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Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes

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Abstract

Interacting effects of high light and drought on the performance of sun and shade phenotypes were experimentally undertaken following survival, chlorophyll fluorescence and gas exchange in 2-year-old saplings of four Mediterranean trees (*Quercus ilex* and *Q. coccifera* as water-saving species, and *Pistacia lentiscus* and *P. terebinthus* as water-spending species). Half of the saplings were grown in full sunlight and the other half in the shade (6% sunlight). Half of each combination of species–phenotype was exposed to high light during a simulated late-summer drought. Light absorptance and gas exchange were scaled up to the whole plant with the 3-D geometrical model, Y-Plant. *Quercus* species were more plastic and tolerated high light and water stress better than *Pistacia* species, surviving longer and in drier soils, and exhibiting a less pronounced photoinhibition. There was no evidence of disadvantage for shade phenotypes under high light with increasing drought. By contrast, shade phenotypes survived longer despite larger initial decreases in photochemical efficiency and higher sensitivity to drought than sun phenotypes. The enhanced control of transpiration during drought in water-saving versus water-spending species (and also in shade versus sun phenotypes in three out of the four species) allowed extended survival. Photoinhibition reduced whole crown carbon gain in high light by *c.* 3% and affected significantly more the shaded leaves of a given plant (reducing their carbon gain by up to 7%) than those exposed to direct sunlight. Despite this apparently minor impact, whole plant carbon gain reduction by photoinhibition negatively correlated with survival and drought tolerance. The implications for succession and forest regeneration in arid environments, particularly under a global change scenario, are discussed.

Introduction

In tropical and moist temperate forests, late successional species typically establish in the shade due to the limited availability of open environments as well as because of their shade-tolerant physiological and morphological characteristics (Bazzaz and Pickett, 1980; Emborg, 1998). Although it is less-well documented, this pattern also occurs in arid environments (Flores and Jurado, 2003; Valladares, 2003). The current paradigm for secondary succession in arid habitats is that shade-tolerant, but relatively more drought-sensitive species, establish under, and ultimately replace, high light-demanding but relatively more drought-tolerant species (Broncano *et al.*, 1998; Rodá *et al.*, 1999; Maestre *et al.*, 2003). The conceptual framework underlying this paradigm is the importance of facilitation under high abiotic stress (Bertness and Callaway, 1994; Callaway and Pugnaire, 1999). This framework agrees well with a number of observations in arid environments (Pugnaire and Luque, 2001; Flores and Jurado, 2003; Ludwig *et al.*, 2003; Pugnaire *et al.*, 2004). However, the picture of succession and plant–plant interactions in arid ecosystems is still far from clear. On the one hand, competition can occur under both high and low abiotic stress conditions (Maestre and Cortina, 2004). On the other hand, interactions

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between light and water availability can restrain succession (Zavala *et al.*, 2000). Alternatively, neighbours can facilitate establishment and therefore succession by providing shade and reducing light and temperature stresses, while at the same time causing greater drought stress due to increased below-ground competition for water (Tschaplinski *et al.*, 1998; Tielborger and Kadmon, 2000*a*; Valladares and Pearcy, 2002). Furthermore, seasonal and inter-annual environmental variation can tip the balance between facilitation and interference in plants from arid sites (Tielborger and Kadmon, 2000*b*). The difficulties of unveiling successional processes in arid ecosystems are further exacerbated by a limited understanding of plant responses to multiple, co-occurring stresses (Valladares and Pearcy, 1997; Nogues and Alegre, 2002; Sack *et al.*, 2003; Valladares, 2003).

Although shade limits photosynthesis and growth of plants, it can also be beneficial in hot arid environments in terms of reduced leaf temperatures, transpiration and photoinhibition (Valladares and Pearcy, 1997; Flores and Jurado, 2003; Gómez-Aparicio *et al.*, 2004). Overstorey plants creating the shade can, however, extract soil moisture, resulting in at least some cases of significantly lower water potentials and greater water stress effects for plants in shaded compared with open environments (Abrams and Mostoller, 1995; Valladares and Pearcy, 2002). Maximizing carbon gain in shaded environments requires shade acclimation, which in turn increases susceptibility to photoinhibition (Anderson and Osmond, 1987; Tognetti *et al.*, 1994; Valladares *et al.*, 2002*b*; Niinemets *et al.*, 2003). Moreover, species-specific shade tolerance can be significantly influenced by water availability, as observed in pine and oak seedlings (D Sánchez-Gómez, MA Zavala, F Valladares, unpublished data). Thus, there are clearly complex interactions between drought and shade involving physiological capabilities and plasticities as well as competitive factors (Niinemets and Valladares, 2004; Prider and Facelli, 2004; Sack, 2004).

