Ecophysiological Traits Associated with Drought in Mediterranean Tree Seedlings: Individual Responses versus Interspecific Trends in Eleven Species

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Abstract: Species differ regarding their drought tolerance and individuals of a given species can modify their morphology and physiology in response to drought. However, since evolutionary and ecological selective pressures differ, individual and interspecific responses to drought might not match. We determined summer survival and a number of ecophysiological variables in two factorial experiments with seedlings of eleven tree species present in Mediterranean ecosystems, grown under slowly imposed water stress and control conditions. Plants experiencing drought exhibited reduced growth, low specific leaf area, chlorophyll content, and photosynthetic rate when compared to the controls, and species-specific drought tolerance was associated with an analogous set of trait values. However, while species with high leaf area ratio and shoot-root ratio exhibited greater drought tolerance, drought induced the reversed response within species. Contrary to expectations, water use efficiency was lower in drought-tolerant species and decreased in water-stressed individuals compared to the control plants. There was a distinctive phylogenetic signal in the functional grouping of species, with oaks, pines, and other genera being clearly different from each other in their drought tolerance and in their functional responses to drought. However, all relationships between ecophysiological variables and drought tolerance were significant after accounting for phylogenetic effects, with the exception of the relationship between drought tolerance and photochemical efficiency. Our results show that drought tolerance is not achieved by a single combination of trait values, and that even though evolutionary processes and individual responses tend to render similar results in terms of functional traits associated with drought, they do not necessarily match.

Key words: Drought tolerance, functional traits, Mediterranean plants, phylogeny, water use efficiency, woody seedlings.

Introduction

Plant species segregate along natural gradients of water availability according to their capacity to withstand drought. However, species from different biogeographic origins and with contrasting ecological requirements coexist at transition zones. The Mediterranean region has played this role of transition zone over geological time scales and many species of the Mediterranean flora are Tertiary relicts or remnants from vegetation shifts during glaciations (Suc, 1984; Palamarev, 1989). In fact, this role as a transition between tropical and cool-temperate zones is an important reason why the Mediterranean region is currently a biodiversity hotspot (Médail and Quézel, 1997; di Pasquale et al., 2004). But one key characteristic of Mediterranean ecosystems is a dry period over the summer, and to what extent Mediterranean plants are fully adapted to this drought remains unclear.

Species coexisting in Mediterranean habitats differ remarkably in their ecophysiological traits and in their responses and tolerances to water limitations (Cabrera, 2002; Gulías et al., 2002; Ogaya and Peñuelas, 2003). In a comparative study of nine woody species coexisting in a Mediterranean forest, it was shown that species like *Ilex aquifolium, Phillyrea latifolia,* and *Juniperus oxycedrus* were rather tolerant of drought-induced cavitation, while other species such as *Quercus ilex, Arbutus unedo,* and *Acer monspessulanum* were much more vulnerable to cavitation (Martinez-Vilalta et al., 2002). There was a close relationship between this vulnerability and the actual water potentials experienced by each species under drought conditions in the field, indicating that even species coexisting in a given forest do not experience the same level of water stress. There are several alternative strategies to cope with drought, and Mediterranean species have been classified as drought-tolerant, when they are able to maintain photosynthetic activity under severe water stress as certain evergreen oaks do, and drought avoiders, when they either shed leaves or close stomata during the dry periods, as certain pines do (Martinez-Ferri et al., 2000; Ferrio et al., 2003; Valladares et al., 2005). Drought tolerance is in general associated with an efficient use of water (Ferrio et al., 2003), but contrasting results have been shown regarding water use efficiency under water stress in Mediterranean ecosystems (Reichstein et al., 2002). Comparisons of survival under common environmental conditions of species with contrasting strategies are scant and, despite the common occurrence of drought in Mediterranean

zones, our understanding of ecology and evolution of Mediterranean species under limited water is still fragmentary.

An important source of complexity arises from the time scale of the ecophysiological processes involved in coping with drought, that is, evolutionary vs. ecological time, or, from a plant perspective, adaptation vs. acclimation processes (Valladares, 2004; Thompson, 2005). It is well-known that species differ regarding their drought tolerance, and a number of features have been associated with this differential tolerance, which are linked to evolution. Analogously, it is well-known that individuals of a given species modify their morphology and physiology in response to drought, which is linked to ecological performance. However, since evolutionary and ecological drivers can significantly differ, inter- and intraspecific traits associated with water stress might not match and, to our knowledge, no experimental study has been specifically designed to address this point.

The objectives of the present study were: i) to identify functional traits associated with drought tolerance of seedlings in eleven tree species present in Mediterranean ecosystems; ii) to quantify morphological and physiological responses of seedlings of these species to a realistic water stress; and iii) to explore the agreement between inter- and intraspecific traits and responses associated with drought. To achieve these goals, we have determined survival over the summer, as an estimator of species-specific drought tolerance, together with a number of functional variables in two factorial experiments with seedlings grown under water stress and control conditions to estimate intraspecific responses. The study was based on the working hypothesis that functional interspecific differences associated with drought tolerance should match intraspecific responses to drought. The agreement is expected to be high with regard to physiological parameters, but not with regard to morphological parameters, where phylogeny typically exerts an important influence (Ackerly et al., 2000). The study also aimed to test the following more specific hypotheses: i) water use efficiency increases with water stress and is higher in drought-tolerant than in drought-intolerant species (Reichstein et al., 2002; Ferrio et al., 2003); and ii) drought tolerance and responses to water stress involve reduced light harvesting by both reduced chlorophyll content and leaf area to reduce photochemical stress and transpiration, respectively (Valladares and Pearcy, 1998; Balaguer et al., 2002), and an increased allocation to roots to enhance water uptake (Hunt and Nicholls, 1986; Lloret et al., 1999).

