{\text{soil}}$  and predawn leaf water potential (Kavanagh et al. 2007).

Table 1. The study species, their distribution characteristics, their wood type and average meteorological data for the sampling sites. Distribution<sup>(1)</sup>: stenomed, semi-arid areas, in the zone of thermophilus shrublands of evergreen broadleaves (mostly phrygana); med, zone of thermophilus shrublands of evergreen broadleaves (mostly maquis) and smed, zone of thermophilus deciduous forests of *Quercus* spp. Wood type: Dp, diffuse-porous; Rp, ring-porous; sRp, semi ring-porous and Vb, xylem arranged in vascular bundles. Study sites: Alt, Altitude (m); AR, annual rainfall (mm); SR, sum of summer rainfall (June–August) (mm);  $T_{avg}$ , mean annual temperature (°C);  $T_{min}$ , mean temperature of the coldest month (°C) and  $T_{max}$ , mean temperature of the warmest month (°C).

Species and functional type	Distribution <sup>(1)</sup>	Wood type	Study sites														
			Low altitude sites						High altitude sites								
			Alt	AR	SR	$T_{avg}$	$T_{min}$	$T_{max}$	Alt	AR	SR	$T_{avg}$	$T_{min}$	$T_{max}$			
Drought semi-deciduous																	
<i>Phlomis fruticosa</i> L.	Stenomed	Dp	69	1064	44	17.54	8.31	27.48	566	1138	106	14.4	5	25			
<i>Cistus creticus</i> L.	Stenomed-med	Rp	64	1064	44	17.54	8.31	27.48	620	1364	85	13.56	4.16	23.39			
Sclerophylls																	
<i>Arbutus unedo</i> L.	Stenomed-med	sRp	As <i>C. creticus</i>						As <i>C. creticus</i>								
<i>Phillyrea latifolia</i> L.	Stenomed-med	Dp	As <i>C. creticus</i>						793	1621	135	12.5	3.7	21.7			
Deciduous																	
<i>Quercus frainetto</i> Ten.	Smed	Rp	84	1345	64	17.41	8.61	26.58	873	1465	121	12.5	3.7	21.7			
<i>Carpinus orientalis</i> Mill.	Smed	Dp	As <i>Q. frainetto</i>						662	1364	85	13.56	4.16	23.39			
<i>Pyrus amygdaliformis</i> Vill.	Med-stenomed	sRp	As <i>P. fruticosa</i>						As <i>P. fruticosa</i>								
Herbaceous																	
<i>Helleborus odoratus</i> L. subsp. <i>cyclophyllus</i> (A. Braun) Strid	Smed	Vb	807	1197	81	10.89	1.31	20.9	1388	1471	150	9.34	0.00	20.46			
<i>Geranium molle</i> L.	Smed	Vb	As <i>Q. frainetto</i>						480	1138	106	14.4	5	25			

<sup>(1)</sup>According to Pignatelli (1982) and Oberdorfer (1990).

### Xylem anatomy

For the woody species, perennial branches with basal diameter about 1 cm were collected from 5 to 6 individuals per species and site (one branch per individual) in close vicinity with the ones used for the ecophysiological measurements and of similar age, during the summer of 2006. Leaves were removed from the branches and leaf area (LA) was measured. A 4–5 cm stem segment was cut from the base of each branch, boiled until all air trapped in the segment was removed and placed in a solution of 70% alcohol and 30% glycerol. Transverse sections about 40 µm thick were prepared using a sliding microtome (Leica SM2000R, Wetzlar, Germany). Sections were stained with toluidine blue to increase contrast and were mounted on slides using a xylene-based medium (Entellan new, Merck).

Sections were examined using a microscope (Leica DMLS, Wetzlar, Germany) equipped with a digital camera (Olympus 5050Zoom, Tokyo, Japan). Digital images at a magnification of 25X were used for the determination of transverse section of the entire xylem area and at 100X for vessel diameter and density measurements. For each transverse section, 1–3 representative sectors extending from the vascular cambium to the pith, occupying > 15% of the section's xylem area, were selected. Within this area, the inner diameter ( $D$ ) of each individual vessel was calculated as the

mean of its maximum and minimum diameter. Vessels were distinguished from fibres visually. Finally, vessels with diameter smaller than 10 µm were excluded from measurements.

Subsequently, the following parameters were calculated for each section: (a) mean vessel diameter ( $D_{mean}$ ); (b) hydraulically weighted mean diameter ( $D_h$ ), as  $\Sigma d_i^5 / \Sigma d_i^4$ , where  $d_i$  is the diameter of the  $i$  vessel measured in sampling sectors (Sperry et al. 1994); (c) vessel density (VD), as the number of vessels per unit xylem area and (d) theoretical leaf specific conductivity (LSCt), according to the equation  $LSCt = K_h/LA$ , where  $K_h$  is the predicted hydraulic conductivity according to the Hagen–Poiseuille law ( $K_h = (T/t)\Sigma(d_i/2)^4$ , in which  $T$  is the xylem area of the section and  $t$  is the area of the sampling sectors) and  $LA$  is the leaf area of the branch (Zimmerman 1983).

Concerning herbaceous species, 5–9 leaf blades per species and site were used for anatomical measurements of the xylem. Sections were made by hand and all vessels with mean diameter exceeding 10 µm were measured using 400X magnification. Measurement of VD was not possible for herbaceous species as the xylem was arranged in vascular bundles.