In this study the impact of combined late summer drought and high light was examined on saplings of four late successional, Mediterranean, evergreen trees. Juveniles of these species have been shown to exhibit contrasting responses to drought, with *Quercus ilex* and *Q. coccifera* being classified as water-savers and *Pistacia lentiscus* and *P. terebinthus* being classified as water-spenders on the basis of their tight or loose stomatal down-regulation of transpiration, respectively (Vilagrosa *et al.*, 2003). The objectives were (i) to determine species differences in the extent of photoinhibition under high light and drought and its impact on gas exchange and survival of sun and shade phenotypes; (ii) to understand the roles of structural avoidance of excessive light in minimizing photoinhibition; and (iii) to evaluate, by scaling from leaves to whole crowns with a 3-D dimensional architecture model, Y-Plant, the impacts of photoinhibition and drought on whole crown performance in these species. Since aridity is expected to

increase in Mediterranean ecosystems (IPCC, 2001), understanding how water and light availabilities influence the performance of sun and shade phenotypes of late-successional Mediterranean plants is crucial in predicting the impact of climate change on natural forest regeneration, reforestation–afforestation practices, and rural land uses.

Materials and methods

Plant material and experimental design

All plants used in this experiment were grown in a nursery at Viveros Barbol, Torremocha del Jarama (40°50′ N, 3°29′ W, Madrid, Spain). The nursery is 710 m asl (above sea level) and the region has a typical continental Mediterranean climate with hot and dry summers and cold winters (9.5 °C mean minimum and 19 °C mean maximum annual temperatures). Most precipitation (250–350 mm of an annual total of 350–500 mm) occurs in spring and autumn (Instituto-Nacional-de-Meteorología, 2003). Local air temperature and photosynthetic photon flux density (PPFD) were registered every 5 min during the whole growth period with a data logger (HOBO model H08-006-04, Onset, Pocasset, MA, USA) with external cross-calibrated sensors. Mean daily solar radiation flux over the system during the summer was 42 mol m^{-2} d^{-1}.

Seedlings of four tree species (*Quercus ilex* L. subspecies *ballota* (Desf.) Samp, *Q. coccifera* L., *Pistacia lentiscus* L., and *P. terebinthus* L.) were grown for 2 years in the nursery. The first year they were grown in 3.0 l forest containers and then in the intervening winter they were transplanted to 4.0 l pots. The soil was a 75:25 (v:v) mixture of Vriezenveen PP1 A soil (Potgrond Vriezenveen bv, Westerhaar, Netherlands), and washed river sand, and had a pH of 6.5. Three kg m^{-3} of Guanumus Angibaud fertilizer (3/35/2 N/P/K) (Angiplant, La Rochelle Cedes, France) and 2 kg m^{-3} of Plantacote mix 4 M fertilizer (15/10/15 N/P/K) (Aglukon Spezialdünger GMBH & Co. KG, Dusseldorf, Germany) were added to the soil mixture. Plants were grown in two different light environments (full sunlight and 6% sunlight). The 6% sunlight environment was created by using layers of neutral shade cloth supported by metal frames. Air temperatures were similar (within ±2 °C) in the different light environments.

At the end of the second growth period (early summer, 2003), saplings of each species were transferred to a glasshouse at CCMA-CSIC (Madrid) where they were first acclimated under the same light environments (open bench versus shade cloth enclosure) as in the nursery for one month before initiating the experiment. This was done to maintain the sun and shade type phenotypes established during growth in the nursery. The glasshouse environment was set to mimic late-summer, Mediterranean climate conditions (relative humidity of the air <55%, air temperature 26–34 °C). The experiment was initiated by randomly selecting and moving plants so that half of each species–phenotype combination was exposed to high light during the six central hours of the day by supplementing the natural light with light from sodium-halide lamps. The other half of each species–phenotype combination received only low light under the shade cloth. The supplemented high-light treatment gave a leaf-level PPFD of 2100–2350 μmol m^{-2} s^{-1} giving a total of 30–40 mol m^{-2} d^{-1}, whereas the low-light treatment gave a maximum PPFD of 210 μmol m^{-2} s^{-1} and a daily a total of 2–3 mol m^{-2} d^{-1}. Thus, the factorial experiment had three factors with a total of 16 combinations (4 species, 2 phenotypes, and 2 light treatments) and 3–5 replicates.

