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Research paper

Differences in functional and xylem anatomical features allow *Cistus* species to co-occur and cope differently with drought in the Mediterranean region

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A significant increase in drought events frequency is predicted for the next decades induced by climate change, potentially affecting plant species mortality rates and distributions worldwide. The main trigger of plant mortality is xylem hydraulic failure due to embolism and induced by the low pressures at which water is transported through xylem. As the Mediterranean basin will be severely affected by climate change, the aim of this study was to provide novel information about drought resistance and tolerance of one of its most widely distributed and common genera as a case study: the genus *Cistus*. Different functional and anatomical traits were evaluated in four co-occurring *Cistus* species in the Mediterranean Montado ecosystem. Soil water availability for each species was also assessed to evaluate if they show different ecological niches within the area. Results showed physiological and xylem anatomical differences between the four co-occurring species, as well as in the soil water availability of the sites they occupy. Despite the significant differences in embolism resistance across species, no trade-off between hydraulic safety and efficiency was observed. Interestingly, species with narrower vessels showed lower resistance to embolism than those with higher proportions of large conduits. No correlation, however, was observed between resistance to embolism and wood density. The four species showed different water-use and drought-tolerance strategies, occupying different ecological niches that would make them cope differently with drought. These results will allow us to improve the predictions about the expected changes in vegetation dynamics in this area due to ongoing climate change.

Keywords: climate change, embolism, hydraulic safety margins, water use, xylem anatomy.

Introduction

In Europe, and especially in the Mediterranean region, the two main expected effects of anthropogenic climate change are an increment in the mean annual temperature and a decrease in the mean annual precipitation, with considerable changes in the patterns of regional and seasonal rainfall events (IPCC 2007). These variations in climate will increase not only the frequency and severity of drought events, but also the occurrence of intense precipitation episodes that will accentuate the climatic seasonality of this area. On a global scale, the frequency of these events has already increased during recent decades, thereby

affecting forest productivity and plant mortality rates (Boisvenue and Running 2006, van Mantgem et al. 2009, Allen et al. 2015). Different studies have already reported substantial growth reductions and mortality events induced by drought stress not only at global scale (Cailleret et al. 2016), but also specifically in the Mediterranean basin for species such as *Quercus ilex*, *Pinus brutia* and *Pinus sylvestris* (Peñuelas et al. 2001, Corcuera et al. 2004, Lloret et al. 2004, Sarris et al. 2007), generating growing concern about the effects of climate change on the distribution and composition of the Mediterranean forests (Martínez-Vilalta et al. 2011).

Hydraulic failure of the water transport system of the plant is now seen as one of the causes of these changes in species distribution since it is the main mechanism leading drought-induced plant mortality (Brodribb and Cochard 2009, Barigah et al. 2013, Salmon et al. 2015, Anderegg et al. 2016). Plant hydraulic failure is mostly due to the formation of gas bubbles (emboli) in the xylem conduits that break the continuum of the water column and lead to a reduction in hydraulic conductance. Embolisms form when xylem pressure is low enough to aspirate air into the functional vessels through the pit membranes (Salleo et al. 2000, Tyree and Zimmerman 2002). Although plants possess physiological and structural adaptations that allow them to minimize this risk of embolism formation (Cochard and Delzon 2013), under drought conditions, reduction in plant water availability decreases xylem pressure (i.e., to more negative values) and, therefore, increases the probability of embolism formation. As the number of embolized conduits increases, the capacity of the plant to move water though the xylem decreases. In fact, if the percentage of these losses in hydraulic conductivity (PLC) reaches 50% for conifers or 88% for angiosperms ('point of no return' or 'lethal level of embolism'; Brodribb and Cochard 2009, Urli et al. 2013) they can cause the death of the plant. Evaluating the resistance from embolism and its linkage to xylem anatomy will provide, therefore, relevant information to predict how drought episodes can affect plant mortality and species distribution in the future.

Different adaptive water-use strategies and xylem functional features have been observed in co-occurring Mediterranean species from different families and genera that allow them to coexist in the same habitat (Bombelli and Gratani 2003, Galle et al. 2011, Vilagrosa et al. 2014). Further related to drought resistance, De Micco et al. (2008) reported a gradual adaptation in different Mediterranean trees and shrubs species to severe drought periods after evaluating their wood anatomical traits along a mesic-xeric gradient. However, how these strategies and xylem features, including resistance to embolism, vary between co-existing Mediterranean species but within a single genus has been poorly evaluated.

The xylem structure is a compromise between efficiency of water transport, safety from embolism and mechanical support (Pittermann et al. 2006, Gleason et al. 2016). Previous studies on Mediterranean species have already highlighted the importance of some xylem traits as wood density as an indicator of drought tolerance for large numbers of species (Jacobsen et al. 2007). Lower embolism resistances have been observed for species with both higher areas of pit membranes connecting vessels ('rare pore' hypothesis, Wheeler et al. 2005) and lower wood densities (Hacke and Sperry 2001, Domec et al. 2010). As the Hagen-Poiseuille Law states, lumen conductance increases with radius to the fourth power. This makes species with many narrow xylem conduits less efficient on water transport than those with fewer larger conduits (Zimmermann 1983). Therefore, it would be expected that species with different tolerances to drought also show differences in their xylem anatomies that allow them to hold these trade-offs.

