Physiological differences explain the co-existence of different regeneration strategies in Mediterranean ecosystems

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Summary

- Fire and drought are selective driving forces in Mediterranean plants, and thus their ability to resprout or recruit after these disturbances is of paramount importance. The contrast in regeneration niche between resprouters and seeders, and each group's different root characteristics, suggest that they are subjected to different degrees of environmental stress and, consequently, to different evolutionary forces.
- We compared leaf traits, xylem traits related to hydraulic efficiency and vulnerability to cavitation, and the physiological response to an imposed drought between seedlings of resprouters and seedlings of seeders. We used 12 species co-existing in Mediterranean basin ecosystems.
- Major differences were found in the xylem architecture and leaf traits, and in the response to drought conditions. Seeders were more efficient at transporting water to leaves but formed, in turn, a safer xylem network. They also presented higher photosynthesis and transpiration rates, and earlier stomatal closure with drought, but good leaf dehydration tolerance.
- Seeders and resprouters can be considered two syndromes whose different functional characteristics are related to water availability and drought responses. These characteristics, together with their differences in rooting habits, account for their distinct regeneration niches and, thus, their co-existence.

Introduction

Fire is an ancient ecological process in many terrestrial ecosystems (Pausas & Keeley, 2009; Pausas & Schwilk, 2012), and it has determined many aspects of the ecology and evolution of Mediterranean plants (Keeley *et al.*, 2011, 2012). The abilities to resprout and to recruit after fire are two extremely important traits for persistence in fire-prone ecosystems (Bond & van Wilgen, 1996; Pausas *et al.*, 2004; Keeley *et al.*, 2012), and they define three life histories: obligate resprouters, obligate seeders (nonresprouters), and facultative species. After a fire, obligate seeders die and recruit profusely from the seeds stored in the seed bank (Keeley & Fotheringham, 2000; Paula & Pausas, 2008; Moreira & Pausas, 2012; Moreira *et al.*, 2012). By contrast, resprouters survive after fire and their above-ground tissues regenerate from protected (often below-ground) buds by using stored carbohydrates (Verdaguer & Ojeda, 2005; Moreira *et al.*, 2012). Facultative species not only recruit profusely after fire, but are also able to resprout. In fact, seeders and resprouters have different regeneration niches (Grubb, 1977; Silvertown, 2004): the seedling regeneration of obligate resprouters is not linked to fire, and they recruit during the inter-fire period under sheltered conditions (i.e. under vegetation cover), while the seedling regeneration of seeders occurs in open post-fire environments (Keeley, 1998; Pausas *et al.*, 2004; Keeley *et al.*, 2012). Given the marked difference in water availability between microsites under vegetation and microsites open to the sun under Mediterranean conditions, the seedlings of resprouters and seeders are subjected to different water-stress conditions. Despite these differences, resprouters and seeders co-exist, are often well mixed on local and landscape scales (Verdú & Pausas, 2007; Ojeda *et al.*, 2010), and represent the two main types of post-fire regeneration strategies in Mediterranean ecosystems (Bond & Midgley, 2001; Keeley *et al.*, 2012).

Mediterranean species display a wide array of functional responses in relation to efficient carbon assimilation, water use and overall resistance to drought conditions (Acherar & Rambal, 1992; Vilagrosa *et al.*, 2003a,b; Hernández *et al.*, 2010; West *et al.*, 2012), and previous studies have indicated that some of these responses are related to the regeneration strategy. It has been suggested that seeders are less vulnerable to cavitation (Jacobsen *et al.*, 2007; Pratt *et al.*, 2010, 2012) and display better leaf structural resistance to drought, greater water-use efficiency at the leaf level (Paula & Pausas, 2006; Saura-Mas & Lloret,

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2007), a more efficient root system (Paula & Pausas, 2011), and a higher carbon gain during periods of high water availability (Hernández *et al.*, 2011) than co-existing resprouters. Conversely, resprouters survive fire and starvation by using their carbohydrates stored in roots (Moreira *et al.*, 2012). However, leaf and root traits are not the only indicators of plant performance, as xylem traits and their hydraulic structure are vitally important because they are closely connected to the water-use strategy and performance of a plant in a given environment (e.g. Gleason *et al.*, 2012 and references therein). Xylem resistance to cavitation is considered a key mechanism of plant adaptation to dry environments and to periods of intense drought conditions (Brodribb & Cochard, 2009; Vilagrosa *et al.*, 2010; Lens *et al.*, 2013) because it is strongly related to resistance to drought-induced embolism (Carlquist, 2012; Scholz *et al.*, 2013). In addition, hydraulic traits impose fundamental constraints to the gas exchange of plants under well-watered conditions, but also under drought conditions (Sperry *et al.*, 1998; Oliveira, 2012). Therefore, plant responses to water shortage can determine the chances of seedling survival within the framework of the current knowledge (i.e. xylem cavitation and starvation; McDowell, 2011; McDowell *et al.*, 2011; Plaut *et al.*, 2012). Nonetheless, they are also related to the efficiency of carbon gain and water use (Galmés *et al.*, 2007; Medrano *et al.*, 2008).

Our hypothesis is framed in the context that differential regeneration niche and the diversity of functional adaptive traits may promote co-existence of different functional groups (Grubb, 1977; Ackerly, 2004a; Silvertown, 2004). Specifically, we hypothesize that the different physiological characteristics for capturing resources and for overcoming drought stress at early stages between resprouters and seeders allow the co-existence of these two strategies in Mediterranean ecosystems. As seeders recruit in post-fire gaps (open conditions), seedlings and saplings are subject to strong oscillations in water availability and to high incident sunlight. Despite these harsh conditions, they need to grow quickly and to mature early in order to avoid the risk of immaturity (*sensu* Zedler, 1995); that is, to ensure seed production before the next fire (Pausas *et al.*, 2004). By contrast, obligate resprouters recruit under more favourable conditions (non-post-fire periods), where vegetation acts as a shelter by ameliorating hard abiotic conditions such as high light intensity and oscillations in water availability (Keeley, 1998). In addition, resprouters allocated more resources to below-ground organs with deep roots systems, while seeders allocated resources mainly to above-ground growth with shallow root systems (Pausas *et al.*, 2004; Verdaguer & Ojeda, 2005). This large allocation of resources by resprouters to the root system allows not only access to more stable deep water resources, but the accumulation of reserves to sustain resprouting after disturbance (Ackerly, 2004b; Verdaguer & Ojeda, 2005; Moreira *et al.*, 2012). In contrast, shallow-rooted species are subjected to major water oscillations all year long (Ackerly, 2004b). Consequently, we predict that seeders should possess functional and physiological traits that relate to greater resistance to water oscillations than resprouters. By contrast, obligate resprouters should show physiological traits that focus on water-use efficiency as they are able to gain more reliable access to deep soil water by minimizing water oscillations. In order to test our hypothesis, we compared plant functional traits related to the efficiency and safety of the use of water, and to the capacity to maintain a suitable water status, between the resprouting and seeding species that co-exist in Mediterranean ecosystems. We also analysed the leaf functional traits deriving from pressure–volume (*P*–*V*) curves (cell–water relationships) to compare the leaf hydration and stomatal dynamics of the two groups. Finally, we subjected seedlings from both groups to a drought cycle, during which we monitored the key variables relating to gas exchange, water potential and degree of leaf hydration. Understanding different plant functional responses to drought in co-existing species would provide important insights enabling the prediction of the fate of Mediterranean communities in a world subjected to global warming.