Materials and Methods

General experimental design

Two experiments simulating summer drought were carried out with species from two different biogeographic regions. In experiment I, four species from the cool temperate Mediterranean transition zone were studied: *Quercus robur* L., *Quercus pyrenaica* Willd., *Pinus sylvestris* L., and *Pinus pinaster* Ait. In experiment II, seven Mediterranean species were studied: *Quercus faginea* Lam., *Quercus ilex* subsp. *ballota* (Desf.) Samp., *Quercus coccifera* L., *Pistacia lentiscus* L., *Pistacia terebinthus* L., *Arbutus unedo* L., and *Viburnum tinus* L. *Quercus robur,* a species that is occasionally present in certain Mediterranean zones as a relict from older climates, was introduced again in

experiment II as an internal control species across the two experiments. Emphasis was placed on simulating realistic summer conditions, so even control plants experienced a certain level of water stress, coupled with the natural summer heat and high light. Water stress was slowly imposed and soil water content was adjusted by using differential irrigation for each plant species to compensate for species differences in evaporative rate. The resulting water availability for the plants was:

Experiment I: *control,* watered every other day to reach $69.5 \pm 1.7\%$ vol. soil water content, and allowed to dry down to $28.2 \pm 3.1\%$, equivalent to -0.5 MPa soil water potential; *water stress,* watered twice a week to reach 46.5 ± 5.2 vol. water content and allowed to dry down to $12.5 \pm 4.0\%$, equivalent to -4.5 MPa soil water potential.

Experiment II: *control,* watered twice a week to reach $50.5 \pm 1.7\%$ vol. soil water content, and allowed to dry down to $25.5 \pm 1.1\%$, equivalent to -1.0 MPa soil water potential, respectively; *water stress,* watered once a week to reach 46.3 ± 5.2 vol. water content, and allowed to dry down to $7.4 \pm 1.0\%$, equivalent to -5.5 MPa soil water potential.

Material and experimental setting

Seeds were collected from the following Spanish localities: *Quercus robur* from Galicia, *Q. pyrenaica* from Sierra de Guadarrama, Madrid, *Q. faginea* from Torrelaguna, Madrid, *Q. ilex* subsp. *ballota* from Sierra Morena, Jaén, *Q. coccifera* from Cádiz, *Pinus sylvestris* and *P. pinaster* from Sierra de Gredos, Ávila, *Pistacia lentiscus* from Levante, *P. terebinthus* from Andalucía, *Arbutus unedo* from Ávila, and *Viburnum tinus* from Moratalla, Murcia. Seeds were germinated in forest multi-pots ($330\,cm^3$ each pot) over the winter and seedlings were transplanted to 3-l containers in early spring. Seedlings were grown at a commercial nursery (Viveros Barbol, Torremocha del Jarama, Madrid, Spain). The study area was located at 40°50′N, 3°29′W, and at 710 m a.s.l. Climate was continental Mediterranean, with hot and dry summers and cold winters; mean maximum and minimum temperature were 19 °C and 9.5 °C, respectively, with absolute maximum temperatures of up to 40 °C. Most annual rainfall (350–500 mm) is received during spring and autumn (250–350 mm) (Instituto-Nacional-de-Meteorología, 2003). Soil substrate (pH 6.5) was a standard mixture used in native plant nurseries for seedling production. This substrate consisted of a 3 : 1 volume mixture of peat Vriezenveen PP1 (Potgrond Vriezenveen B.V., Westerhaar, the Netherlands), and washed river sand. We also added $3\,kg/m^3$ of Guanumus Angibaud fertilizer ($3/35/2\,N\,P^{-1}\,K^{-1}$, Angiplant, La Rochelle Cedex, France) and $2\,kg/m^3$ of Plantacote mix 4 M fertilizer ($15/10/15\,N\,P^{-1}\,K^{-1}$, Aglukon Spezialdünger GMBH and Co. KG, Düsseldorf, Germany).

Local air temperature and available photosynthetic photon flux density (PPFD) were recorded every 5 min during the whole growing season with a data logger (HOBO model H08-006-04, Onset, Pocasset, MA, USA) and external sensors cross-calibrated with a Li-Cor 190SA sensor (Li-Cor, Nebraska, USA). Mean daily PPFD over the summer was $42\,mol\,m^2\,d^{-1}$.

Watering levels were established by adjusting irrigation intensity. Differences in water availability across treatments in experiment I were monitored by estimation of soil volumetric

water content with an Aquaterr Moisture meter (model EC-200, Aquaterr Instruments, Fremont, CA), a capacitance probe which measures the dielectric constant of the soil–air–water combination. Differences in water availability across treatments in experiment II were monitored with a portable moisture measurement instrument based on the TDR method, TRI-ME-FM (Imko Micromodultechnik GMBH, Ettlingen, Germany), connected to a P2 probe. Soil water potential was estimated from the relationship between volumetric water content of the soil and water potential of Whatman No. 42 disks (Whatman plc, Kent, United Kingdom) following the filter paper technique (Deka et al., 1995).

A total of 352 healthy seedlings were selected for each experiment. 44 seedlings of each species were allocated to each irrigation regime (control and water stress treatment) in experiment I, while 22 seedlings of each species were allocated to each irrigation regime in experiment II. Seedlings were grown in the open. Almost no rain was experienced during the growth period but plastic shelters were temporarily used to exclude water from drought-stressed plants during storms.

Survival, growth, and allometric measurements

Survival of each seedling was evaluated at the end of each of the 5–6 censuses carried out during the growing season. Individuals that had lost all their aerial structure, did not have any photosynthetically active leaf (i.e., green and flexible leaves), and exhibited loss of stem flexibility at the upper 1/3, were recorded as dead. No resprouting was observed in any individual labelled as dead. Seedlings were sprayed with a fungicide solution (50% Carbendazyme, Fungicida Polivalente, COMPO Agricultura SL, Barcelona) twice during the experiment in order to control fungal infections. None of the mortality events showed signs of a death mediated by fungal infection or pest damage.