Assessment of plant and leaf-level performance during drought

A drought cycle designed to mimic late summer conditions was initiated at the end of August 2003. Survival, soil water potential,

chlorophyll fluorescence, and leaf gas exchange were registered every 2–3 d during the progress of desiccation in sun and shade individuals of each species exposed to either high or low light. A sapling was considered dead when all their leaves were dry (giving no fluorescence signal) and their stems, branches, and petioles were stiff and fragile instead of flexible. Soil volumetric water content was measured in all pots every other day with a Theta Probe ML2x and a HH2 moisture meter (Delta T Devices, Cambridge, UK). Soil water potential was estimated from the relationship between volumetric water content of the soil and water potential of Whatman No. 42 discs (Whatman plc, Kent, UK) following the filter-paper technique (Deka *et al.*, 1995). The photochemical efficiency of photosystem II (F_v/F_m) of fully expanded leaves was determined with a pulse-modulated fluorometer (FMS2, Hansatech Instruments, Norfolk, UK). In the early morning and again in the late afternoon 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 F_v/F_m measurements made. Photosynthetic response to irradiance was measured in one fully-expanded, current-year leaf of 6–11 plants per species per phenotype 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, estimated transpiration and dark respiration were measured weekly until gas exchange signals were too low for reliable measurements. Instantaneous water-use efficiency (*WUE*) was calculated as the ratio of net photosynthetic rate over transpiration at saturating light.

Modelling of canopy architecture and whole crown gas exchange and photoinhibition

The 3-D crown architecture model, Y-Plant (Pearcy and Yang, 1996), was used to scale the effects of drought and photoinhibition measured at the single leaf level to the whole plant crown level. The version used is an update of the original model presented in 1996. The modifications include (i) a leaf energy balance model for computation of leaf temperature and transpiration rates, (ii) the Ball–Woodrow–Berry model (BWB model; Ball *et al.*, 1986) model of stomatal conductance, (iii) the Farquhar–von Caemmerer–Berry model (FvCB model; Farquhar *et al.*, 1980) for CO_2 assimilation, and (iv) the Ögren and Sjöström (1990) model for photoinhibition as implemented by Werner *et al.* (2001). The coupled analytical solution of Baldocchi (Baldocchi, 1994) for stomatal conductance and assimilation rate was used.

The crown architectural information required by Y-Plant was obtained from measurements on three individual plants per species and phenotype (a total of $3 \times 4 \times 2 = 24$ plants randomly chosen from >100 plants). At each node within the crown, the internode and petiole angles and azimuths, the angle and azimuth of the leaf surface normal, and the azimuth of the midrib were recorded with a compass-protractor. Leaf, petiole, and internode lengths were measured with a ruler and petiole and internode diameters were measured with digital callipers. A node or internode in Y-Plant are not identical to an actual node or internode since true nodes were skipped if the leaves had been shed and if no branching occurred at them. An internode may therefore contain one or more actual true nodes. The nodes were numbered proceeding from the base to the top of the plant and along each branch. By recording the mother node (the node from which a subsequent node arises) for each node, the proper topology of the shoot could be reconstructed by Y-Plant using the geometrical information. Leaf shape was established from *x*, *y* coordinates of the leaf margins. Leaf size was then scaled in the crown reconstruction from the measured leaf length.

Leaves were assigned physiological characteristics including a maximum light-saturated assimilation rate (A_{max}) a dark respiration rate (R_d), leaf absorptance (*a*), a curvature factor (θ), and quantum yield (ϕ) required for simulating the light response of CO_2 assim-

ilation with the rectangular hyperbola model of Thornley (1976). The equation for this model is:

$$A(I) = \frac{\phi I + A_{max} - \sqrt{(\phi I + A_{max})^2 - 4\theta I A_{max}}}{2\theta} - R_d \qquad (1)$$

where *A* is the assimilation rate and *I* is the PPFD. Values of the maximum carboxylation capacity (V_{cmax}) and electron transport capacity (V_{jmax}) required for the FvCB model of leaf assimilation were also assigned. Values of V_{cmax} and V_{jmax} were derived from assimilation versus CO_2 dependence (A/C_i) curves. Different values for these parameters were assigned for different stages in the drying cycle according to the measured physiological responses. The FvCB model was used primarily to calculate A_{max} at light saturation and the current intercellular CO_2 pressure and leaf temperature, and then it was applied to the rectangular light response model. The advantage of this approach is that it gives much better fits in the transition from light limitation to saturation than the FvCB model alone, which often has an unrealistically sharp transition.