Materials and Methods

Plant material

We selected 12 common species that co-exist in fire-prone ecosystems in the western Mediterranean basin, including seeders and resprouters (Table 2). The resprouting and post-fire seeding abilities of each species were assigned according to the BROT database (Paula *et al.*, 2009; www.uv.es/jgpausas/brot) and personal observations. All the seeders were unable to resprout after disturbance (i.e. obligate seeders), and accumulated a seed bank in the soil; they presented fire-stimulated germination and recruited profusely after fire (Paula *et al.*, 2009; Moreira *et al.*, 2010). By contrast, none of the resprouters generated a fire-persistent seed bank (i.e. obligate resprouters). For reasons of simplicity, facultative species (seeders with resprouting ability) were not considered. Seeders and resprouters not only differ in terms of their regeneration mechanism, but also display other vegetative and flower trait differences, and can be associated with the nonsclerophyllous and sclerophyllous syndromes (respectively) defined by Verdú & Pausas (2013; see also Pausas & Verdú, 2005). These two regeneration strategies account for the range of persistence types found in the Mediterranean basin, where seeders present a smaller phylogenetic range and poorer morpho-functional diversity than resprouting species (Pausas *et al.*, 2004; Pausas & Verdú, 2005; Verdú & Pausas, 2013).

Seeds collected from wild populations from south-eastern Spain were cultivated for 2 yr under nursery conditions in full sunlight. Approximately 50–75 seedlings per species (some 900 plants) were grown in the Santa Faz forest nursery (Regional Forestry Services, Generalitat Valenciana; 38º23′N; 0º26′W; Alicante, 80 m asl; mean annual rainfall 353 mm; mean annual temperature 18°C) for the first year in standard forestry trays with a 300 cm^3 volume, and were watered according to demand (about twice a week). At the beginning of the second year, seedlings were individually transplanted into 3.5-l pots containing a mixture of peat and coconut peat (1 : 1, v/v), and were fertilized with 1 g of slow-release fertilizer (N : P : K 14 : 8 : 14) per litre of substrate. After 3 months, seedlings were placed in a glasshouse (mean 30°C day : 15°C night temperatures during a natural

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photoperiod) and were well watered for 1 month. Then 20–30 seedlings were taken per species to measure xylem traits and vulnerability to cavitation; five seedlings were selected to determine cell–water relationships, while 20 seedlings were used in an imposed drought experiment.

Xylem traits and vulnerability to cavitation

Hydraulic conductance was measured in the entire shoots of five seedlings per species in the laboratory using a high-pressure flow meter (HPFM; Dynamax, Houston, TX, USA), as described in Tyree *et al.* (1995). Seedlings were well watered the night before measurements were made. Hydraulic conductance was measured early in the morning with moderate irradiance. Shoots were cut under water at the root collar level and a water-filled tube was attached immediately to seedling shoots. Samples were perfused with distilled and degassed ultrapure water, and were filtered through a 0.1-μm water filtration membrane in the transient mode with pressure applied at a rate of 5–10 kPa s^{-1}. Hydraulic conductance was calculated from the slope of the linear region in the regression of flow (F) against the applied pressure every few seconds (ΔP). The perfusion water temperature was measured to correct for possible effects of viscosity. Hydraulic conductance was measured with leaves, and after removing all the leaves; then, it was scaled by considering the xylem cross-sectional area to obtain xylem-specific hydraulic conductance with (K_{s_l}) and without (K_s) leaves.

The critical pressure potentials for xylem cavitation (P_{crit}), a proxy for xylem resistance to cavitation, were measured in three to five plants per species with a Schölander chamber (PMS Instrument Company, Corvallis, OR, USA). P_{crit} is the water potential at the inflection point where embolism is initiated, and is considered the minimum water potential that permits steady-state water transport (Pratt *et al.*, 2007). Some of our species were very resistant to cavitation and presented functional characteristics, such as long vessels, that might promote errors when determining vulnerability curves (see Cochard *et al.*, 2010). For these reasons, we decided to use P_{crit} as the proxy for resistance to cavitation in each species. Any water potential below P_{crit} results in some hydraulic failure as a result of conduit cavitation, with associated changes in either xylem hydraulic conductance or the root-to-leaf gradient of the water potential (Vilagrosa *et al.*, 2003a). In fact, P_{crit} is related to hydraulic failure, and the more resistant species are to cavitation, the more negative P_{crit} is (Pratt *et al.*, 2007). However, in order to ensure the validity of our interpretations, we compared P_{crit} with the vulnerability to cavitation at a 12% loss conductance (P_{12}), as it is also considered an indicator of the xylem pressure at which embolism begins (Sparks & Black, 1999; Wortemann *et al.*, 2011). The regression among nine Mediterranean species, from which we obtained data for both P_{crit} and P_{12}, showed that the two parameters correlated positively ($P = 0.0029$; $R^2 = 0.74$; see Supporting Information Fig. S1).

For the determination of $P_{crit,}$ the branches of each species were flushed with a degassed and filtered (0.22 μm) 10 mM KCl solution for 15–30 min to eliminate embolisms. For each species, stems of 1.5 times the maximum vessel length (previously determined; see next paragraph) were attached to the pressure chamber (*c.* 10 cm of the basal part of the stem inside the chamber, with the rest protruding outside the chamber). Then, pressure was slowly increased (3–5 bars min^{-1}) and P_{crit} was determined by visually observing the bubbles emerging from the opposite end of the shoot under water. These measurements were carried out under relaxed tensions of xylem to avoid possible artefacts (Cochard *et al.*, 2013; Wheeler *et al.*, 2013).