We carried out a total of five mortality censuses leading to four regularly spaced time intervals from June to October. One extra census was carried out in mid-September with plants of experiment II to obtain more detailed dynamics of the mortality. By the end of the summer, growth ceased even in control plants and seedling maximum height and main stem diameter (near the contact point with the root) were recorded for each individual plant. Stem diameter was measured with callipers with an accuracy of 0.01 mm. A slenderness index was calculated as plant height (in cm) divided by stem diameter (in mm). Then, plants were harvested and divided into leaves, stems, and roots. A subset of the leaves of each plant was scanned with a PC-compatible desk scanner at 600 dots per inch resolution, and the area of each individual leaf was estimated using UTHSCSA Imagetool 2.00alpha (C. Donald Wilcox, S. Brent Dove, W. Doss McDavid and David B. Greer, Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio, TX, USA; ddsdx.uthscsa.edu). This subset of leaves was dried for a minimum of 48 h at 65 °C and weighed to calculate the specific leaf area (SLA). With the dry mass of leaves, stems, and roots of each individual plant, total plant mass and leaf area, leaf area ratio (LAR), and shoot-root ratio were calculated. All measurements were carried out in seedlings surviving up to the last census.

### Physiological measurements and pigment determinations

Physiological measurements were carried out at the end of the summer (last week of August till mid-September) in 10 randomly chosen plants per species and treatment. The maximum photochemical efficiency of photosystem II (Fv/Fm) of fully expanded leaves was determined with a pulse-modulated fluorometer (FMS2, Hansatech Instruments, Norfolk, United Kingdom). The leaves were first darkened for 30 min with leaf clips containing a shutter that could be opened when the fluorometer probe was attached, and minimal (Fo) and maximal (Fm) fluorescence were measured under dim red light and after a short and saturating pulse of actinic light, respectively. These values were used to calculate Fv/Fm and were also used to calculate non-photochemical quenching (NPQ = Fm – Fm'/Fm; where Fm' is maximal fluorescence without a period of dark adaptation) (Maxwell and Johnson, 2000).

Net photosynthetic rate and stomatal conductance were measured in one fully-expanded, current-year leaf of 6–11 plants per species per treatment with a portable open gas exchange system (ADC LCA4, Analytical Development Co., Hoddesdon UK) following the procedures described in Valladares et al. (2000). Maximum net photosynthetic rate, stomatal conductance, and estimated transpiration, using the parameters provided by the manufacturer for the standard cuvette, were recorded by the end of the summer in control and water-stressed plants of each species and experiment at saturating light ($1600 \pm 50 \, \mu mol \, m^{-2} \, s^{-1}$ PAR), ambient CO_2 (370 ± 30 ppm), and 30 ± 2 °C. Instantaneous water use efficiency (WUE) was calculated as the ratio of maximum net photosynthetic rate over transpiration at saturating light. Intrinsic water use efficiency (maximum photosynthetic rate divided by stomatal conductance, as in Gulías et al. [2002] was found to be linearly and significantly correlated with transpiration ($r^2 = 0.92$, $p < 0.0001$). Gas exchange measurements were carried out only in experiment II.

Chlorophylls were extracted from leaf disks of healthy, fully expanded leaves of 10 plants per species and treatment using dimethyl sulfoxide (DMSO) for 2.5 h at 65 °C. Absorptance of the extracts was determined with a UV-visible light spectrophotometer (Spectronic 2000, Bausch and Lomb, USA), and leaf chlorophyll and carotenoid concentrations were calculated according to Barnes et al. (1992). Pigment analyses were only carried out in experiment I.

Data analysis

The Kaplan-Meier product-limit method was used to estimate the survival function directly from the survival times in our mortality data set. Independent analyses were used for each species and water combination. All selected variables (plant dry mass, total leaf area, LAR, SLA, Fv/Fm, non-photochemical quenching, caroteniod and chlorophyll content, maximum photosynthetic rate, and instantaneous water use efficiency) were analyzed with ANOVA to assess differences between treatments and species. The Fisher LSD-test was used for post hoc analysis. Prior to ANOVA, data were checked for normality and homogeneity of variances, and were log-transformed when needed to correct deviations from these assumptions. Linear regression analysis was used to test for specific relationships between final survival and the variables selected. The ex-
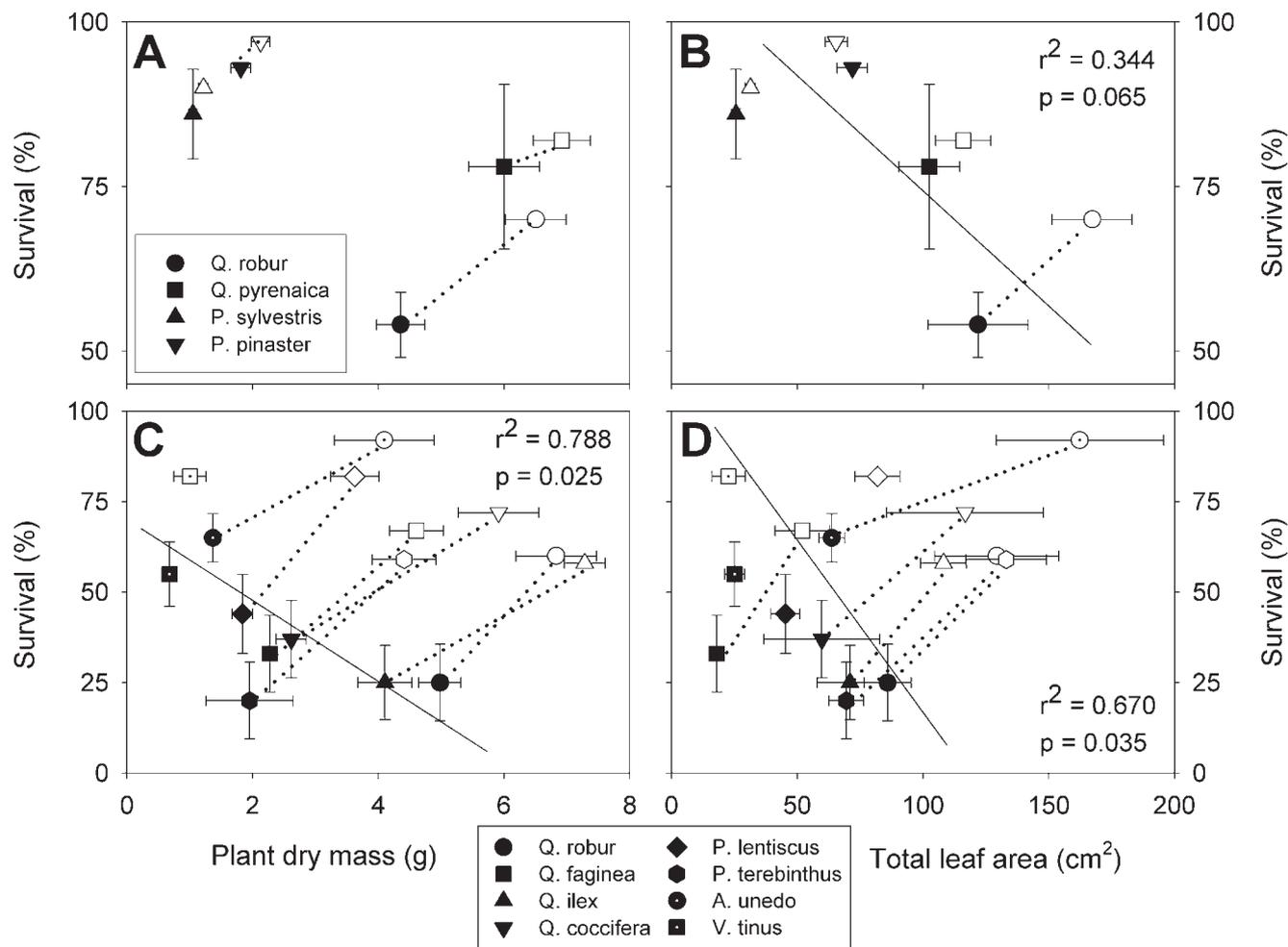
Fig. 1 Survival over the summer of water-stressed (closed symbols) and control plants (open symbols) vs. plant dry mass (**A, C**) and total leaf area (**B, D**) under water stress (experiment I, 4 species, **A, B**; experiment II, 8 species, **C, D**). Each data point is the mean of 10 – 15 plants of a given species and water treatment ± SD. Survival was determined from an initial number of 44 and 22 seedlings per species and water treatment in experiments I and II, respectively. Continuous lines indicate the regression line for water-stressed plants. Dotted lines connect water-stressed and control plants of the same species; they are shown only when differences between the two treatments were significant (ANOVA, $p < 0.05$).