This study focused on the Montado ecosystem in Portugal in which shrubs are seen now as a key element for (i) preventing soil erosion due to the accumulation of organic material (Andreu et al. 1998) and (ii) the improvement of water and nutrients levels. Among these shrubs, Cistus species are the major components of both the Montado ecosystem and the Mediterranean vegetation as a whole (Carlier et al. 2008), and Cistus ladanifer L., Cistus monspeliensis L., Cistus populifolius L. and Cistus psilosepalus Sweet are some of the most common species co-occurring in this ecosystem. Interesting adaptive strategies haven been reported already for many Mediterranean shrubs species, and especially for the Cistus genus, for which a seasonal dimorphism has been described for some them, such as Cistus incanus L. subsp. incanus (Aronne and De Micco 2001, De Micco and Aronne 2009) or Cistus salvifolius L. (Simões et al. 2012), while others are closer to evergreens, such as C. ladanifer (Simões et al. 2012). The main objective of this study was to evaluate some of the most relevant functional and xylem anatomical traits related to drought tolerance and xylem hydraulic efficiency (Table 1) to determine how the four most common Cistus species in Montado

Symbol/abbreviation	Definition	Units employed
d _h	Hydraulically weighted vessel diameter	μm
g _s	Stomatal conductance	mol m ⁻² s ⁻¹
Kp	Potential specific hydraulic conductance	kg m ⁻¹ MPa ⁻¹ s ⁻¹
P ₁₂	Pressure at which 12% loss of conductivity occurred	MPa
P ₅₀	Pressure at which 50% loss of conductivity occurred	MPa
P ₈₈	Pressure at which 88% loss of conductivity occurred	MPa
PLC	Percentage loss of conductivity	%
SWC	Soil water content	%
$T_{\rm W} D_{\rm T}^{-1}$	Thickness-to-span ratio of vessels = intervessel wall thickness (T_W) divided by vessel lumen diameter (D_T)	-
VF	Vessel frequency	vessels mm ⁻²
V _G	Vessel grouping index	_
Ψ_{mid}	Midday water potential	MPa
ρ	Wood density	$g cm^{-3}$

Table 1. List with major variables evaluated with definition and units employed.

differ in (i) ecological niche, (ii) anatomical traits and (iii) physiological performance, and (iv) how the different traits and performance correlate within this genus. The aim was to provide key information on the drought resistance and water-use strategy of one of the most widely distributed genera in the Mediterranean region.

Materials and methods

Plant material and study area

The study was focused on four *Cistus* species co-occurring in the Montado ecosystem: *C. ladanifer* (semi-ring-porous,



Figure 1. (A) Monthly mean air temperatures (T, solid line), rainfall (columns) and vapour pressure deficit (VPD, dashed line) values from March to November 2012 recorded for the study area; (B) soil water content (SWC, %) at 0–10 cm and 10–20 cm soil depths beneath shrub canopies of the different *Cistus* species measured during the year 2012. Columns indicate mean \pm standard error (n = 4). Means for the same date and depth followed by different letters are significantly different (P < 0.05).

multistem although some individuals show a short main stem), C. monspeliensis (diffuse-porous, multistem), C. populifolius (diffuse-porous, multistem) and C. psilosepalus (diffuseporous, multistem). The Montado is an agro-silvo pastoral ecosystem located in the Mediterranean basin and dominated by cork and holm oak trees (Quercus suber L. and Quercus rotundifolia Lam., respectively), shrubs and grasses. The dominant plant formation of the area of study is a scrubland belonging to Hyacinthoido-Quercetum cocciferae. The scrubland patches are dominated by C. salvifolius and C. ladanifer, which account for >70% of the community cover. The subordinate species in the community, which account around 10-20%, are mostly evergreen sclerophylls and drought semi-deciduous shrubs, including C. monspeliensis C. populifolius and C. psilosepalus. Its high rainfall inter-annual variability results in frequent occurrence of drought events (Pereira and Paulo 2004) that strongly constrain the growth and productivity of this ecosystem (Mooney et al. 1974, Gratani and Varone 2006).