### Water-stress-induced vulnerability curves

Vulnerability curves were developed according to the dehydration method (Sperry and Sullivan 1992). Four to seven

branches from each species were collected during summer from the high altitude site of each species (Table 1). Branches with basal diameter 1–2 cm and length about 1.5 m (or the maximum possible in the case of the small shrubs *P. fruticosa* and *C. creticus*) were chosen with special care to include shoots capable of giving segments that fit into the apparatus used for the conductance measurements (see below). Branches were immediately transported to the laboratory and placed in water overnight in order for their water content to equilibrate. After equilibration, the water potential of each branch was determined by measuring three excised shoots with a pressure chamber. Accordingly, different manipulations were followed to achieve different water potentials. If initial water potential was higher than desired (usually this was the case) the branch was left to dry on a bench for a period varying from 0.5 to 48 h (depending on the water potential we wanted to achieve) and then wrapped in a plastic bag for 2 h to equilibrate again. If initial water potential was lower than desired (because of native or artificial embolism caused by branch collection and transportation to the laboratory), the branch was flushed with distilled water at 50–70 kPa for a period varying from 1 to 2 h to increase its water potential by dissolving xylem embolism (Tognetti et al. 1998, 1999). Then, for each branch, water potentials were remeasured, as described above and three unbranched segments, 0.5 cm in diameter and 2.5–3.5 cm in length, were cut under water to avoid causing additional embolism. The segments were fitted to a ‘Sperry tubing apparatus’ to measure their percentage loss of hydraulic conductance (PLC) due to xylem embolism following a method fully described by Sperry et al. (1988). Briefly, a deionized, filtered (0.2  $\mu\text{m}$ ), acidified (pH 2, HCl; Sperry et al. 1994) solution was passed through the segments under 4–7 kPa hydrostatic pressure. Initial hydraulic conductance ( $K_i$ ) was calculated by measuring the mass flow rate of the solution and dividing it by the hydrostatic pressure causing the flow. Then the segments were flushed at a positive pressure of 175 kPa for 10 min, to remove all emboli, and maximum hydraulic conductance ( $K_{\text{max}}$ ) was calculated as before. Percentage loss of hydraulic conductance was determined as  $\text{PLC} = 100(K_{\text{max}} - K_i)/K_{\text{max}}$ . Water potential and PLC values were plotted on the same graph to produce the vulnerability curves. Concerning the use of the very acidic solution described above, it has to be noted that in recent papers (i.e., Li et al. 2009) the use of a low concentration KCl solution is preferred, which may be considered as more meaningful from a physiological point of view.

Vulnerability curves could not be obtained for herbaceous species because of the nature of their stems and petioles. Soft stems with large pith areas or even hollows, in the case of *G. molle*, made the measurement of hydraulic conductance impossible with the method described above. Additionally, it has to be noted that for some species (i.e., *C. orientalis* and *P. amygdaliformis*, see Figure 3) replication of vulnerability curves was lower compared to the rest of the species.

Table 2. Xylem anatomical characteristics of the studied species from the low and high altitude sites (see also Table 1) and the significance of difference between the two sites. Each value is the average ( $\pm$ SD) of 5–9 measurements ( $n = 5$ –9) (Sig., \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , ns: not significant).  $D_h$ , mean vessel diameter;  $D_{\text{mean}}$ , mean vessel diameter;  $D_h$ , hydraulically weighted mean diameter; VD, vessel density; LSCt, theoretical leaf specific conductivity.

Species and functional type	$D_{\text{mean}}$ ( $\mu\text{m}$ )		$D_h$ ( $\mu\text{m}$ )		VD (vessels/ $\text{mm}^2$ )		LSCt ( $\times 10^3$ ( $\mu\text{m}^2$ ))		Sig.
	Low	High	Low	High	Low	High	Low	High	
Drought semi-deciduous									
<i>P. fruticosa</i>	20.53 $\pm$ 0.81	19.8 $\pm$ 1.45	32.08 $\pm$ 1.43	31.58 $\pm$ 4.09	349.63 $\pm$ 13.21	265.33 $\pm$ 49.86	170.39 $\pm$ 44.72	119.03 $\pm$ 52.89	ns
<i>C. creticus</i>	16.97 $\pm$ 0.94	17.26 $\pm$ 0.63	23.51 $\pm$ 2.35	25.57 $\pm$ 0.92	252.97 $\pm$ 26.1	299.22 $\pm$ 67.67	33.03 $\pm$ 12.53	54.24 $\pm$ 13.61	*
Sclerophylls									
<i>A. unedo</i>	18.65 $\pm$ 1.03	18.17 $\pm$ 0.56	25.58 $\pm$ 1.55	24.84 $\pm$ 1.31	462.22 $\pm$ 152.66	523.54 $\pm$ 41.93	39.3 $\pm$ 18.16	38.91 $\pm$ 12.88	ns
<i>P. latifolia</i>	15.86 $\pm$ 0.97	16.72 $\pm$ 0.79	20.05 $\pm$ 1.58	21.8 $\pm$ 2.04	272.6 $\pm$ 70.85	305.2 $\pm$ 52.84	9.97 $\pm$ 4.37	16.7 $\pm$ 8.39	ns
Deciduous									
<i>Q. frainetto</i>	33.13 $\pm$ 1.6	31.86 $\pm$ 3.02	73.03 $\pm$ 12.48	59.57 $\pm$ 9.11	84.58 $\pm$ 18.92	106.81 $\pm$ 25.4	101.55 $\pm$ 54.84	54.97 $\pm$ 13.18	ns
<i>C. orientalis</i>	23.48 $\pm$ 1.59	22.59 $\pm$ 1.9	35 $\pm$ 1.61	33.04 $\pm$ 3.3	169.53 $\pm$ 51.11	176.85 $\pm$ 74.12	27.84 $\pm$ 13.04	22.67 $\pm$ 7.36	ns
<i>P. amygdaliformis</i>	15.41 $\pm$ 2.05	15.11 $\pm$ 0.95	18.02 $\pm$ 2.83	18.03 $\pm$ 1.82	219.96 $\pm$ 109.29	249.04 $\pm$ 74.94	28.62 $\pm$ 21.31	26.02 $\pm$ 12.21	ns
Herbaceous									
<i>H. odoratus</i> subsp. <i>cyclophyllus</i>	20.39 $\pm$ 1.97	17.48 $\pm$ 1.05	28.88 $\pm$ 3.78	23.46 $\pm$ 2.5	–	–	11.53 $\pm$ 6.41	6.43 $\pm$ 3.44	ns
(A. Braun) Strid									
<i>G. molle</i>	15.63 $\pm$ 0.79	16.14 $\pm$ 0.68	18.88 $\pm$ 1.54	19.74 $\pm$ 1.34	–	–	5.45 $\pm$ 1.54	5.44 $\pm$ 1.28	ns