istence of functional groups within the species was explored by principal component analysis; varimax rotation was used to maximize the segregation of the species according to the first two factors. The nine variables used in the principal component analysis were survival, specific leaf area (SLA), leaf area ratio (LAR), photochemical efficiency (Fv/Fm), shoot-root ratio, total leaf area, final plant biomass, slenderness index, and stem diameter. All the statistical analyses were performed using STATISTICA 6.0 (Statsoft Inc., Tulsa, USA). The phylogeny of the species studied was reconstructed using Phylomatic, software developed by Webb and Donoghue (2002), which uses the molecular phylogenetic information of Soltis et al. (2000). The final phylogenetic tree was assembled by pruning the Soltis et al. (2000) tree to the genus level and by using additional, more specific information to solve distances among congeneric species. Phylogenetic relations under the genus level were obtained from Manos et al. (1999) and Liston et al. (1999) for molecular distances and from López-González (2001) for detailed taxonomic information to solve polytomies due to the absence of molecular data (the case of *Quercus pyrenaica*). With the phylogenetic information of the species, phylogenet-

ic-independent contrasts (Felsenstein, 1985) were carried out to remove the influence of phylogeny on the relationships between the ecophysiological response variables. The software PDAP (Phenotypic Diversity Analysis Programs Version 6.0, Copyright March 2002 by Garland, T. Jr., Midford, P.E., Jones, J.A., Dickerman, A.W., and Diaz-Uriarte, R.), which is described in Garland et al. (1993), was used. The independent contrasts were carried out with the modulus PDTREE (Garland et al., 1999). PDTREE allows the user to enter and edit a phylogenetic tree and associated phenotypic data for the species at its tips. A Brownian motion model of evolution was assumed. Felsenstein's pairwise independent differences (contrasts) were standardized by dividing each contrast by the standard deviation of the contrast (i.e., square root of sum of the lengths of the branches of the phylogenetic tree).

Results

Species differed significantly in their survival over the summer. Survival over the summer was particularly low under water stress, with most species exhibiting final survivorships be-
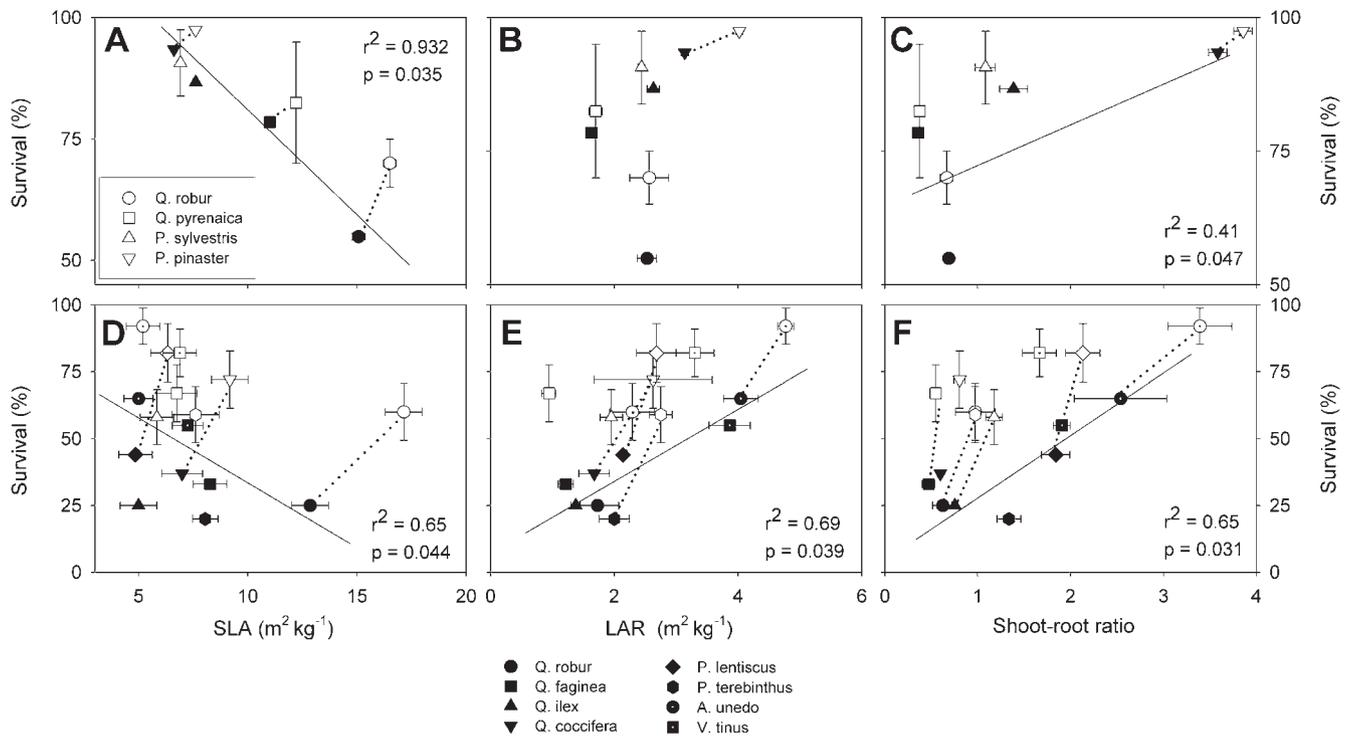
Fig. 2 Survival over the summer of water-stressed (closed symbols) and control plants (open symbols) vs. specific leaf area (SLA, **A, D**), leaf area ratio (LAR, **B, E**), and shoot-root ratio (**C, F**) under water stress (ex-