Maximum vessel length was measured in the same pool of seedlings by the air infiltration method along the entire length of large stems (Ewers & Fisher, 1989). Five to eight branches longer than 0.4 m were cut and their basal end was attached to a tubing system. They were then flushed with the above-described solution for the K measurements, at *c.* 0.12 MPa for 15–30 min, to remove embolisms. Each branch was then infiltrated with compressed air at 0.05 MPa at its basal end, while its apical end was immersed in water. Branches were successively shortened by 20-mm portions from their apical end until air bubbles were observed, indicating that at least one vessel was open at both ends. The remaining sample length was considered equal to the maximum vessel length of the sample (Zimmermann & Jeje, 1981).

For the xylem anatomy measurements, cross-sections (*c.* 15–30 μm) from three stem segments per species were cut using a sliding microtome. Stem cross-sections were stained with safranin and astra-blue, rinsed in water and mounted onto slides. Then samples were examined under a light microscope (Olympus BX50; Olympus Austria Corp., Vienna, Austria). Images were directly captured from a digital camera and analysed using image analysis software (IMAGEJ; National Institutes of Health, Bethesda, MD, USA). At least 40 conduits from each cross-section were measured. Mean vessel diameter (d), mean hydraulic diameter (d_h), vessel density (VD), vessel lumen fraction (VLF; i.e. the cross-sectional xylem area used for water transport) and conduit implosion resistance (CIR) were measured to characterize each seedling's xylem anatomy. d_h was calculated on the basis of vessel contribution to hydraulic conductance using this formula: $d_h = \sum d^5 / \sum d^4$ (Sperry *et al.*, 1998). CIR was measured as $(t/b)_h^2$ following Hacke *et al.* (2006), where t refers to the wall thickness between vessels, and b to the vessel lumen diameter.

Cell–water relationships

A pressure–volume (P–V) analysis was performed to determine the following functional leaf traits: relative water content at the turgor loss point (RWC_{tlp}), water potential at the turgor loss point (Ψ_{tlp}), osmotic potential at full turgor (Π_o) and bulk modulus of elasticity (ε_{max}). As Mediterranean species have very short petioles, P–V curves were determined from terminal leafy shoots with five to six leaves attached. Shoots were carefully cut and submerged in distilled water until full rehydration was achieved. Next they were wrapped in plastic seal and left overnight in a refrigerator (12 h at 4°C). Curves were obtained according to Tyree & Hammel (1972) and after considering the recommendations of Kubiske & Abrams (1991) to prevent leaf oversaturation. Finally, shoots were oven-dried at 70°C for 24 h and weighed at a resolution of 1 mg to calculate the *P*–*V* parameters cited above.

Physiological responses to drought conditions

A set of 20 seedlings per species was subjected to an experimental drought for 2 months. All the plants were watered to field capacity the night before the drought period started. Throughout the desiccation period, the predawn water potential, the relative water content of leaves (RWC) and the gas exchange variables (photosynthesis, stomatal conductance and transpiration) were monitored every 2–3 d in three to five individuals of each species. Predawn water potential was determined before sunrise in a Schölander chamber (PMS Instrument Company). As our species' leaves were small in size, small twigs rather than single leaves were considered. The RWC was determined after the water potential determinations by collecting and weighing twig leaves (giving the fresh weight, FW). Then leaves were rehydrated for 24 h at 4°C in a freezer, and were re-weighed (giving the turgid weight after rehydration, TW). Finally, samples were oven-dried (70°C for 24 h) to constant weight (giving the dry weight (DW)) and the RWC was calculated as (FW − DW)/(TW − DW).

Leaf gas exchange rate measurements were taken between 6 a.m. and 9 a.m. (solar time) under saturating light in a portable infrared gas analyser (Li-6400; Li-Cor Inc., Lincoln, NE, USA) at the following settings: 55 ± 5% relative humidity, saturating light intensity (1500 μmol photon m^{-2} s^{-1}), 25.6 ± 0.5°C mean temperature and 400 μmol mol^{-1} CO_2 concentration. For each seedling, we measured the rates of net photosynthesis (A), stomatal conductance (g_s) and transpiration (E). Intrinsic water-use efficiency (IWUE), that is, the A/g_s ratio, was used to make a direct comparison of the intrinsic physiological characteristics (Galmés *et al.*, 2007). All the variables were expressed on a leaf area basis, which was calculated by scanning individual leaves and analysing images with specific software (WinRhizo; Régent Instruments Inc., Quebec, Canada).

Data analysis

Data analysis was carried out for the purpose of comparing functional traits between resprouting and seeding post-fire strategies. For each measured variable, the difference between the two strategies was analysed by a mixed-effect model using species as a random factor nested in resprouting ability, tested using the likelihood ratio (LR) test (cross-species analysis). All the leaf traits were $\log_{10}$-transformed before the statistical analysis in order to improve normality and homoscedasticity. The differences between regeneration strategies were also subjected to an analysis that included species relatedness, to account for similarities resulting from common ancestry. For this purpose, we used the generalized estimating equation (GEE) procedure with the mean species values. The GEE procedure applies a Generalised linear model (GLM) approach by incorporating the phylogenetic relatedness among species as a correlation matrix in the model (Paradis & Claude, 2002). Phylogenetic relatedness was obtained by assembling a phylogenetic tree with the help of the Phylomatic database implemented in PHYLOCOM 3.41 (Webb *et al.*, 2008), and with additional information from Guzmán *et al.* (2009). For the drought experiment, the differences in A, g_s, RWC and IWUE between regeneration strategies were also analysed using a mixed-effect model with species as the random factor. As the water potential varies during the drought experiment, and as it may vary differently between the two strategies, the water potential was introduced into the analysis as a covariable; the interaction between the water potential and the regeneration strategy was also analysed. The results of this analysis were corroborated by a phylogenetic-controlled test. To do this, the data were split into four water potential ranges (–MPa ranges of 0–1, 1–2, 2–3 and 3–4) and differences between resprouters and seeders were analysed using the GEE approach for each range (as above). Mixed-effect model analyses and pylogenetically controlled analyses were performed using the 'nlme' and the 'ape' library, respectively, of the R software (Paradis *et al.*, 2004; Pinheiro *et al.*, 2011).