The study was carried out in southern Portugal, close to Évora, (38°32'N; 8°01'W; 240 m above sea level). The area has the typical winter-wet, summer-dry pattern of the Mediterraneantype climate, with a mean annual rainfall of 609.4 mm a mean annual temperature of 15.9 °C (climatological normals for 1971–2000, Figure 1) and a dry period that lasts up to 5 months (Simões et al. 2008). In terms of bioclimatic classification (Rivas-Martínez et al. 2004), it is located in the MesoMediterranean, lower dry to subhumid belt of the Mediterranean pluviseasonaloceanic bioclimate and biogeographically stands as the Lusitan-Extremadurean Province of the Mediterranean region. The landscape is gently undulating with slopes varying from 3 to 8% and the geological substratum consists of granites and gneisses (Carvalhosa et al. 1969). The soils are developed from granites and correspond to dystric Leptosols and dystric Cambisols (WRB 2006), with loam sandy to sandy loam texture (Driessen et al. 2001). A weather station was installed in the study area for measurements of dry and wet bulb temperatures with a hygro-Thermo Transmitter, Germany and rainfall with a tipping Bucket Rain Gauge (model 52203, R. M. Young Company, Traverse City, Michigan, USA). Average values taken over 60 min were recorded with a CR10 data logger (Campbell Scientific, Logan, UT, USA). Air vapour pressure deficit was calculated from dry and wet bulb temperatures.

The individuals selected for this study were all between 10 and 15 years old at the time of the study. The distance between individuals of the same species was between 0.5 and 1 m, whereas between individuals of different species was between 50 and 500 m. The mean height and diameter of the canopy for those individuals selected for the study were 142.50 ± 19.12 and 89.50 ± 8.06 cm, respectively. Soil water content (SWC, %) was determined gravimetrically (Rundel and Jarrell 1989) in April, June, September and October 2012. On each sampling date, three soil samples were collected beneath four different

individuals of each *Cistus* species at 0–10 and 10–20 cm soil depth. Sample cores were of 4.5 cm (diameter) \times 10 cm (length) = 158.96 cm³ (volume). Each sample was then weighed to determine the wet soil mass (wm), dried at 105 °C during 48 h, and weighed again to determine the dry soil mass (dm). The SWC was then calculated on a dry weight basis:

$$SWC = \frac{(wm) - (dm)}{(dm)} \times 100$$
(1)

Xylem anatomical traits

Different xylem anatomical traits were evaluated in three mature stems (dolichoblasts) from three different individuals per species sampled in December 2013. All samples were between 0.5 and 1.0 cm in diameter and were the same ones used for determining the vulnerability to embolism formation (more details about their sampling below). Semi-thin transverse sections (~20 μ m thick) were prepared from the central part of each xylem sample (i.e., at 14 cm far from the samples ends) and using a rotating microtome (Leica RM2165 and R35 type blades, Leica Biosystems, Nanterre, France). Sections were stained by dual staining with Safranin and Astra Blue (Chaffey 2002) and observed under an optical microscope (transmitted light, Zeiss Axioplan 2, Zeiss, Jena, Germany) with x40 magnification. Images were recorded using a digital camera (AxioCam HR, Zeiss) with AxioVision digital imaging software. By using the 'mosaic' tool, a single image per sample was constructed by joining images with the same magnification. After spatial calibration, anatomical measurements were performed by image analysis. Automatic segmentation enabled isolation of the vessels, and vessel frequency (VF, vessels mm^{-2}), vessel grouping index ($V_{\rm G}$), hydraulically weighted vessel diameter ($d_{\rm h}$, μ m) and potential specific hydraulic conductance (K_p , kg m⁻¹ MPa⁻¹ s⁻¹) were determined for three cross-sections per species using Adobe Photoshop CS2 9.0 (Adobe Systems Incorporated, San Jose, CA, USA) and ImageJ software (Rasband 2015). $V_{\rm G}$ is defined as the ratio of total number of vessels (N_{vessels}) to total number of vessel groupings (including solitary and grouped vessels, N_{groupings}) and it was calculated according to Carlquist (2001) as follows:

$$V_{\rm G} = \left(\frac{N_{\rm vessels}}{N_{\rm groupings}}\right) \tag{2}$$

 $K_{\rm p}$ (sensu Tyree and Ewers 1991) was calculated by adding up the conductivities of the conduits found in the cross-section, using the Hagen–Poiseuille equation to calculate the conductivity of every single conduit:

$$K_{\rm p} = \frac{\pi r^4}{8\eta} \tag{3}$$

where *r* is the internal radius of the conduit and η the dynamic viscosity of water taken as 10^{-9} MPa s at 20 °C. $K_{\rm p}$ values were normalized by the cross-section area (pith and bark were removed from the computation when present) and converted to sample volume by multiplying them with 0.998 g cm⁻³, which is the density of water at 20 °C. The vessel-diameter distribution per species was determined after classifying the vessels into bin diameters (diameter size classes of 2 µm width). A minimum of 400 vessels were analysed per cross-section. The thickness-tospan ratio was also estimated as a measure of vulnerability to implosion as opposed to vulnerability to embolism. It corresponds to $(t_w b^{-1})$, where t_w is the double-wall thickness and b the vessel lumen diameter. The double-wall thickness was determined from the shared walls of at least 15 and up to 30 vessels per species. Fibre wall thickness was measured in a minimum of 30 fibres per section.