periment I, 4 species, **A – C**; experiment II, 8 species, **D – F**). For information on lines, error bars, and number of samples see legend of Fig. **1**.

tween 20% and 50%. Kaplan-Meier analyses revealed species with significantly higher survivorships under water stress, such as *Arbutus unedo* and *Viburnum tinus,* versus species like *Pistacia terebinthus,* with the lowest survival rate. The two pine species studied were relatively tolerant of water stress, while oaks exhibited a relatively wide range of survival under water stress. Values of survival for each species and water treatment can be seen in Figs. **1 – 5**.

By the end of the summer, seedlings exposed to water stress were significantly smaller than the control plant in all species except three (*Viburnum tinus* and the two pines), which exhibited relatively small plants in both control and water stress treatments. This limited growth was revealed in a number of variables, from basal stem diameter (data not shown) to total leaf area and plant dry mass (Fig. **1**). The same trend was observed across species, so survival under water stress was negatively related to plant mass in experiment II and to leaf area in both experiments (Fig. **1**). Plants exposed to water stress exhibited significantly lower specific leaf area (SLA) under drought and the same trend was observed across species. However, the two allometric variables, leaf area ratio (LAR) and shoot-root ratio, exhibited different trends within species, with lower values under water stress, as compared to trends across species, with higher values in species exhibiting higher survival under water stress (Fig. **2**). These trends were clear although not completely consistent for all the combinations of variables, experiments, and species.

Maximal photochemical efficiency (Fv/Fm) was lower under water stress (experiment II), and there was a tendency (borderline significant regression) for drought-tolerant species towards higher values of Fv/Fm under water stress than those from less tolerant species (Fig. **3**). Nevertheless, the significance of the regression between survival under limiting water and Fv/Fm vanished when phylogenetic relatedness among species was considered. Non-photochemical quenching and carotenoid content did not exhibit a clear pattern either within or between species. By contrast, chlorophyll content exhibited a significant pattern: both water-stressed individuals within a species and those species relatively more drought-tolerant had lower chlorophyll content than their counterparts (Fig. **4**). Maximum photosynthetic rate (Amax) and instantaneous water use efficiency (WUE) were lower under water stress, and drought-tolerant species exhibited lower values of Amax and WUE than less tolerant species (Fig. **5**). All relationships between ecophysiological variables and drought tolerance were significant after considering the influence of phylogeny, with the exception of the relationship between drought tolerance and Fv/Fm.

Principal component analysis of survival under water stress, together with the ecophysiological variables that were measured in both experiments, segregated three main functional groups among the eleven species (Fig. **6**). One group included the most drought-tolerant species (the two pine species plus *A. unedo* and *V. tinus*), which had high shoot-root ratios and LAR, and a wide range of values for the slenderness index and Fv/Fm under water stress. Another group included species with high growth rates (leading to large plants over the summer) and SLA, and was made up of deciduous and semi-deciduous oaks of moderate tolerance to water stress. And a third group included the evergreen oaks and the two *Pistacia* species, which exhibited low to moderate tolerance to water
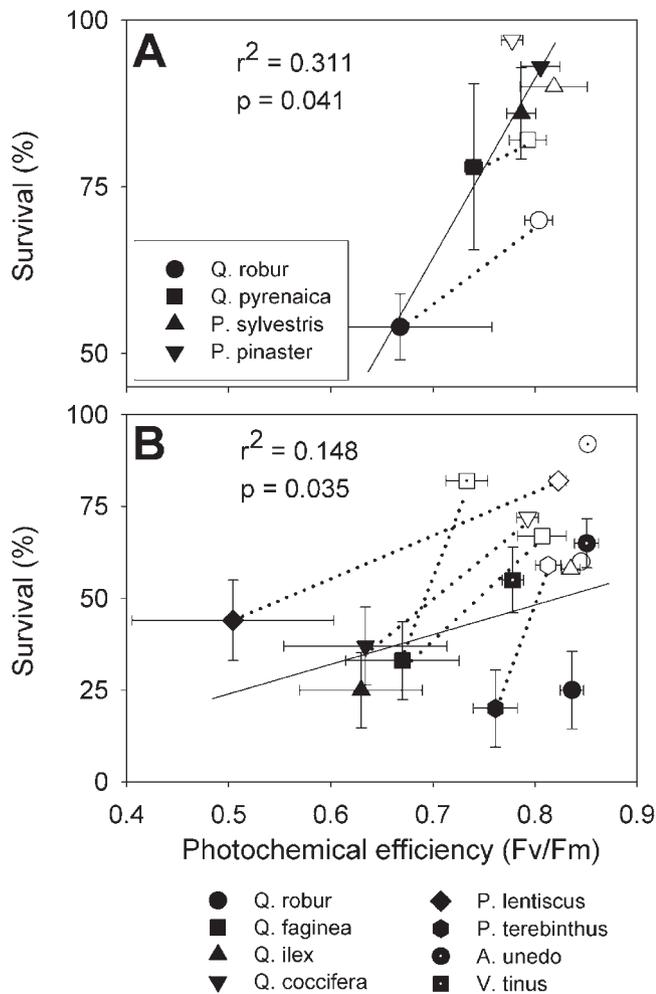
Fig. 3 Survival over the summer of water-stressed (closed symbols) and control plants (open symbols) vs. photochemical efficiency under water stress (experiment I, 4 species, **A**; experiment II, 8 species, **B**). For information on lines, error bars, and number of samples see legend of Fig. **1**.