Results

The hydraulic characteristics of xylem differed between regeneration strategies (Fig. 1). K_{s_l}, P_{crit}, VD, VLF and CIR were significantly greater in seeders than in resprouters. The mean vessel diameter (d) and hydraulic diameter (d_h) were also significantly greater in seeders than in resprouters, although differences were partially attributable to their phylogenetic affiliation (i.e. marginally significant when accounting for relatedness; Fig. 1, Table 1).

The cell–water relationships (*P*–*V* curves) also differed between the two groups of plants (Fig. 2). Resprouters lost turgor at water potentials of *c.* −3.0 ± 0.1 MPa, while seeders did so at −1.9 ± 0.1 Mpa; Π_o was higher in seeders than in resprouters. Similarly, the ε_{max} values in resprouters corresponded to relatively nonelastic cell walls, with high water potential gradients being associated with slight losses in cell volume. Conversely, the ε_{max} in seeders was associated with both elastic cell walls and higher tolerance to loss of symplastic water. In fact, RWC_{tlp} remained lower for seeders than for resprouters at the turgor loss point, with values of 79 ± 3% and 87 ± 1% for seeders and resprouters, respectively (Fig. 2, Table 2).

During the drought experiment, seeders maintained significantly higher gas exchange rates when water was readily available (Table 3, Fig. 3). Stomatal conductance (g_s) and transpiration (E) were also higher (approximately twofold higher) in seeders, while intrinsic water-use efficiency (IWUE) was higher in resprouters. The water stress experienced by both groups during the drought period sufficed to cause stomatal closure and to bring about changes in all the analysed variables. A and g_s dropped to zero as drought progressed, at *c.* −4 MPa (Fig. 3). The transpiration (E) data showed the same tendency as g_s (data not shown). As water stress increased, both variables sharply dropped in seeders, while a slight decrease in resprouters was observed, to the extent that they achieved similar values at a water potential of *c.* −2 MPa

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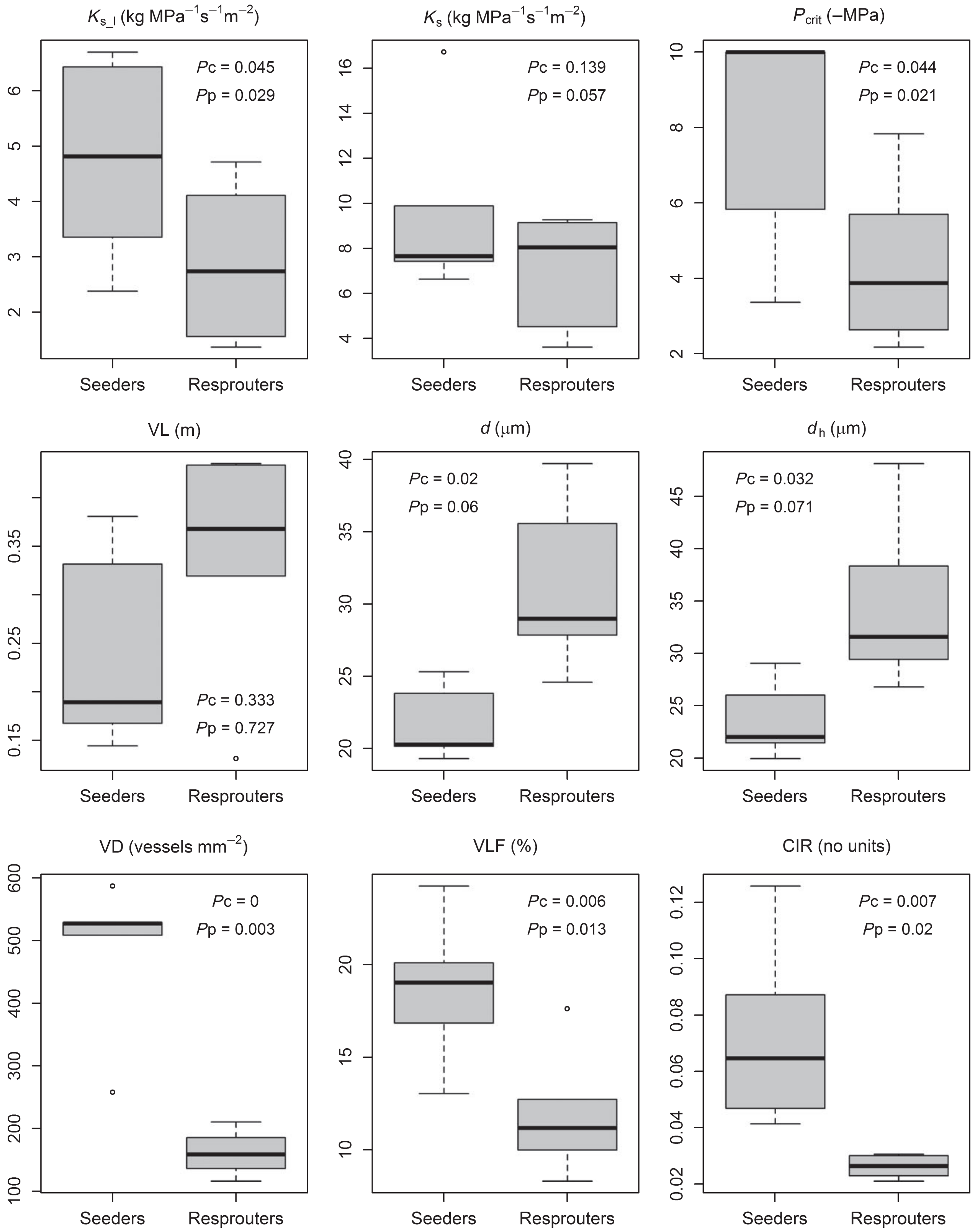

Fig. 1 Comparison of shoot hydraulic conductance, xylem characteristics and vulnerability to cavitation between seeders and resprouters (see Table 1 for abbreviations). P-values of the statistical comparison are shown for the cross-species analyses (P_c) and the phylogenetically controlled tests (P_p). Boxplots indicate the median (horizontal line), the first and third quartiles (box), the range that excludes outliers (i.e. 1.5 interquartile range; whiskers), and the outliers (points). Detailed results by species are shown in Supporting Information Fig. S2. See Table 2 for a list and the main regeneration traits of species used in the present study.