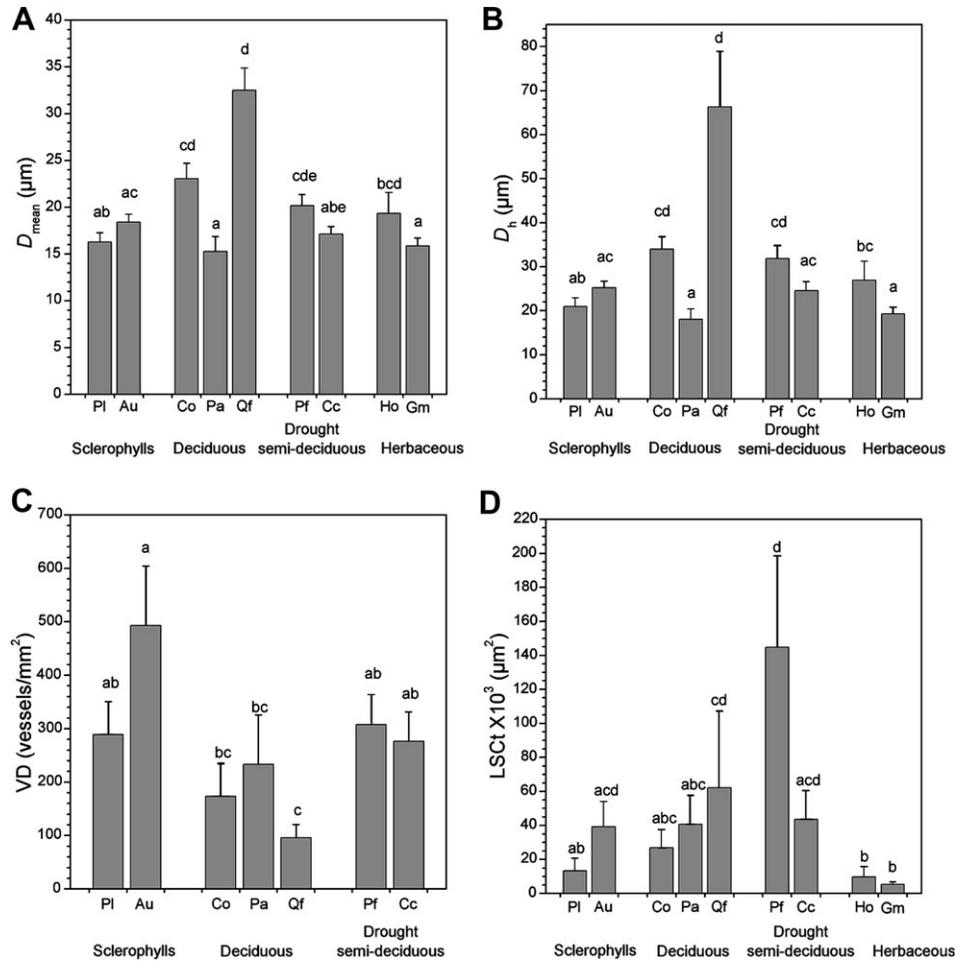


Figure 1. Xylem anatomical parameters of the studied species grouped according to their functional type, i.e., sclerophylls, deciduous, drought semi-deciduous and herbaceous. (A)  $D_{mean}$ , mean vessel diameter; (B)  $D_h$ , hydraulically weighted mean diameter; (C) VD, vessel density; and (D) LSCt, theoretical leaf specific conductivity. Each bar represents species average of pooled data from both sites ( $n = 10-14$ ) and error bars represent SD. Different letters among bars indicate significant differences among species at  $P < 0.05$ . PI, *Phillyrea latifolia*; Au, *Arbutus unedo*; Co, *Carpinus orientalis*; Pa, *Pyrus amygdaliformis*; Qf, *Quercus frainetto*; Pf, *Phlomis fruticosa*; Cc, *Cistus creticus*; Ho, *Helleborus odoros* subsp. *cyclophylus* and Gm, *Geranium molle*.

However, our purpose was to complete these measurements during the maximum summer stress period (i.e., mid to end of the summer) to capture the most extreme responses for all species. Under this consideration and given the timely nature of the experimental procedure, no further measurements were performed after the onset of the autumn rains, to avoid any possible seasonal change in embolism vulnerability (Kolb and Sperry 1999, Jacobsen et al. 2007).

*Statistical analysis*

Site effect on the anatomical characteristics of the species (Table 2) was statistically evaluated for each species using *t* test or Mann–Whitney test, when data deviated from normality. To explore the differences among species (Figure 1), data from both sites were pooled for each species and evaluated using a non-parametric Kruskal–Wallis test (since normality and equality of variances prerequisites were not met)

followed by Dunn’s multiple comparison post hoc analysis. Spearman correlation analysis was performed for the relationships between safety and efficiency parameters (Table 3). Finally, linear and nonlinear regression models were used to describe the relationships among the studied parameters (Figures 2–6), and their  $r^2$  and  $P$  values are shown in the graphs and their legends. Especially for vulnerability curves (Figure 3) the physiological meaningful function proposed by Pammenter and Vander Willigen (1998) was used, except when a model with higher  $r^2$  value was found.