stress and had a low slenderness index and Fv/Fm under water stress. There was a clear phylogenetic signal in the functional grouping of the eleven species. In fact, phylogenetic distances among species explained most of the grouping except for the case of the evergreen oaks (*Q. ilex* and *Q. coccifera*), which were functionally closer to the two *Pistacia* species than to the other oaks (Fig. **6**).

Discussion

Plants experiencing drought exhibited reduced growth, low specific leaf area, chlorophyll content, photosynthetic rate, and water use efficiency when compared to their well-watered counterparts. Analogously, species-specific drought tolerance was associated with a low growth rate, chlorophyll content, photosynthetic rate, and water use efficiency. However, while species with high leaf area ratio and shoot-root ratio exhibited greater drought tolerance, drought induced the reversed response within species. Thus, the main working hypothesis was basically confirmed: while physiological traits of the more drought-tolerant species matched physiological responses to

water stress, certain morphological traits did not follow the same trend across species as within species (i.e., in individual responses to drought). Higher leaf area ratio and shoot-root ratio are in general not associated with drought tolerance, since drought-tolerant plants tend to minimize transpiration and maximize water uptake through an extended root system (Ludlow, 1987). Our findings regarding these two variables thus might be explained by phylogenetic constraints on allometry and dry mass allocation, with drought-sensitive species such as the deciduous oaks having inherent patterns of high allocation to roots and leaf area, which are not related to evolution under limiting water. We argue that morphological traits are more influenced by phylogeny and thus less flexible in their response to water stress than physiological traits, particularly at the seedling stage. In other words, plants of a given phylogenetic group have a given architecture and morphology with a relatively narrow margin for environmentally induced changes, while gas exchange and pigment composition are broadly shared among distantly related species and are more strongly affected by environmental conditions.

Phylogeny is always involved in any comparative study, particularly when dealing with contrasting species co-occurring in transitional zones, such as here with trees and shrubs found in the Mediterranean region (Cabrera, 2002; Webb et al., 2002). In fact, there was a distinctive phylogenetic signal in the functional grouping of species, with oaks, pines, and other genera being clearly different from each other in their drought tolerance, in their functional responses to drought, and in their ecophysiological traits (Fig. **6**). However, the strong phylogenetic signal in our data did not invalidate the relationships between ecophysiological variables and drought tolerance, which were significant after accounting for phylogenetic effects, with only one exception, the relationship between drought tolerance and photochemical efficiency. Our results show that even though evolutionary processes (adaptation and natural selection) and individual responses (acclimation and tolerance) tend to produce similar results in terms of functional traits associated with water stress and drought tolerance, they do not necessarily match.