Table 1 The variables, units and symbols used in the study

Symbols	Variables	Units
Ψ	Water potential	−MPa
Ψ_{tlp}	Water potential at a turgor loss point	−MPa
Π_o	Osmotic potential at full turgor	−MPa
RWC	Relative water content	%
RWC_{tlp}	Relative water content at a turgor loss point	%
ε_{max}	Bulk modulus of elasticity	MPa
A	Net photosynthesis on a leaf area basis	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$
g_s	Stomatal conductance	$mol\ H_2O\ m^{-2}\ s^{-1}$
E	Transpiration	$mol\ H_2O\ m^{-2}\ s^{-1}$
IWUE	Intrinsic water use efficiency (A/g_s)	$\mu mol\ mol^{-1}$
$K_{s\text{-}l}$	Xylem-specific conductance with leaves	$kg\ MPa^{-1} s^{-1}\ m^{-2}$
K_s	Xylem-specific conductance without leaves	$kg\ MPa^{-1} s^{-1}\ m^{-2}$
P_{crit}	Critical xylem cavitation pressure potential	−MPa
VL	Vessel length	m
d	Average vessel diameter	µm
d_h	Hydraulically weighted mean vessel diameter	µm
VD	Vessel density	$vessels\ mm^{-2}$
VLF	Vessel lumen fraction	%
CIR	Xylem implosion resistance $(t/b)_h^2$	No units

(Fig. 3, Table 4). Differences between the two groups were seen for the RWC of leaves: while the RWC values of resprouters remained close to 90% for most of the dehydration period (until the water potential reached −3 MPa), seeders showed continuous reductions in parallel with drops in the water potential. The two groups reached *c*. 60% of the RWC for the water potentials at *c*. −4 MPa, and they showed similar responses in IWUE during the drought period.

Discussion

Co-existing resprouters and seeders clearly differ in terms of their water relations, with marked differences in their hydraulic and xylem characteristics, in the leaf traits involved in cell–water relationships, and in their response to drought. These differences, together with those observed between the two groups in other traits (Pausas, 1999; Pausas & Verdú, 2005; Paula & Pausas, 2006, 2008, 2011; Pratt *et al.*, 2007, 2008, 2010, 2012; Hernández *et al.*, 2011), support the idea that they are distinct syndromes with different functional characteristics at the whole-plant level. Our findings also suggest that the physiological responses of juveniles to drought are the key differences between these two syndromes, and that these differences allow the partition of their regeneration niches. Seedlings of obligate seeders are physiologically better adapted to strong water oscillations under post-fire conditions than seedlings of resprouting species. By contrast, seedlings of resprouters do not recruit under open post-fire conditions but in sheltered microsites during the interfire periods (i.e. conditions that favour germination and development). In fact, we cannot consider that one of the syndromes is better adapted to Mediterranean conditions than the other, as they represent two different strategies able to co-exist in fire- and drought-prone Mediterranean ecosystems (Keeley *et al.*, 2012). This regeneration niche partitioning between the two syndromes contributes to explaining their co-existence, and has important physiological implications.

Seeders exhibit greater hydraulic efficiency than resprouters (Fig. 1), with hydraulic conductance (K_{s_l}) almost double that in resprouters; this is in agreement with the higher gas exchange rates obtained in seeders (Table 3; see also Pratt *et al.*, 2008 for Californian *Rhamnaceae* species). Better hydraulic efficiency can be attained by increasing the number of vessels, enlarging the vessel diameter, altering the vessel lumen fraction or decreasing resistance to water flow (Zanne *et al.*, 2010). Our results reveal that seeder species showed greater vessel density (VD; approximately three times greater in seeders) and a higher vessel lumen fraction (VLF) than resprouters. This larger number of vessels per unit of sapwood counteracts the shorter vessel diameter (d and d_h) observed in seeders, and allows these species to maintain their high capacity to supply leaves with water. Therefore, seeders are more efficient at conducting water than resprouters; hence they are better adapted to take full advantage of periods with good water availability in order to sustain high gas exchange rates, as observed in the drought experiment (Fig. 3; see also Hernández *et al.*, 2011). In addition, their better efficiency at conducting water to leaves prevents sharp drops in the water potential, allows continued carbon gain under high water demand conditions (e.g. at midday), and reduces the risk of cavitation events during periods of relatively low water stress (Sperry *et al.*, 1998; Sparks & Black, 1999).

The existence of a trade-off between the efficiency of the water-conducting system and safety against vessel cavitation is often assumed in water-limited ecosystems (Hacke *et al.*, 2006; Gleason *et al.*, 2012). However, we found a positive relationship between K_{s_l} and P_{crit} ($r = -0.62$; $P = 0.032$; Fig. 4), and the more efficient group in terms of transporting water to leaves (i.e. the seeder species) also had a safer conduit system. Seeders presented lower P_{crit} values, higher CIR and narrower vessels, and all these traits are related to higher drought tolerance (i.e. the safety component). Seeders also showed good water transport efficiency as a result of VD, K_{s_l} and VLF being higher than in resprouters. When all the species were considered together, CIR and VLF were positively correlated ($r = 0.77$; $P < 0.001$) and d_h and P_{crit} were negatively correlated ($r = -0.62$; $P = 0.03$; Fig. 4). Thus, our results agree with those of recent studies, which have observed a lack of a trade-off between the efficiency of water conduction and vulnerability to cavitation (Bhaskar *et al.*, 2007; Chave *et al.*, 2009; Peguero-Pina *et al.*, 2011). In fact, in their world-wide study, Olson & Rosell (2012) discovered that vessel size is not related to the rainfall regime, but to plant size, and in such a way that vessels are narrower in dryland communities because plants are smaller. Hence, not only vessel characteristics but also the network structure of xylem would affect stem hydraulic properties (Carlquist, 2012).