**Results**

*Xylem anatomy*

Values of the anatomical parameters for all studied species and sites, as well as the corresponding statistical results, are presented in Table 2. Most parameters do not show any

Table 3. Correlation coefficients ( $r$ ) for the relationships between safety and efficiency parameters of static or dynamic nature.  $D_{\text{mean}}$ ,  $D_{\text{h}}$ , VD and LSCt anatomical parameters are presented in Table 2. Minimum  $K_{\text{plant}}$  and minimum  $\Psi_{\text{leaf}}$  represent the seasonal minimum values throughout the 3-year study period,  $\Psi_{50}$  values were calculated from vulnerability curves (Figure 3). Asterisks denote significant correlations at the 0.01 level.

Safety		Efficiency				
		Static				Dynamic
		$D_{\text{mean}}$	$D_{\text{h}}$	VD	LSCt	Minimum $K_{\text{plant}}$
Static	$\Psi_{50}$	-0.143	-0.143	-0.429	-0.536	-0.847*
Dynamic	Minimum $\Psi_{\text{leaf}}$	0.092	0.092	0.018	0.268	-0.798*

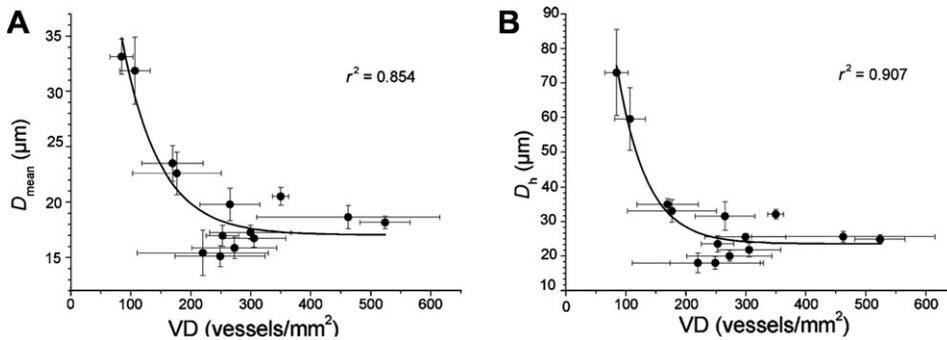


Figure 2. Relationship between VD and  $D_{\text{mean}}$  (A) and  $D_{\text{h}}$  (B) for all species except herbaceous. Each dot represents data for one species and site ( $n = 5-6$ ) and error bars represent SD. For both cases, data were fitted by an exponential decay equation and regressions are highly significant ( $P < 0.0001$ ).

significant differences between the low and high altitude site for each species. The only significant differences concern  $D_{\text{mean}}$  and  $D_{\text{h}}$  for *H. odorus* (lower values in the high site), VD for *P. fruticosa* (lower values in the high site) and LSCt for *C. creticus* (higher values in the high site).

Since in most cases differences between low and high sites were not significant and to evaluate differences between species and possible grouping according to their functional groups, values from the two sites were merged for each parameter and species. As shown in Figure 1, all parameters are highly variable between species, but no clear grouping is evident for any of them.

Despite the high among-species variability in the anatomical parameters, there is a good negative relationship between VD and  $D_{\text{mean}}$  ( $r^2 = 0.85$ ) and  $D_{\text{h}}$  ( $r^2 = 0.91$ ) (Figure 2). As expected (Dickison 2000), species with wide vessels show lower VD and vice versa.

#### Vulnerability curves

Vulnerability to xylem embolism varied markedly among species (Figure 3). As in the case of anatomical parameters, no common pattern was found among species belonging to the same functional group. On the contrary, the two extremes were found in the same functional group, i.e., sclerophylls, with *P. latifolia* being the most resistant ( $\Psi_{50} = -7.00$  MPa) and *A. unedo* being the most vulnerable ( $\Psi_{50} = -0.46$  MPa) to water stress induced embolism among all studied species.

Furthermore, different vulnerability patterns were found between the two semi-deciduous. *Cistus creticus* ( $\Psi_{50} = -5.2$  MPa) is capable of sustaining high values of relative conductance in a range of xylem tension between 0 and  $-4$  MPa, whereas *P. fruticosa* ( $\Psi_{50} = -2.48$  MPa) gradually loses about 70% of its conductance in the same range. However, below  $-4$  MPa *C. creticus* shows an abrupt loss of conductance and reaches 100% PLC at  $-6.17$  MPa, whereas *P. fruticosa* maintains a small proportion of conductive xylem even at  $-8$  MPa (the measuring limit of our pressure bomb).

The deciduous species showed the most homogeneous vulnerability patterns. *Pyrus amygdaliformis* ( $\Psi_{50} = -3.29$  MPa) was most vulnerable to xylem embolism, followed by *C. orientalis* ( $\Psi_{50} = -4.35$  MPa) and *Q. frainetto* ( $\Psi_{50} = -4.56$  MPa).

#### Hydraulic conductance

To approach a more ecophysiological expression of the safety–efficiency trade-off, transpiration ( $E$ ),  $\Psi_{\text{soil}}$  and  $\Psi_{\text{leaf}}$  were measured seasonally in the field for 3 years, including three summer stress periods and for all the studied species and sites. From these measurements,  $K_{\text{plant}}$  was calculated according to Eq. (1) and the data obtained between March and September of 2005–2007, i.e., under favourable and water limiting conditions, were used to investigate the existence of a common pattern between species as a response to water shortage (Figures 5 and 6, see below). Winter data