Wood density (ρ , g cm⁻³) was measured in a 3-cm long and 0.5–1.0 cm diameter stem segment per individual, in seven individual per species. Samples were debarked and submersed in water in order to measure its volume displacement according to Archimedes' principle. The displacement weight was converted to sample volume by multiplying it by density of water at 20 °C (i.e., 0.998 g cm⁻³). Samples were then stored at 75 °C for 48 h, and their dry weight was measured afterwards. The ρ was calculated as the ratio of dry weight to fresh volume.

Water potential and stomatal conductance

Midday leaf water potential (i.e., at midday solar time, Ψ_{mid}) was measured on a monthly basis, from April to November 2012, with a Scholander-type pressure chamber (PMS 1000, PMS Instruments, Albany, Oregon, USA). A total of 12 leaves were measured per species (three leaves per plant and four different plants per species). All leaves were taken in the south-facing side of the crown and at similar heights above the ground to minimize variability due to any possible heterogeneity in light conditions, and immediately placed in a plastic bag to prevent further transpiration before the measurements that were carried out just after the sampling.

Leaf gas exchange measurements were carried out to determine the stomatal conductance (g_s , mol m⁻² s⁻¹) for each species. Measurements were done with a portable steady-state photosynthetic system (Li-6400; Li-Cor, Lincoln, NE, USA) in three seasons of 2012: spring (in April/May, i.e., before the drought period), mid-summer (in August, i.e., during the drought period) and autumn (in November, i.e., after the drought period) on six current-year and fully expanded leaves from six different individuals per species (one leaf per individual). All measurements were done during the morning (i.e., from 9:00 to 10:00 am) and under natural environmental conditions.

Embolism resistance

For determining the resistance to embolism of the four Cistus species, 1-m long leafy shoots (one shoot per individual in three individuals per species) were cut in the air early in the morning and immediately wrapped in wet paper and enclosed in airtight plastic bags to stop transpiration. Samples were shipped to the INRA-PIAF laboratory (Clermont-Ferrand, France) where they were stored at 5 °C and processed within 1 week. Once in the laboratory, 280-mm long segments were excised from shoots under water and both ends debarked and trimmed with a fresh razor blade. Sample diameter was measured in three places, at both ends and halfway along each stem segment. Vulnerability to embolism was assessed with the Cavitron technique (Cochard et al. 2005). Briefly, the technique uses centrifugal force to decrease the water pressure in the sample while, at the same time, the decrease of its hydraulic conductance is measured. It is now well-documented that the presence of open vessels causes artifactual results with all centrifuge methods (Cochard et al. 2010, 2013, Torres-Ruiz et al. 2014, Choat et al. 2016). To avoid biased results, we removed the open vessels from the analysis by infiltrating the samples with air at low pressure (0.15 MPa). This empties (i.e., fills with air) any possible vessel that could be opened at both sample ends. To check if the amount of functional vessels after the air-infiltration were high enough for an accurate determination of the resistance to embolism, branches were placed in an X-ray microtomograph (Nanotom 180 XS, GE, Wunstorf, Germany) at the INRA-PIAF laboratory and scanned at their middle part. X-ray scans provided 3D images of the internal structure of the sample that allowed us to determine the number of vessels that remained conductive after air infiltration (see Figure S1 available as Supplementary Data at Tree Physiology Online for more details). Percentages of functional vessels were higher than 85% in for all the samples, ensuring the accuracy of the results obtained with the Cavitron technique. For computing the vulnerability curves, the maximum sample conductivity (K_{max}) was first measured at low speed and relatively high xylem pressure (-1.0 MPa). The xylem pressure was then decreased stepwise by increasing the rotational velocity, and the conductivity (K) measured at each pressure step. Sample loss of conductivity (PLC, %) was computed as follow:

$$PLC = 100 \times \left(1 - \frac{K}{K_{max}}\right)$$
(4)

The relationship between PLC and the water xylem pressure induced by centrifugation can be described by the following sigmoidal equation (Pammenter and Vander Willigen 1998):

$$PLC = \frac{100}{(1 + e^{a/25(P - P_{50})})}$$
(5)

where a is the slope of the curve at the inflection point, and P_{50} represents the pressure at which 50% loss of conductivity

occurred. Following Domec and Gartner (2001) we estimated the P_{12} value (i.e., xylem pressure at PLC = 12%), which is considered as the xylem pressure at which embolism formation is initiated, and the P_{88} value, considered to be the point of no return or lethal xylem pressure for angiosperms (i.e., xylem pressure at PLC = 88%; Anderegg et al. 2012, Urli et al. 2013).