Plants respond to intense drought by making adjustments in net assimilation and transpiration rates, because differences in the rate or shape of the response curves might confer competitive advantages (Acherar & Rambal, 1992; Oren *et al.*, 1999). We also observed two types of response that are linked to the two regeneration strategies. Seeders had the highest gas exchange

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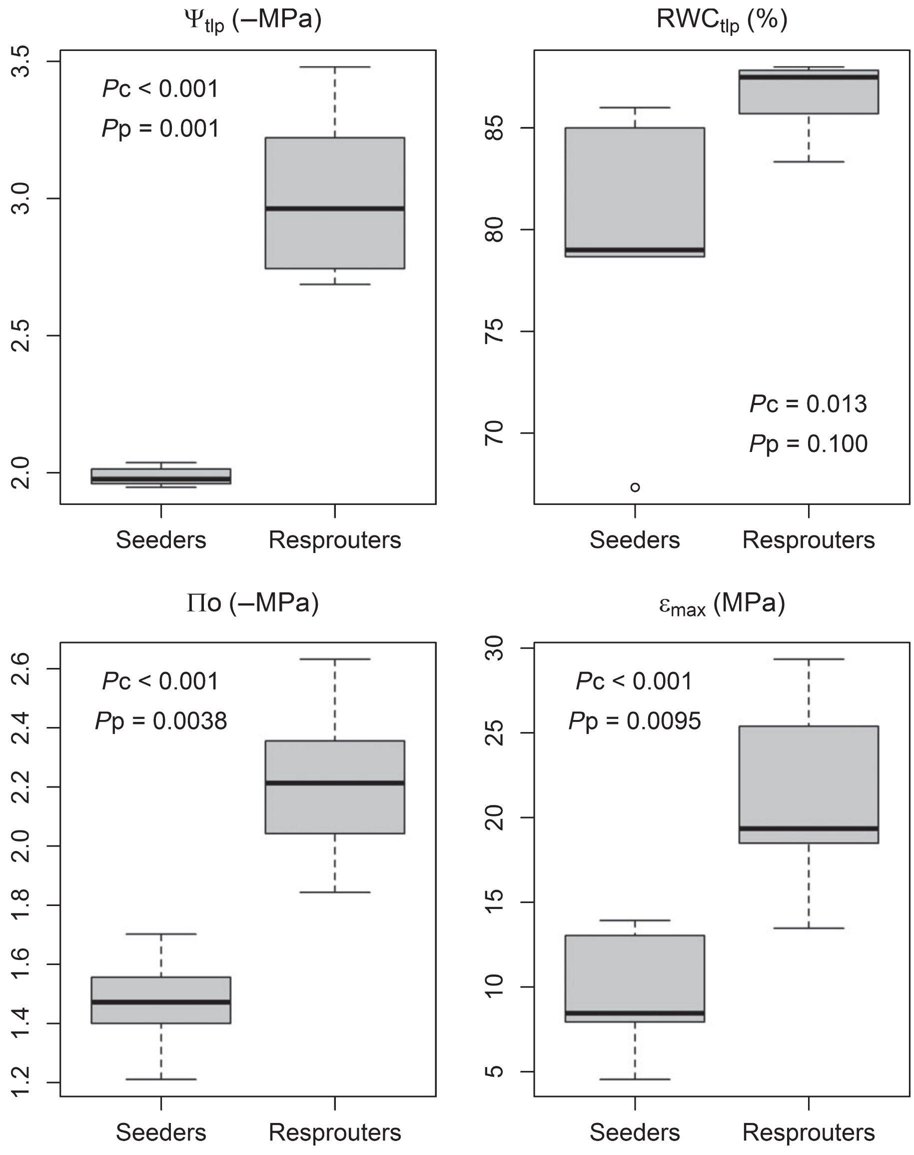

Fig. 2 The cell–water relationship parameters (pressure–volume (*P*–*V*) traits) for seeders and resprouters (see Table 1 for abbreviations). *P*-values of the statistical comparison are shown for the cross-species analyses (P_c) and the phylogenetically controlled tests (P_p). Boxplots indicate the median (horizontal line), the first and third quartiles (box), the range that excludes outliers (i.e. 1.5 interquartile range; whiskers), and the outliers (points). See Table 2 for a list and the main regeneration traits of species used in the present study.

Table 2 The species included in the study, their taxonomic family and their regeneration strategy

Species	Abbreviation	Family	Regeneration strategy
Arbutus unedo L.	*Au*	Ericaceae	Resprouter
Myrtus communis L.	*Mc*	Myrtaceae	Resprouter
Phyllirea angustifolia L.	*Pa*	Oleaceae	Resprouter
Pistacia lentiscus L.	*Pl*	Anacardiaceae	Resprouter
Quercus coccifera L.	*Qc*	Fagaceae	Resprouter
Quercus ilex L.	*Qi*	Fagaceae	Resprouter
Rhamnus alaternus L.	*Ra*	Rhamnaceae	Resprouter
Cistus albidus L.	*Ca*	Cistaceae	Seeder
Cistus clusii L.	*Cc*	Cistaceae	Seeder
Cistus monspeliensis L.	*Cm*	Cistaceae	Seeder
Lavandula latifolia Medic	*Ll*	Lamiaceae	Seeder
Rosmarinus officinalis L.	*Ro*	Lamiaceae	Seeder

values, but also showed greater sensitivity to drought conditions ($r = 0.80$; $P = 0.001$). In fact, the gas exchange rates of seeders dropped sharply as the water potential decreased (Fig. 3c), and stomatal closure occurred at between −2 and −3 MPa (Figs 2a, 3a,c). High gas exchange rates with low WUE and high water transport efficiency to leaves allow the seedlings of seeders to take full advantage of rainy periods (i.e. especially in the early post-fire years). This response pattern is typical of early successional species (Galle *et al.*, 2011), species displaying ruderal and advantageous competitive behaviour (Galmés *et al.*, 2007), and those that are capable of rapid resource acquisition (Diaz *et al.*, 2004; Hernández *et al.*, 2010). In parallel, seeders can overcome strong water stress conditions thanks to their high resistance to xylem cavitation. In fact, water potentials lower than −5 MPa have been reported in seeders under field Mediterranean summer conditions (Clemente *et al.*, 2005; Gabarrón-Galeote *et al.*, 2012). By contrast, resprouters exhibit a conservative use of resources and lower gas exchange values, but are more stable during prolonged water

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Table 3 Net photosynthesis, stomatal conductance, transpiration, intrinsic water-use efficiency and relative water content of the leaves recorded under higher water availability conditions (> −1 MPa) in the two regeneration groups (seeders and resprouters), and the *P*-values of the statistical comparison for the cross-species and phylogenetically controlled analyses

Trait (units)	Resprouters	Seeders	*P*-value, cross-species	*P*-value, phylogenetically controlled
A (µmol CO_2 m^{-2} s^{-1})	6.7 ± 0.2	14.2 ± 1.0	**0.0001**	**0.00369**
g_s (mol H_2O m^{-2} s^{-1})	0.081 ± 0.003	0.219 ± 0.011	**<0.0001**	**0.00151**
E (mol H_2O m^{-2} s^{-1})	1.6 ± 0.1	3.8 ± 0.1	**<0.0001**	**0.0017**
IWUE (µmol mol^{-1})	91.2 ± 2.2	67.4 ± 6.6	0.0592	0.082
RWC (%)	87.6 ± 0.7	84.5 ± 1.5	0.368	0.090

Mean ± SE for three to five individuals in each species. Numbers in bold indicate statistical differences between the two groups. *A*, net photosynthesis on a leaf area basis; g_s, stomatal conductance; *E*, transpiration; IWUE, intrinsic water use efficiency; RWC, relative water content.