An important plant trait involved in coping with drought is water use efficiency (WUE), which has been reported to increase with water stress in both ecological (Ferrio et al., 2003; Adams and Kolb, 2004; Pagter et al., 2005) and agronomical studies (Romero et al., 2004; Turner, 2004; Liu et al., 2005). However, we have observed a significant decrease in instantaneous water use efficiency, with both drought tolerance across species and water stress within a species. This decreased WUE under drought was due to drastically reduced net photosynthesis resulting from very limiting stomatal conductances combined with high respiration rates, and also to reduced light harvesting and utilization efficiency due to low chlorophyll content and photoinhibition. Also in contrast to expectations, whole ecosystem WUE has been shown to decrease in Mediterranean forests exposed to severe drought and both increased respiration and decreased photosynthetic activity were responsible for the negative carbon balance over a drought period (Reichstein et al., 2002). Even though we have not measured dark respiration, there are several indications pointing to respiration as one possible explanation for our findings regarding WUE. Respiration seems to exhibit a biphasic response to water stress, with an initial decrease followed
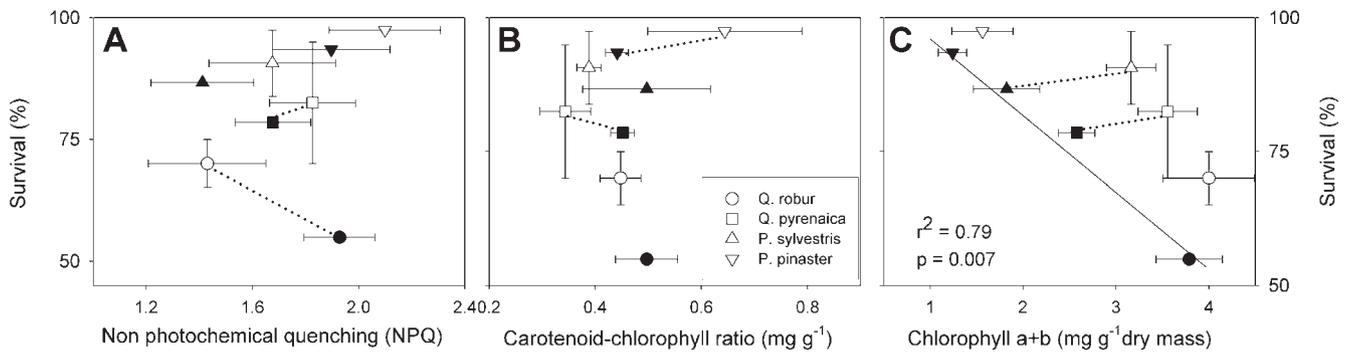
Fig. 4 Survival over the summer of water-stressed (closed symbols) and control plants (open symbols) vs. non-photochemical quenching (NPQ, **A**), carotenoid to chlorophyll ratio (**B**), and chlorophyll a+b con-tent (**C**) under water stress (experiment I, 4 species). For information on lines, error bars, and number of samples see legend of Fig. **1**.
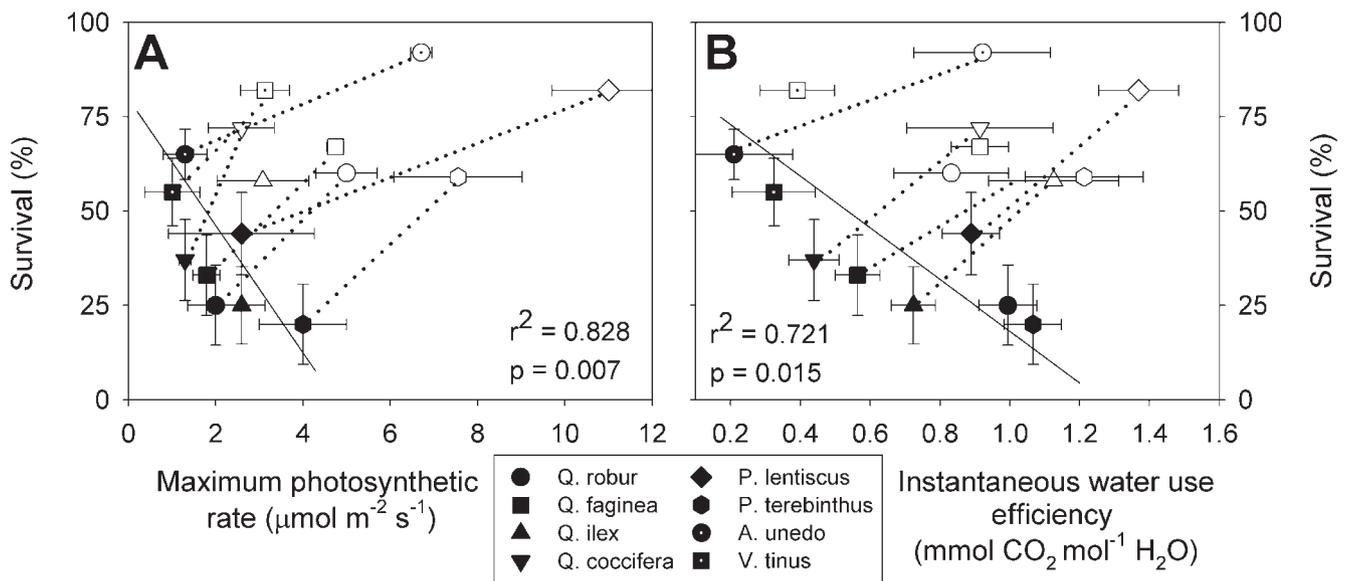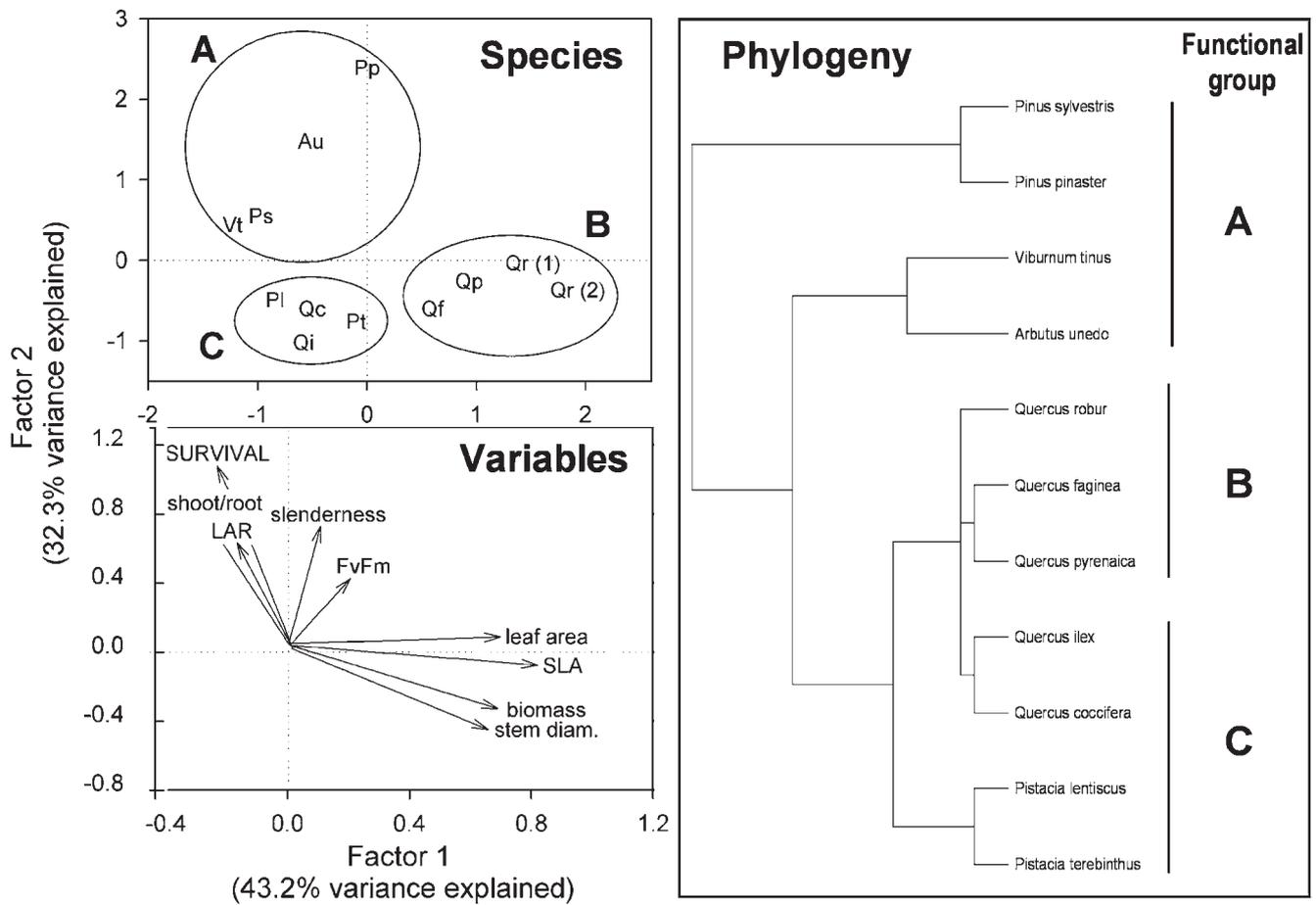