Statistical analyses

To evaluate differences in xylem structure and physiological traits amongst the four Cistus species and due to the reduced sample size, a Mann-Whitney U test was used to compare VF, $K_{\rm p}$, $(t_{\rm w} b^{-1})^2$ and fibre wall thickness between species. A oneway analysis of variance (ANOVA) was used to compare ρ between species and for comparing differences in SWC between the areas occupied by the different species within each evaluated month. When the differences were significant, a multiple comparison of means (post hoc Tukey honest significant difference test) was carried out. For minimum mean Ψ_{mid} and g_{s} , comparisons were made with two-way ANOVA testing for month, species and month x species effects. The two-sample Kolmogorov-Smirnov test (Sokal and Rohlf 1995) was used to check possible differences in the vessel-diameter distributions of the four Cistus species. P_{12} , P_{50} and P_{88} values were considered significantly different between species when their 95% confidence intervals did not overlap. To evaluate the patterns of correlations between traits and performance, linear regression analyses and Pearson correlation coefficients were used to quantify association between pairs of variables.

Results

The time courses of all the climate variables during the experimental period were typical for the area, with a wet, mild season from October to April and a hot, dry one from mid-May to September (Figure 1A). SWC varied significantly among the sites occupied by the different *Cistus* species (Figure 1B), with *C. populifolius* occupying areas that remained wetter, even during the dry season, than those occupied by the other *Cistus* species.

Results from the xylem anatomical traits analyses reported no significant differences in VF between the four *Cistus* species evaluated (Figure 2). *Cistus populifolius* and *C. psilosepalus* showed similar ρ values between them (0.67 ± 0.01 and 0.65 ± 0.01 g cm⁻³, respectively) but different from *C. ladanifer* and *C. monspeliensis* (0.76 ± 0.01 and 0.75 ± 0.01 g cm⁻³, respectively) (Figure 2). Whereas all species showed similar thickness-to-span ratio, they showed significant differences in their fibre wall thickness, with *C. monspeliensis* and *C. psilosepalus* showing the highest (4.91 ± 0.16 µm) and lowest (2.37 ± 0.13 µm) mean values, respectively. Xylem vessel-diameter distributions showed that most of the vessels for each species showed small diameters (between 6 and 15 µm in diameter),



Figure 2. Mean \pm standard error values for vessel frequency (VF, n = 3), mean wood densities (ρ , n = 7), fibre wall thickness (n = 3), thickness-to span-ratio (($t_w b^{-1}$)², n = 3), vessel grouping index (V_G , n = 3, a minimum of 90 vessels were measured per sample) and potential specific hydraulic conductance (K_{ρ} , n = 3) per species. Different letters indicate statistical differences between species (P < 0.05). n.s. = no significant differences between species.

with only very few vessels having a diameter greater than 30 μ m (Figures 3 and 6). Significant differences were obtained in vessel-diameter distribution between the four *Cistus* species (*P* < 0.001), with *C. monspeliensis* showing an important proportion of vessels in between 16 and 26 μ m in diameter. Despite this, all species showed similar V_G, *d*_h and *K*_p (Table 2 and Figure 2).

The $\Psi_{\rm mid}$ displayed the typical seasonal trend with the lowest values recorded between July and August, showing significant differences across species. Cistus ladanifer, C. monspeliensis and C. populifolius reached minimum mean Ψ_{mid} values below -4.0 MPa, whereas C. psilosepalus was able to maintain its minimum mean value at -2.6 ± 0.1 MPa (Figure 4A). All the species recovered their water status after the first rainfall events in September, showing water potential values ≥ -1.5 MPa, i.e., similar to those registered during the spring before the dry period evaluated. Despite the fact that all the species showed mean $\Psi_{\rm mid}$ values within a narrow range of values (from -1.6 to -2.3 MPa) before the dry period, they already showed significant differences in g_s between them (Figure 4B). Thus, C. ladanifer and C. psilosepalus showed higher mean g_s values (0.46 and 0.54 mol m⁻² s⁻¹, respectively) than *C. monspeliensis* and *C.* populifolius (0.02 and 0.09 mol $m^{-2} s^{-1}$, respectively). In August, during drought, all the species experienced a strong



Figure 3. Xylem vessel diameter distribution per species. Different letters in brackets indicate statistical differences between species (P < 0.001). A minimum of 400 vessels were analysed per cross-section, in three different sections per species.

reduction in g_s , showing values below 0.01 mol m⁻² s⁻¹. In November, after the drought period and once all the species showed mean Ψ_{mid} values between -1.5 and -1.1 MPa, *C. ladanifer* and *C. psilosepalus* still showed higher g_s values than *C. monspeliensis*

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Table 2. Mean \pm standard error values of vessel grouping index (V_G, n = 3), hydraulically weighted vessel diameter (d_{h} , n = 3), potential specific hydraulic conductance (K_{p} , n = 3) and minimum mean leaf water potential (Ψ_{mid} , n = 12) for each species. The xylem pressure at which embolism formation is initiated (P_{12}), water potential corresponding to 50% loss of conductivity (P_{50}) and the pressure causing a drastic hydraulic failure (P_{88}) were calculated from the vulnerability curves obtained for each species. SM indicates the hydraulic safety margins for each species (i.e., minimum $\Psi_{mid} - P_{50}$). Asterisks indicate significant differences (SD) between species (P < 0.05 for Min Ψ_{mid} , and non-overlapping confidence intervals for P_{12} , P_{50} and P_{88}). n.s. = non-significant differences between species.