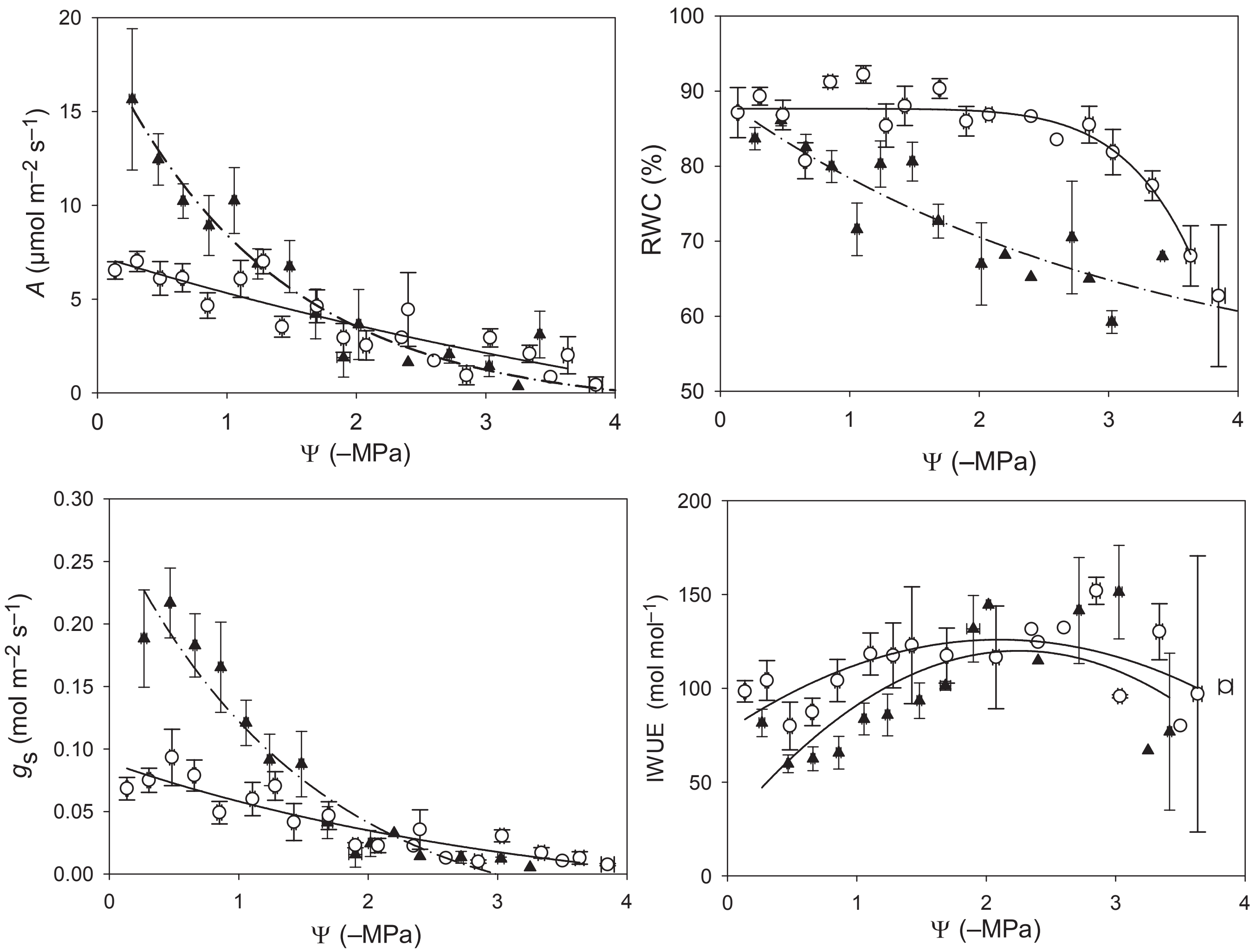

Fig. 3 Changes in net photosynthesis (*A*), leaf relative water content (RWC), stomatal conductance (g_s) and intrinsic water-use efficiency (IWUE) in relation to the predawn water potential (Ψ) reached by all plants and species during the drought experiment. For visualization purposes, data were grouped in water potential intervals (± 0.2 MPa). The statistical results (considering the entire raw data set) are presented in Table 4. The phylogenetically controlled analysis suggests differences between seeders and resprouters in *A*, g_s and IWUE for values of between 0 and −1 MPa (*P* values = 0.083, 0.007 and 0.027, respectively), and for RWC for values of between −1 and −2 MPa. Triangles, seeders; circles, resprouters. Values are mean ± SE. See Table 2 for a list and the main regeneration traits of species used in the present study.

shortage (i.e. stomatal closure and loss of cell turgor occur at *c.* −4 MPa; Figs 2a, 3a,c). Given that summers are often long in Mediterranean conditions, maintaining open stomata at such low water potential might provide fitness benefits (e.g. a high survival rate). These results indicate marked differences in physiological responses between the different regeneration strategies during desiccation conditions, and they are consistent with the water-saver (resprouters) and water-spender (seeders) mechanisms

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Table 4 Statistical significance of the difference in response to the imposed drought between seeders and resprouters in relation to several physiological parameters (A, g_s, RWC and IWUE; see Fig. 3)

	A		g_s		RWC		IWUE	
	LR	P	LR	P	LR	P	LR	P
Ψ (–MPa)	119.0	**<0.0001**	68.6	**<0.0001**	104.8	**<0.0001**	15.5	**0.0001**
RS	5.1	**0.024**	11.2	**0.001**	4.5	**0.034**	4.97	**0.026**
RS × Ψ	40.4	**<0.0001**	32.2	**<0.0001**	19.7	**<0.0001**	3.54	0.059

Values are the sequential likelihood ratio (LR) and the associated P-value; first we fitted the water potential (Ψ), and then we tested the regeneration strategy (RS) and the interaction of the two factors. The results of the phylogenetically controlled comparisons made between seeders and resprouters are provided in the legend of Fig. 3. Numbers in bold indicate statistically significant differences between the two groups. A, net photosynthesis on a leaf area basis; g_s, stomatal conductance; RWC, relative water content; IWUE, intrinsic water use efficiency.