Simulations with Y-Plant gave the diffuse and direct light interception assimilation rate, transpiration rate for each leaf in the crown, and then by integration the corresponding whole crown values. Simulations were carried at 30 min intervals between sunrise and sunset for a late summer day (7 September) at 42° N latitude. Clear sky conditions were simulated and a standard overcast sky (SOC) distribution of diffuse solar radiation was assumed. The direct solar beam radiation was calculated from an assumed solar constant (2450 μmol photons m^{-2} s^{-1}) and the solar elevation angle as determined by the date, time, and latitude. A clear sky atmospheric transmission coefficient of 0.8 was assumed which gave maximum diffuse plus direct solar radiation fluxes of 2000 μmol m^{-2} s^{-1} at noon. Plant water-use efficiency was calculated as daily carbon gain divided by daily transpiration both estimated for the whole crown with Y-Plant.

Potential photoinhibition was estimated by sampling the absorbed PPFD received on 2 mm^2 at the centres of 20 target leaves giving representative sample of all leaves on the shoot. This approach was used since it gave a more accurate representation of the temporal distribution of PPFD, and especially the magnitude and duration of exposure to high PPFD, than the whole-leaf values, since the latter frequently included portions that were sunlit with the remaining parts shaded. The sub-sampled PPFD was then used to compute the weighted average PPFD for the previous 6 h according to the procedure of Ögren and Sjöström (1990). Weighted PPFD over the last 6 h is a good estimator of the reduction in F_v/F_m under natural conditions (Ögren and Sjöström, 1990; Valladares and Pearcy, 1999). The weighted PPFD was computed with Y-Plant for the specific leaves on which F_v/F_m measurements were made; these data were used to establish the relationship between weighted PPFD and the reduction in F_v/F_m. These relationships were then used to simulate the reduction in F_v/F_m for the target leaves throughout the day. A scaling factor, $S = (F_v/F_m)/(F_v/F_{mmax})$ was then calculated where F_v/F_{mmax} is the maximum value in the absence of photoinhibition (Werner *et al.*, 2001), and this scaling factor was applied to the quantum yield for CO_2 uptake and the curvature factor. These reduced values were then applied in equation 1 to estimate the impact of photoinhibition on carbon gain. A high correlation has been found between the reduction in F_v/F_m and the reduction in the apparent quantum yield of photosynthesis in a number of studies (Demmig and Bjorkman, 1987). Calculations of whole crown carbon gain with and without photoinhibition were carried for two physiological states: at the beginning of the drought experiment (soil water potential > -1 MPa) and at an intermediate drought (soil water potential > -4.5 MPa). The photosynthesis model was parameterized at different times during the drought cycle. The simulations of photoinhibition only reflected the effects of short-term 'dynamic' photoinhibition, but by

parameterizing the model at different points in the drought cycle any 'chronic' photoinhibition, which would be affected by other effects such as stomatal conductance decreases, were also included.

Statistical analyses

Three-way analysis of variance (ANOVA, Tukey test, SYSTAT 9.0 Windows version 2000, SPSS Inc. Chicago IL, USA) was used to test for differences among species, sun–shade phenotypes and light treatments, and interactions. In all cases, the data met the assumptions of normality and homoscedasticity. 95% confidence intervals for the mean obtained with ANOVA are presented in most figures. Significance threshold is $P < 0.05$ unless stated otherwise. Linear and non-linear regression analysis was used to explore the relationships between photochemical efficiency and soil water potential and weighted PPFD dose, and between carbon gain and mortality and soil water potential.