Species	<i>d</i> _h (μm)	Min $\varPsi_{\sf mid}$ (MPa)	P ₁₂ (MPa)	Р ₅₀ (МРа)	P ₈₈ (MPa)	SM (MPa)
C. ladanifer	18.5 <u>+</u> 3.2	-4.6 ± 0.1a	-7.9a	-8.9a	-9.9a	4.3
C. monspeliensis	23.3 <u>+</u> 1.3	-4.5 <u>+</u> 0.4a	-8.3a	-10.2b	-12.0b	5.7
C. populifolius	22.8 <u>+</u> 0.9	-4.2 <u>+</u> 0.1a	-7.2b	-8.1c	-9.0c	3.9
C. psilosepalus	22.2 <u>+</u> 1.2	-2.6 ± 0.1b	-4.9c	-6.5d	-8.1d	3.9
SD	n.s.	*	*	*	*	



Figure 4. Time courses of (A) minimum leaf water potential measured at midday (Ψ_{mid}) and (B) stomatal conductance (g_s) measured in spring, mid-summer and autumn from 9:00 to 10:00 am. Each data point represents an average of nine and six values for Ψ_{mid} and g_s , respectively; vertical bars represent \pm the standard error. Asterisks and different letters indicate significant differences between species (P < 0.05). n.s. = no significant differences.

and *C. populifolius*, although differences were only significant between these two former species and *C. psilosepalus*. Thus, and for g_s and Ψ_{mid} , the two-way ANOVA not only reported significant

Table 3. Correlations in *Cistus* species between pairs of functional and anatomical traits with different parameters related with embolism resistance. Correlations are at individual level for vessel frequency (VF) and K_p are at individual level (n = 12), and at species level for the minimum mean Ψ_{mid} value (n = 4) and wood density $(\rho, n = 4)$. Values indicate the Pearson correlation coefficients (r) and the level of significance (P), respectively. Bold values indicate significant correlations at P < 0.05.

Variables	P ₁₂	P ₅₀	P ₈₈
VF (vessels mm ⁻²)	0.744; 0.256	0.902; 0.098	0.970; 0.03
$K_{\rm p} ({\rm kg m^{-1} MPa^{-1} s^{-1}})$	-0.114; 0.886	-0.269; 0.731	-0.386; 0.614
Min mean, $arPsi_{mid}$ (MPa)	0.982; 0.018	0.877; 0.123	0.709; 0.291
$\rho \text{ (g cm}^{-3})$	-0.842; 0.158	-0.869; 0.131	-0.821; 0.179

differences between species and months, respectively (P < 0.01 in both cases, see Table S1 available as Supplementary Data at *Tree Physiology* Online), but also a significant interaction between month and species (P < 0.01).

The four *Cistus* species were highly resistant to embolism, with P_{50} values below -6.5 MPa. Substantial significant differences were observed between them, with P_{50} values ranging from -6.5 to -10.2 MPa for *C. psilosepalus* and *C. monspeliensis*, respectively (Table 2 and Figure 5). Similarly, both P_{12} and P_{88} also showed considerable differences between species with values that ranged between -4.9 and -8.3 MPa, and between -8.1 and -12.0 MPa, respectively. The differences between species both in P_{50} and minimum mean Ψ_{mid} resulted in important differences in hydraulic safety margins, i.e., the difference between both traits. Thus, *C. monspeliensis* showed the widest hydraulic safety margin (5.70 MPa) whereas *C. psilosepalus* and *C. populifolius* showed the narrowest one (3.90 MPa for both species) (Table 2) (Figure 6).

The correlations between xylem traits and performance showed how those species in which embolism formation starts at higher xylem tensions (i.e., lower P_{12} values) are able to reach lower Ψ_{mid} during the drought season (Table 3). In fact, the safety margins considering P_{12} instead of P_{50} ranged from 2.3 to 3.9 MPa for *C. psilosepalus* and *C. monspeliensis*, respectively. A significant correlation was also observed between the hydraulic safety margin and lethal water potential



Figure 5. Vulnerability curves to embolism for each species represented as the percentage loss of conductivity (PLC, %) versus xylem pressure (MPa). Data points represent the mean value of three samples; vertical bars represent \pm standard error. Each curve is fitted according to Eq. 5. Red areas indicate the 95% confidence intervals for each curve.



Figure 6. Xylem anatomical cross-sections of *C. ladanifer* (A), *C. populi-folius* (B), *C. psilosepalus* (C) and *C. monspeliensis* (D). Scale bar represents 100 µm.