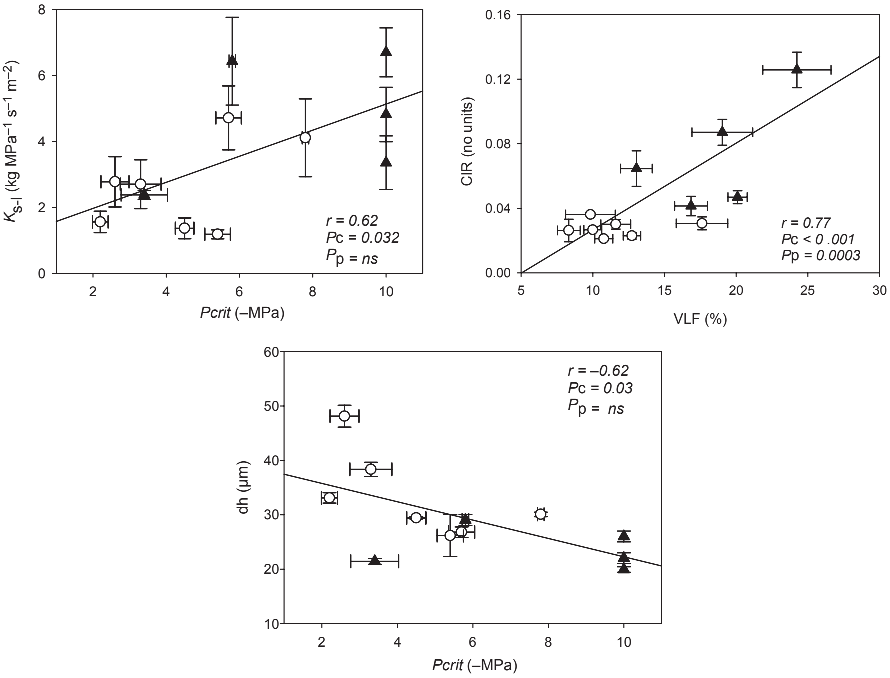

Fig. 4 Relationships between variables related to the efficiency of the water-conducting system and safety against vessel cavitation. Triangles, seeders; circles, resprouters; $K_{s\text{-}l}$, xylem-specific conductance with leaves; CIR, xylem implosion resistance; d_h, hydraulically weighted mean vessel diameter; VLF, vessel lumen fraction; P_{crit}, critical xylem cavitation pressure potential. Values are mean ± SE. See Table 2 for a list and the main regeneration traits of species used in the present study.

reported for Mediterranean species (Correia & Catarino, 1994; Vilagrosa *et al.*, 2003b). The co-existence of species with contrasting strategies for the use of water is common within Mediterranean species (Galmés *et al.*, 2007; Medrano *et al.*, 2008; Moreno-Gutiérrez *et al.*, 2012) and reflects the diversity of functional groups living in these ecosystems (Thompson, 2005; Verdú & Pausas, 2013).

Different functional characteristics were also observed at the cell level (*P–V* traits) between the two groups of species, and were similar to the gas exchange results: seeders showed a higher (less negative) Ψ_{tlp}. Furthermore, RWC_{tlp} was lower in seeders than in resprouters, which is consistent with the response to the drought period observed and indicates a high degree of cell dehydration tolerance. This tolerance in seeders should be mediated by an increase in both protective mechanisms and the capacity to recover cell functionality efficiently after damage caused by drought (Müller *et al.*, 2006; Jubany-Marí *et al.*, 2009). In fact, seeders are able to maintain higher photosynthetic rates than resprouters for lower RWC (i.e. as plants become drier; Fig. 3a,b). This cell dehydration capacity and sustained high photosynthetic rates may be related to their shallow rooting habit, which is likely to result in considerable oscillations in water availability throughout the year (Correia & Catarino, 1994; Ramírez *et al.*, 2012). The high cell-wall elasticity (i.e. low ε_{max}) in seeders could, therefore, lead to a positive turgor pressure in cells, by prolonging carbon fixation with no increments in the soil-to-leaf water potential, contrary to what occurs in resprouters (Corcuera *et al.*, 2002). In comparison, high ε_{max} and low Ψ_{tlp} in resprouters are related to drought strategies that avoid water deficit, with high gradients of water tension to pull up water from soil to leaves, to thus maintain a well-hydrated state and to allow quick recovery after drought (Corcuera *et al.*, 2002). This response pattern is commonly observed in a wide range of sclerophyllous Mediterranean species, ranging from trees to shrubs (Corcuera *et al.*, 2002; Vilagrosa *et al.*, 2003b; Quero *et al.*, 2011).

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In conclusion, seeders and resprouters can be considered two separate syndromes, with seedlings possessing different functional characteristics related to hydraulic traits as they display distinct responses to water availability and drought conditions in water economy and carbon fixation terms. Specifically, seeders show a range of traits that allow them to deal better with water-limited and highly variable conditions, but they are also capable of taking full advantage of periods with high water availability. Conversely, resprouter species seem to be adapted to more stable water availability conditions, favoured by their deep root system, but they also display traits that help them resist water shortages in summer months. Our results suggest that the trade-off between allocation to below-ground (in resprouters) and above-ground (in seeders) structures is related not only to faster growth in height and reproduction in seeders (Pate *et al.*, 1990; Pausas *et al.*, 2004) but also to a bigger investment in stem xylem traits to resist intense dry periods. Such differences can prove to be an evolutionary adaptation to different regeneration microsites: post-fire gaps prone to drought (seeders) and sheltered conditions under mature vegetation (obligate resprouters). The tight relationship between the regeneration strategy and the drought response suggests that any changes in the frequency and intensity of droughts might differentially affect the two syndromes, which might have consequences for species recruitment and for the assembly of communities. Consequently, the functional differences between these two syndromes need to be considered in any dynamic vegetation model aiming to predict biodiversity and community composition switches in a changing world.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Relationship between P_{crit} measured in several species and P_{12} calculated form the vulnerability curves in the same species.

Fig. S2 Shoot hydraulic conductance, xylem characteristics and vulnerability to cavitation for all species used in this study and for both regeneration groups, that is, seeders and resprouters.

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