Results

Sun and shade phenotypes of the fours species studied were morphologically different (Table 1). Sun phenotypes exhibited significantly thicker stems than shade phenotypes, but their height exhibited no consistent pattern, and total leaf area was similar although with significant differences across species. Saplings of the four species studied responded very differently to the simulated late summer drought, with the water-spending *Pistacia* species depleting soil water and dying faster than the water-saving *Quercus* species (Fig. 1). Differences in the speed of soil drying were not due to differences in plant leaf area since it did not significantly differ between species and phenotypes (leaf

Table 1. *Morphological data (plant height, root collar diameter, and total leaf area) of sun and shade phenotypes of the four species studied*

Species and phenotypes with different letters exhibited significant differences in ANOVA ($P < 0.05$, Tukey test; $n=12–18$).

| Species	Phenotypes	Height (cm)			Root collar diameter (mm)			Leaf area (cm^2)		
		Mean	SD	Test	Mean	SD	Test	Mean	SD	Test
P. lentiscus	Sun	21.9	3.3	a	5.0	0.8	a	174.3	14.6	a
P. lentiscus	Shade	11.6	1.8	b	2.6	0.4	b	188.5	15.8	a
P. terebinthus	Sun	16.6	2.5	b	4.7	0.7	a	197.3	16.6	a
P. terebinthus	Shade	11.9	1.8	b	3.7	0.6	b	199.5	16.8	a
Q. coccifera	Sun	18.8	2.9	a	4.7	0.7	a	85.2	7.2	b
Q. coccifera	Shade	24.9	3.8	a	4.3	0.7	a	93.1	7.8	b
Q. ilex	Sun	22.4	3.4	a	6.1	0.9	c	125.1	10.5	c
Q. ilex	Shade	27.8	4.2	a	5.6	0.9	a,c	116.9	9.8	c
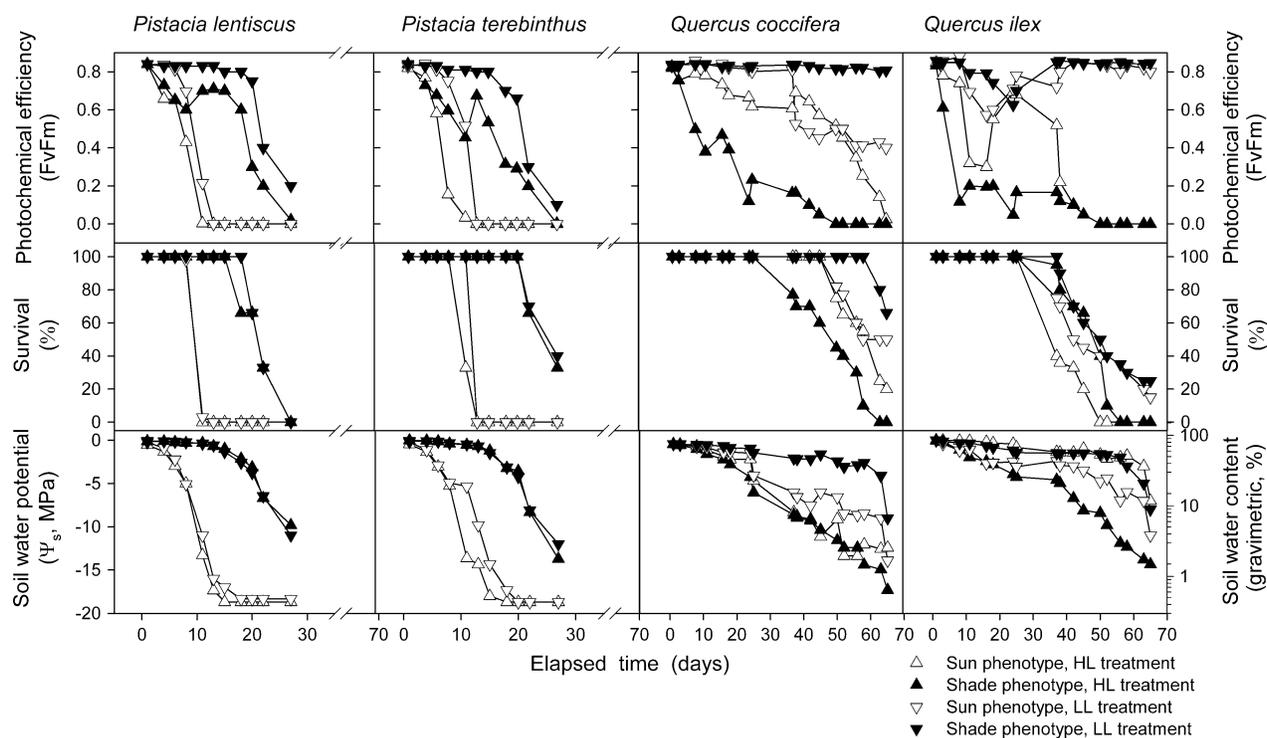
Fig. 1. Changes in soil water (expressed as both soil water potential and gravimetric water content), survival, and photochemical efficiency of 2-year-old saplings of four woody Mediterranean species during a drought cycle. The last watering of 4.0 l pots containing the saplings was on day 0. Each data point is the mean of measurements taken on 3–8 plants Error bars are omitted for clarity.