Table 4. Significant correlations in *Cistus* species between pairs of functional and anatomical traits. Traits: minimum leaf water potential (Ψ_{mid}), xylem pressure at which embolism formation is initiated (P_{12}), lethal water potential (i.e., xylem pressure at PLC = 88%; P_{88} , MPa), hydraulic safety margins and fibre wall thickness. Values indicate the Pearson correlation coefficients (r) and the level of significance (P) respectively.

	P ₁₂ (MPa)	P ₈₈ (MPa)	Fibre wall thickness (µm)
Minimum Ψ_{mid} (MPa)	0.982; 0.018	_	-
Hydraulic safety margin (MPa)	_	-0.961; 0.039	_
P ₈₈ (MPa)	-	-	-0.966; 0.034

(i.e., P_{88}), and between P_{88} and the thickness of the fibre wall. Thus, species with lower P_{88} values showed thicker fibre walls (Table 4).

Discussion

Differences in anatomical and physiological traits between species, as well as in the amount of water available in the sites they occupy, suggest different water-use strategies and ecological niches for the four *Cistus* species that co-occur in the Montado ecosystem. This constitutes key information for improving the predictions about the changes in vegetation dynamics in this area.

Vessel-diameter distributions differed for the four Cistus species evaluated. Although this could induce differences in hydraulic efficiency as well, they all showed similar K_{p} values. This, and the fact that K_p and P_{50} do not show a significant correlation between them, indicates that hydraulic efficiency does not trade-off against the safety of the xylem within or across species. This agrees with recent results reported by Gleason et al. (2016) testing this hypothesis across 335 angiosperm and 89 gymnosperm species. They showed that a considerable number of species show both low efficiency and low safety, which suggests that the xylem safety-efficiency trade-off may not have contributed to the divergence of all plant species. Although our study does not concern the cause of the differences in resistance to embolism across these Cistus species, the lack of a safetyefficiency trade-off suggests that such differences would be probably due to differences, e.g., at the intervessel pit membrane level (e.g., thickness, Li et al. 2016, microstructure or pore diameters, Scholz et al. 2013, Tixier et al. 2014) or in those traits related to mechanical strength (Lens et al. 2011) more than to the vessel sizes. Our results, therefore, do not support the 'rare pits' hypothesis that postulates a link between xylem efficiency and safety in angiosperms, i.e., wider and, therefore, more efficient vessels should be more vulnerable to embolism (Christman et al. 2012). Contrary to this hypothesis, our results in Cistus showed that species with higher percentages of narrower vessels are more vulnerable to embolism and that the one showing the highest proportion of large conduits (C. monspeliensis) was actually the species most resistant to embolism.

Interestingly, resistance to embolism was not related to ρ . The relevance of this finding lies in the fact that a theoretical link between both traits could be expected on the basis of avoiding conduit implosion. Also, as resistance to embolism is closely linked to the thickness of the intervessel pit membrane, species with higher resistances and, therefore, thicker pit membranes (Li et al. 2016), would show higher wood densities. In fact, a significant correlation between them has been already reported for larger species datasets (Hacke and Sperry 2001), although there are also some studies showing that, in angiosperms, wood density is mainly driven by the density of wood outside vessel

lumens (Zieminska et al. 2013, 2015). The lack of a significant correlation in our study could be due to the fact that only four species within the same genus were evaluated, so stronger correlations between traits would be expected when more species and from different genera are evaluated. A significant correlation was, however, observed between the fibre wall thickness and the lethal water potential for the four Cistus species. The important role of the fibre wall thickness on embolism resistance has been previously reported at an interspecific level (Jacobsen et al. 2005) but, to our knowledge, this is the first time that such correlation has been shown within a single genus, which reinforces and enhances its importance as a potential embolism resistance indicator. The good correlation between both traits is probably based on the key role that fibres play in reinforcing conduit walls to avoid conduit implosion or collapse (Cochard et al. 2004, Brodribb and Holbrook 2005). However, recent studies in conifers have shown that xylem collapse is unlikely to occur under field conditions, since the seasonal minimum water potential values are generally less negative than the negative pressure needed to cause tracheid wall implosion (Bouche et al. 2014, 2016). Whether this is also the case for angiosperms remains unknown, requiring further micromorphological analyses to elucidate what is the role of the fibre wall thickness on embolism resistance in both groups of plants.