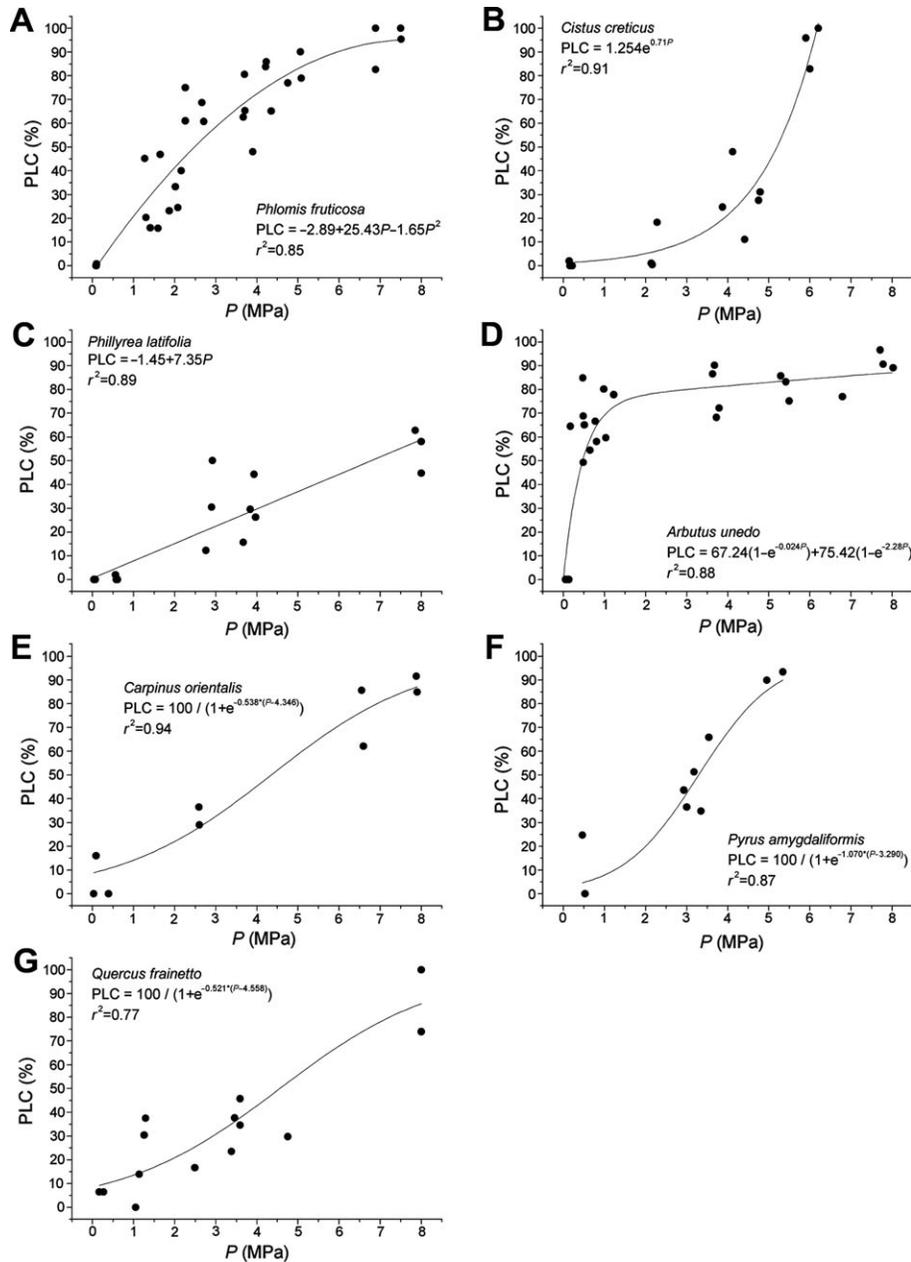


Figure 3. Xylem embolism vulnerability curves of the studied species, expressed as PLC versus xylem tension ( $P$ ) that caused that loss. Each dot represents one shoot measurement. The equations of the fit functions and their  $r^2$  values are shown in the graphs. All regressions are highly significant ( $P < 0.0001$ ).

were not used to avoid low temperature effects as well as water shortage effects due to frost events.

*Safety–efficiency relationships*

To investigate any possible pattern in the safety–efficiency trade-off, correlations between safety (minimum  $\Psi_{leaf}$ ,  $\Psi_{50}$ ) and efficiency (anatomical characters and minimum  $K_{plant}$ ) parameters were examined (Table 3). None of the four anatomical parameters showed any correlation with either of the two safety parameters (minimum  $\Psi_{leaf}$ ,  $\Psi_{50}$ ). However, minimum  $K_{plant}$  showed significant correlations with both safety parameters. A linear relation was found

between seasonal minimum  $K_{plant}$  and embolism vulnerability ( $\Psi_{50}$ ) and an exponential relationship between seasonal minimum  $K_{plant}$  and seasonal minimum  $\Psi_{leaf}$  (Figure 4).

Additionally, when data for all species, seasons and sites were examined, a strong exponential relationship was also found between  $K_{plant}$  and  $\Psi_{leaf}$ , as a result of similar relationships between transpiration and  $\Psi_{soil}$  (see Eq. (1)) with  $\Psi_{leaf}$  (Figure 5). Furthermore, different species occupy different parts of the curve that describes  $K_{plant}$ – $\Psi_{leaf}$  relationship, with species of the same functional group not always following the same pattern (Figure 6).

Herbaceous species, not capable of surviving during the summer stress period, correspond to the vertical linear part

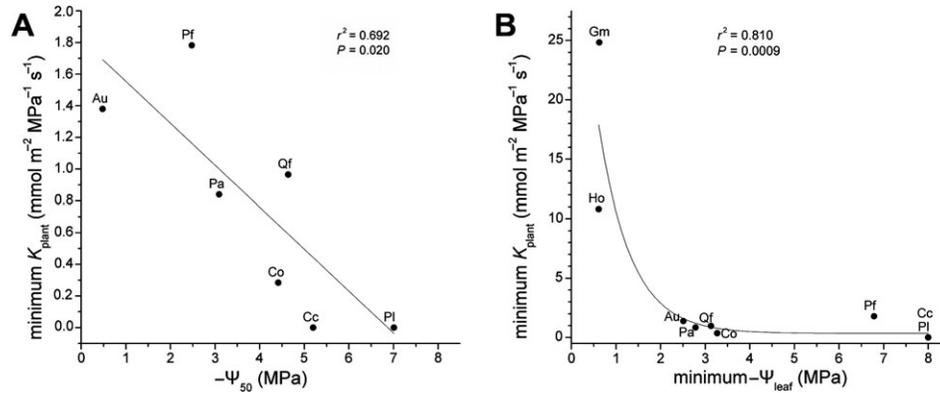


Figure 4. Relationship between soil to leaf hydraulic conductance ( $K_{\text{plant}}$ ) and  $\Psi_{50}$  (A) and leaf water potential ( $\Psi_{\text{leaf}}$ ) (B) for all the studied species. Minimum  $K_{\text{plant}}$  and  $\Psi_{\text{leaf}}$  values from the low sites throughout the 3-year study period were used. Data were fitted with a linear (A) and an exponential decay (B) equation, and  $r^2$  and  $P$  values are shown in the graphs. For species abbreviations see Figure 1.