Fig. 5 Survival over the summer of water-stressed (closed symbols) and control plants (open symbols) vs. maximum photosynthetic rate (**A**) and instantaneous water use efficiency (**B**) under water stress (ex-periment II, 8 species). For information on lines, error bars, and number of samples see legend of Fig. **1**.

by an increase as the stress progresses due to damage repair (Ghashghaie et al., 2001; Flexas et al., 2005). In our case, high leaf temperatures (air temperature above 30 °C with high irra-diance loads and no transpirational cooling) might have sig-nificantly raised respiration under water stress over the sum-mer. Discrepancies between current and previous findings on the effect of drought on WUE are due, at least in part, to differ-ences in the severity of the water stress experienced by study plants. While in previous studies water-stressed plants are typically exposed to – 1 MPa (agronomic studies) down to – 2 or – 3 MPa (ecological studies) of soil water potential, the wa-ter-stressed plants studied here experienced water potentials as low as – 4.5 (experiment I) and – 5.5 (experiment II) MPa. We suggest that there is a biphasic response in WUE, which is analogous to that found in respiration and to some extent trig-gered by the increased maintenance component of respiration reported at severe water stress. Discrepancies could thus be solved by considering that, in many studies reporting an in-crease of WUE with drought, the second phase of decreased

WUE induced by severe water stress might not have been reached.

In a previous study with four of the eleven species studied here, we have observed that, in contrast to current results and in agreement with many previous studies, WUE increased with drought (Valladares et al., 2005). In that study, WUE was scaled up to the whole plant by means of a 3-D geometric re-construction of the crown using the computer model Y-plant (Pearcy and Yang, 1996). This scaling might be involved in the differences between the two studies, since whole plant poten-tial carbon gain and water loss significantly differ from leaf-level photosynthesis and transpiration. Another two reasons to explain the different results are: i) leaf-level physiological values used in Valladares et al. (2005) to calculate WUE were not obtained from the second part of the biphasic response, where respiration increase and stomatal limitations to photo-synthesis make carbon balance more negative, and ii) plants used in each study were of different age, 2-year-old saplings

Fig. 6 Segregation of the eleven species studied according to eight functional variables and survival over the summer under water stress in a principal components analysis. Results from experiments I and II were used jointly. Three main groups of species (A, B, and C) were observed. The phylogeny of the species studied (see "Methods") is shown on the right panel together with the three functional groups. Abbreviations for the species were taken from the genus and the specific epithet; Qr (1) and Qr (2) represent *Quercus robur* from experiment I and II, respectively.

in Valladares et al. (2005) vs. 1-year-old seedlings in the current study, the former having undergone a drought over the summer during their first year of growth.

Drought in nature usually co-occurs with high-light stress, so their interactive effects on plants must be taken into account to understand the ecology and evolution of plants under limiting water (Cornic, 1994; Valladares and Pearcy, 1997). In our study, sensitivity to high-light stress tended to be greater under water stress and in drought-intolerant plants. No trend was found, though, for non-photochemical quenching and carotenoid content, suggesting that photoprotection by energy dissipation was not associated with drought tolerance or individual responses to water stress. In a field study, drought-avoiding and drought-tolerant Mediterranean trees were found to employ different mechanisms for coping with excess light during the midday depression in photosynthesis, which involved different degrees of dynamic photoinhibition as a photo-protective mechanism (Martinez-Ferri et al., 2000). These behaviours suggested different mechanisms to withstand water deficit in drought-avoiding and drought-tolerant species. Our results support the notion that high-light stress under water stress is minimized by reducing light harvesting

(Valladares and Pearcy, 1997). The reduced chlorophyll content in water-stressed plants and in the most drought-tolerant species agrees with observations in plants from arid environments (Balaguer et al., 2002 and references therein).

In a study of two Mediterranean sub-shrubs (rosemary and lavender), the drought-induced decreases in the relative quantum efficiency of PSII photochemistry were attributed to "down-regulation" of electron transport; after the autumn rainfall, photosynthetic capacity fully recovered (Nogues and Alegre, 2002). It was concluded that these two species were well adapted to drought, and that the increased water deficit expected in a global change scenario is unlikely to have a significant impact on the photosynthetic capacity of leaves. However, rosemary and lavender are truly Mediterranean plants, i.e., species that evolved under a Mediterranean climate, and not Tertiary relicts as are species of the genera *Quercus, Arbutus, Viburnum,* and *Pistacia,* or even older species, as it is the case for pines and junipers. All these species that evolved well before the Mediterranean climate was established are currently important components of Mediterranean shrublands and forests (Thompson, 2005). Thus, climate change involving increased aridity will lead to significant changes in species com-

position and dynamics in these forests due to important differences in the drought tolerance and in the strategy to cope with water stress of woody plants currently coexisting in Mediterranean ecosystems (Ogaya and Peñuelas, 2003).

Our results with seedlings cannot be extrapolated to adult trees, since ecophysiological features have been shown to vary with ontogeny in Mediterranean trees (Mediavilla and Escudero, 2004). Nevertheless, ecophysiology of tree seedlings is particularly important because it is linked to the regeneration niche of each species, and the capacity to withstand water limitations at the seedling stage has a direct impact on forest composition and dynamics, particularly in Mediterranean ecosystems (Retana et al., 1999; Sánchez-Gómez et al., 2006). Our results do indicate, however, that drought tolerance is not achieved by a single combination of trait values and that these combinations can differ at different levels of analysis, i.e., in individual responses to drought vs. interspecific comparisons of drought tolerance.

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