The marked differences in plant water status and gas exchange observed during the drought period, even between species occupying places with a similar water availability, and the differences in embolism resistance, suggest different wateruse strategies between the four Cistus species. This is the case, for example, for C. populifolius and C. psilosepalus. While C. populifolius showed a low g_s rate before the drought period despite occupying areas with relatively high SWC, co-occurring C. psilosepalus individuals were able to maintain higher q_s rates and less negative $\Psi_{\rm mid}$ despite being located in areas of with lower SWC. In fact, it can be hypothesized that apparent differences in water-use strategies could explain the differences in the ecological niches they occupy. Contrasting water-use strategies have been already reported both among co-occurring species, including Mediterranean shrubs, from diverse taxonomic groups (Hernandez et al. 2010, Quero et al. 2011, Moreno-Gutierrez et al. 2012, Lázaro-Nogal et al. 2013) as well as within a single genus (David et al. 2007, Tognetti et al. 2007). The coexistence of different strategies within a certain area or ecosystem has been typically related to differences in plant functional traits (Filella and Peñuelas 2003). This is in accordance with our results showing a significant correlation between the minimum $\Psi_{\rm mid}$ reached during the dry season and the xylem pressure at which PLC starts to increase rapidly (P_{12}) . The scaling factor between both traits indicates that an important increase in resistance to embolism is required to allow plants to reach lower $\Psi_{\rm mid}$ during the season. The four species always maintained their $\Psi_{\rm mid}$ well above their P_{12} values during the

season, thanks probably to the strong reductions in g_s observed during the drought period for all the species. Such reductions in g_s indicate a tight control of the g_s to avoid critical decreases in water potential that would increase the degree of xylem embolism and, therefore, induce drastic reductions of their hydraulic function. Avoiding the formation of embolism in the xylem is crucial for plant survival during drought, especially when recent evidence for a lack of xylem refilling under tension has been reported (Charrier et al. 2016). The control of the plant water status through an effective control of the stomatal behaviour has been already observed both in other Mediterranean (e.g., Q. ilex, Mediavilla and Escudero 2003, Olea europaea, Cuevas et al. 2010, Torres-Ruiz et al. 2013, 2015) and non-Mediterranean species (Actinidia deliciosa, Torres-Ruiz et al. 2016). Within the genus Cistus, previous studies also support such tight stomatal control by reporting quick responses of the q_s and other photosynthetic traits in response to water stress for different species such as Cistus albidus (Galle et al. 2011), Cistus incanus (Bombelli and Gratani 2003) and Cistus clusii (Vilagrosa et al. 2014).

Apart from the different water-use strategies, differences in embolism resistance and hydraulic safety margin also suggest a different effectiveness in drought avoidance and tolerance between species. Wide hydraulic safety margins indicate a certain capacity for regulating the transpiration by the plant, allowing them to operate at xylem water potentials far enough from the P_{12} value to prevent runaway xylem embolism (Sperry et al. 2002). During a drought episode, species with larger hydraulic safety margins would have higher chances of survival since they would be more competitive than those with narrow margins, which would succumb first (Martinez-Vilalta et al. 2002, Choat et al. 2012). Our results show how those Cistus species showing lower lethal water potential values also show larger hydraulic safety margins, which make them more efficient in avoiding catastrophic hydraulic failures during periods of particularly severe drought and, therefore, more tolerant to drought. Interestingly, C. monspeliensis showed a wider hydraulic safety margin than C. populifolius, despite occupying sites with lower water availabilities.

The significant correlation between VF and P_{88} (i.e., lethal water potential) across species shows how species with low VF values are more resistance to embolism than those with higher VFs. This highlights how important the xylem organization is for embolism spreading, since higher VF values increase intervessel connections (Loepfe et al. 2007) and, as this favours the spreading of embolisms from vessel to vessel, make the xylem tissue more vulnerable to embolism (Brodersen et al. 2013). This correlation between VF values and P_{88} observed for *Cistus* is particularly relevant considering that previous studies within other plant genera did not find any correlation between both traits (Lens et al. 2011, Scholz et al. 2013, Hajek et al. 2014). These contrasting results indicate that the relevance of VF on resistance to embolism can vary largely among genera, being probably linked to differences in the structure of the vascular

system (i.e., diffuse-porous or ring-porous trees) and, in some cases, the vessel arrangement, although this seems not to be the case for *Cistus* since no differences in $V_{\rm G}$ were observed between species. At a local scale, co-existing species with different water-use strategies usually exhibit spatial or temporal differentiation in microhabitat, resource use or other factors, i.e, different niches for each of them that allow their co-occurrence (Ackerly et al. 2006). Such local differences along with the differences in embolism resistance, lethal water potential, stomatal behaviour and hydraulic safety margins have important implications for species distribution and dynamics.

Conclusions

Different water-use and drought-tolerance strategies were observed for the four co-occurring Cistus species in the Montado ecosystem that would make them face and withstand drought differently. They all showed differences in their vessel diameter distribution despite showing similar hydraulic efficiencies. Different resistances to embolism were observed for each species; however, no trade-off between hydraulic efficiency and safety was observed, suggesting that the xylem safety-efficiency trade-off does not contribute to the species divergence in this genus. Species with lower lethal water potential values showed larger hydraulic safety margins, which also contribute to increase their tolerance to drought. The differences in habitat and functional traits observed for the Cistus species co-existing in the Montado constitute key information for evaluating the link between species responses to drought and species dynamics and, therefore, for predicting changes in vegetation dynamics and determining the best management practices to mitigate the ecological consequences of ongoing climate change.

Supplementary Data

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

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Conflict of interest

None declared.

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