of the curve, showing both the highest  $\Psi_{\text{leaf}}$  values (never falling below  $-1.2$  MPa) and the highest  $K_{\text{plant}}$  values among all species (Figure 6D).

Deciduous species have similar  $\Psi_{\text{leaf}}$  range but their maximum  $K_{\text{plant}}$  values are rather different, with *Q. frainetto* showing the highest values ( $K_{\text{plant}} = 14.18 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ ), followed by *P. amygdaliformis* ( $K_{\text{plant}} = 6.51 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ ) and *C. orientalis* ( $K_{\text{plant}} = 3.29 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$ ). Despite their differences the deciduous species occupy the middle part of the curve, as they do not reach extremely high  $K_{\text{plant}}$  or low  $\Psi_{\text{leaf}}$  values (Figure 6C).

Semi-deciduous show the wider range among all species, extending to all three parts of the curve except for the upper vertical part occupied by herbaceous (Figure 6A). However, at low  $\Psi_{\text{leaf}}$  values *P. fruticosa* tends to show higher  $K_{\text{plant}}$  values than *C. creticus*, whereas at high  $\Psi_{\text{leaf}}$  values the opposite happens. This seems in accordance with the different patterns in the PLC curves among these species, with *P. fruticosa* showing higher PLC than *C. creticus* at high xylem tension values (Figure 3A and B). Additionally, at very low  $\Psi_{\text{leaf}}$  values, *P. fruticosa* seems to sustain some conductance, whereas conductance values for *C. creticus* tend to zero, which may also be related to their different PLC patterns.

On the other hand, the two sclerophyllous species show similar  $K_{\text{plant}}$  range, but remarkably different  $\Psi_{\text{leaf}}$  range, with *P. latifolia* showing much lower water potentials compared to *A. unedo* (Figure 6B). This is in accordance with their different vulnerability to xylem embolism (Figure 3) and their different conductive capacity under drought conditions (Figure 4). Indeed, during August 2005  $\Psi_{\text{leaf}}$  at  $-2.51$  MPa for *A. unedo* and less than  $-8$  MPa for *P. latifolia* were measured at the low site. The corresponding PLC (according to vulnerability curves, Figure 3C and D) was 79.12% and  $\geq 57.33\%$ , respectively. Although *A. unedo* was more embolized, it exhibited higher  $K_{\text{plant}}$  ( $= 1.84 \text{ mmol m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$  for *A. unedo*,  $\approx 0 \text{ mmol}$

$\text{m}^{-2} \text{ MPa}^{-1} \text{ s}^{-1}$  for *P. latifolia*) in accordance with its higher LSCt compared with *P. latifolia* (Table 2).

## Discussion

The ability of plants to survive under variable water availability conditions depends on their efficiency and safety characteristics relating to water management. In this safety–efficiency trade-off, static and dynamic parameters/mechanisms may be distinguished. Anatomical characteristics of the xylem, like the ones examined in this study, may be considered of a static nature, since they are not able to respond to rapid water availability fluctuations. Even though some studies indicate that embolism vulnerability may change seasonally (Kolb and Sperry 1999, Jacobsen et al. 2007), we may assume that vulnerability curves created during summer, as in this study, express the maximum capacity of plants to resist cavitation under water shortage conditions. In that sense, vulnerability curves may also be considered of a static nature for a certain species, since they depict their ability to resist cavitation, but they do not implicate various characteristics and mechanisms, which may directly adjust plant water status under variable conditions. For example, a vulnerability curve for a certain species may specify that at  $\Psi_{\text{leaf}} = -3$  MPa, 60% of its conductive capacity is lost. But does this particular species ever reach such a  $\Psi_{\text{leaf}}$  value under water shortage conditions? On the other hand,  $K_{\text{plant}}$  and  $\Psi_{\text{leaf}}$  integrate the actual functional plant responses under the variable environment and may be considered to have a dynamic nature.

According to the above consideration, when static efficiency parameters (anatomy) were examined against safety parameters of either static ( $\Psi_{50}$ ) or dynamic (minimum  $\Psi_{\text{leaf}}$ ) nature, no relationship was found (Table 3), possibly because xylem anatomy characteristics related with conduit efficiency may not play an important role in embolism

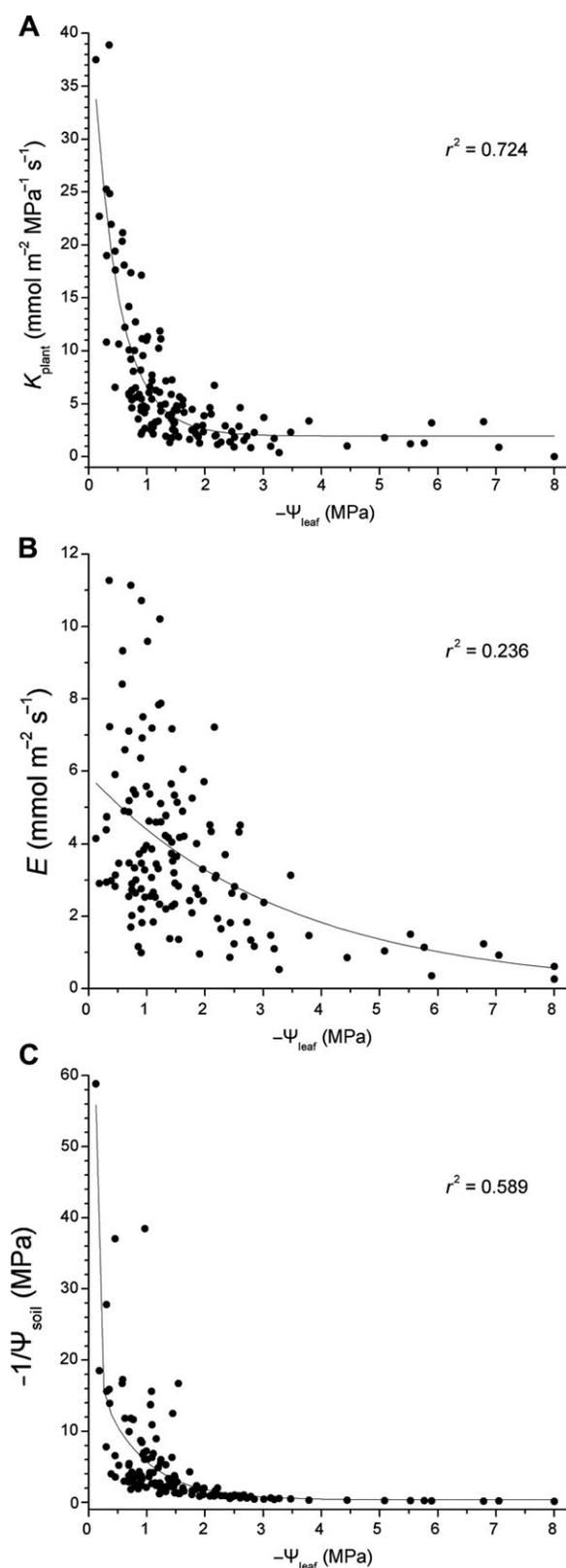


Figure 5. Relationship between soil to leaf hydraulic conductance ( $K_{\text{plant}}$ ) (A), transpiration ( $E$ ) (B) and  $\Psi_{\text{soil}}$  (C) with minimum diurnal leaf water potential ( $\Psi_{\text{leaf}}$ ), for all the studied species. All data are fitted with an exponential decay equation and all regressions are highly significant ( $P < 0.0001$ ).

vulnerability and the ability of plants to survive under water shortage conditions (Sperry and Sullivan 1992, Cordero and Nilsen 2002). On the contrary, when the dynamic efficiency parameter  $K_{\text{plant}}$  was examined, strong relationships were found with both safety parameters (Table 3; Figure 4), since  $K_{\text{plant}}$  may incorporate all the possible characteristics and mechanisms involved in plant water relations.

Consequently, the information provided by anatomical characteristics and vulnerability curves may only be used as rough indicators of the ability of species to survive under water stress conditions. Furthermore, the lack of common characteristics in xylem anatomy (Figure 1) and vulnerability curves (Figure 3) between species belonging to the same functional group may be considered rather reasonable, since different water adjusting mechanisms may be followed by species of the same group. Although more species and functional groups have to be studied, our results are supported by the rather contradictory results about xylem anatomy and cavitation resistance found in relevant papers. For example, some studies assert that deciduous species bear wider vessels than evergreens (Villar-Salvador et al. 1997, Castro-Diez et al. 1998, Gorsuch et al. 2001), while others find no correlation (Cavender-Bares and Holbrook 2001, Martinez-Vilalta et al. 2002). Additionally, it has been reported that gymnosperms are more resistant to xylem embolism than angiosperms, but when angiosperms are divided into evergreens and deciduous, cavitation resistance is similar (Maherali et al. 2004).

Concerning static parameters and in addition to the ones studied in this paper, vessel wall thickness, xylem fibres, root depth, root xylem anatomy and leaf anatomy are considered to be of major importance for interpreting the responses of plants to water availability (Hacke et al. 2000, Jacobsen et al. 2005, Kessler et al. 2007). On the other hand, dynamic parameters, such as stomatal control, osmoregulation and the existence of water reservoirs inside the plant body, may play a major role on plant water status adjustment (Sperry and Pockman 1993, Larcher 2003). Furthermore, recent studies indicate that whole plant hydraulic resistance may be affected by conduit pit structure, which may be rapidly adjusted through changes in ion concentration in the xylem sap (Zwieniecki et al. 2001, 2004, Choat et al. 2008). Additionally, aquaporine activity/expression may also play an important role in plant water balance (Kaldenhoff et al. 2008). Obviously, measuring and understanding the interrelations between all the involved parameters would result in the most accurate conclusion about the safety–efficiency trade-off, but this may prove to be a difficult task (e.g., root depth measurement and direct measurement of pit hydraulic characteristics).

Alternatively, the proposed  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship presented in this study may be used as a tool for determining the capacity of one species for survival under water shortage conditions. On the one hand, whole plant hydraulic conductance is the final result of all the possible mechanisms

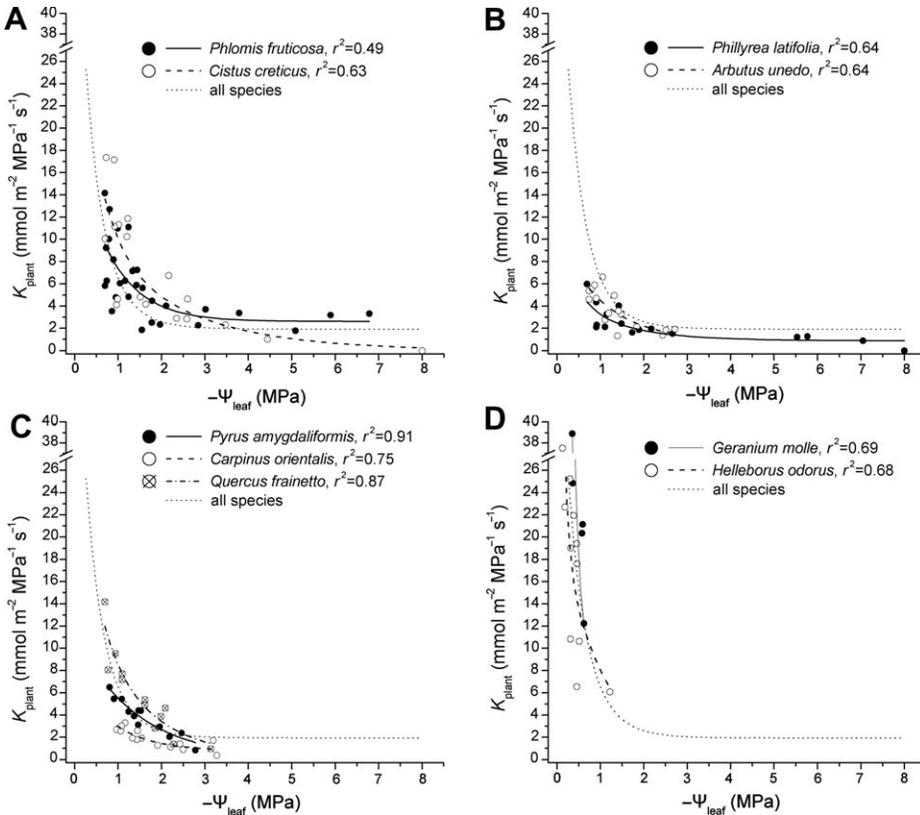


Figure 6. Relationship between soil to leaf hydraulic conductance ( $K_{\text{plant}}$ ) and minimum diurnal leaf water potential ( $\Psi_{\text{leaf}}$ ), for drought semi-deciduous (A), sclerophylls (B), deciduous (C) and herbaceous species (D). The dotted line in all graphs corresponds to the line describing the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship for all species shown in Figure 5A. All data are fitted with an exponential decay equation and all regressions are highly significant ( $P < 0.0001$ ).

(both of static and of dynamic nature) affecting water movement across the soil–plant–atmosphere continuum under the prevailing conditions along seasons and may be considered as an efficiency analogue. On the other hand,  $\Psi_{\text{leaf}}$  reflects the actual water status of the plant under field conditions and may be considered as a safety analogue, especially its seasonal minimum value for a certain species (Hacke et al. 2000, Jacobsen et al. 2007). Accordingly, even though the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship does not determine which particular characteristic(s)/mechanism(s) may play a major role for a certain species, it may be considered as a safety–efficiency analogue.

In support of that view, the following points are worth noting:

1. All the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  data presented in this study (nine species, three seasons, three growing periods and two sites per species) are fit by a single curve (Figure 5A).
2. Every single species corresponds to a particular part of that curve (Figure 6).
3. The position of one species on the curve is indicative of its capacity for survival under water stress conditions. For example, the vertical part of the curve corresponds to species with low capacity (i.e., herbaceous) while the horizontal part corresponds to species well adapted to drought conditions (i.e., semi-deciduals and *P. latifolia*).

4. The range of both  $K_{\text{plant}}$  and  $\Psi_{\text{leaf}}$  values for one species is also indicative of the repertoire of adaptive mechanisms incorporated by that species (i.e., semi-deciduals). Additionally, this range may indicate totally different mechanisms adopted by species growing side by side, even in the case where they belong to the same functional group (i.e., sclerophylls).

According to the above, a clear pattern is evident for the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship among all studied species and this relationship may result in species ranking in accordance or not with functional groups (Figures 5 and 6). Herbaceous, deciduous and semi-deciduous are clearly distinguished since they correspond to the vertical, middle and almost the entire curve accordingly (Figure 6A, C and D). This may be explained by the strategy adopted by each group to cope with water management. Herbaceous species are escaping the drought period (high  $\Psi_{\text{leaf}}$  values) incorporating no adaptive mechanisms to water stress conditions at all, but show high growth rates (high  $K_{\text{plant}}$  values) to complete their life cycle before the onset of the dry period. Deciduous species, being active throughout the dry period, seem to avoid water stress (intermediate  $K_{\text{plant}}$  and  $\Psi_{\text{leaf}}$  values) possibly by developing an extensive and deep root system, offering access to deep water reservoirs (Hacke et al. 2000). Semi-deciduous appear as the most capable to tolerate water shortage (low  $\Psi_{\text{leaf}}$  values) while being highly

effective during the favourable periods of the year (high range of  $K_{\text{plant}}$  values) as a result of a repertoire of morphological, anatomical and physiological mechanisms, associated with partial leaf shedding during summer and leaf dimorphism (Harley et al. 1987, Kypris and Manetas 1993a, 1993b, Grammatikopoulos et al. 1995).

On the other hand, sclerophylls not only are not clearly distinguished from semi-deciduous and deciduous, but they also appear remarkably different from each other (Figure 6B). In that case, the use of the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship indicates that the two studied sclerophylls follow different strategies to cope with water shortage. This is confirmed by the results of several papers studying these species, which show important differences in embolism vulnerability, stomatal control and conductive capacity and also suggest differences in their root system (Martinez-Vilalta et al. 2002, 2003, Bombelli and Gratani 2003, Ogaya and Penuelas 2003).

In conclusion, even though no clear pattern has been found for the studied static parameters related with water management (anatomical characters and embolism vulnerability), a distinct pattern has been evident for the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship between all studied species. This relationship is proposed as a safety-efficiency trade-off analogue, incorporating all the possible parameters and functions of static and dynamic nature that may be involved in plant water relations. In the challenge of clarifying the specific mechanisms and their interactions involved in water stress responses, the  $K_{\text{plant}}-\Psi_{\text{leaf}}$  relationship may prove to be a useful tool. The confirmation of this relationship for more species and functional groups other than the ones of this study would provide a strong support in that direction.

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