area per plant was 143 ± 60 cm^2 mean $\pm$SD). Shade phenotypes of the two *Pistacia* species survived significantly longer than their sun phenotype counterparts. This trend was not as clear in the two oak species since there was a significant phenotype×light.treatment interaction during the drought cycle: Shade phenotypes survived longer than sun phenotypes in low light while the reverse was true in high light (Fig. 1). Shade phenotypes, however, died at higher soil water potential, indicating a higher sensitivity to drought in comparison with their sun phenotype counterparts (Fig. 2).

The slow dehydration of shade phenotypes of *Pistacia* and *Quercus* allowed for acclimation to high light. As a result photochemical efficiency (F_v/F_m) exhibited some recovery after several days of treatment. Although shade phenotypes had a higher initial sensitivity to high light, they died at mean F_v/F_m values similar to those of sun phenotypes (Figs 1, 2). F_v/F_m exhibited a threshold response to soil water potential in low light with a sharp decrease as soil water potential decreased beyond the thresholds (-5 and
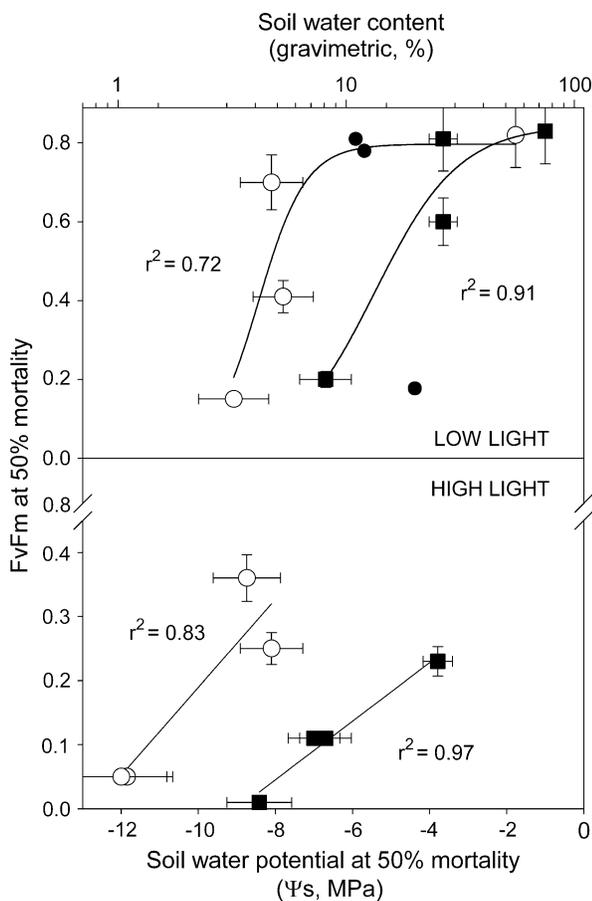
-3 MPa in sun and shade phenotypes, respectively). In high light, F_v/F_m exhibited a linear decrease with decreasing soil water potential in high light (Fig. 2).

There were significant species and sun–shade phenotype differences in whole-crown light absorption and potential carbon gain (Fig. 3). Saplings of *Pistacia terebinthus* displayed >75% of their leaf area to direct sunlight with the remainder being self-shaded by the shoot architecture. By contrast, the two *Quercus* species saplings displayed only 55% of their leaf area to direct sunlight due to greater self-shading. Sun and shade phenotypes of the *Pistacia* species exhibited only minor and not statistically significant differences in whole-crown PPFD absorptance and carbon gain,
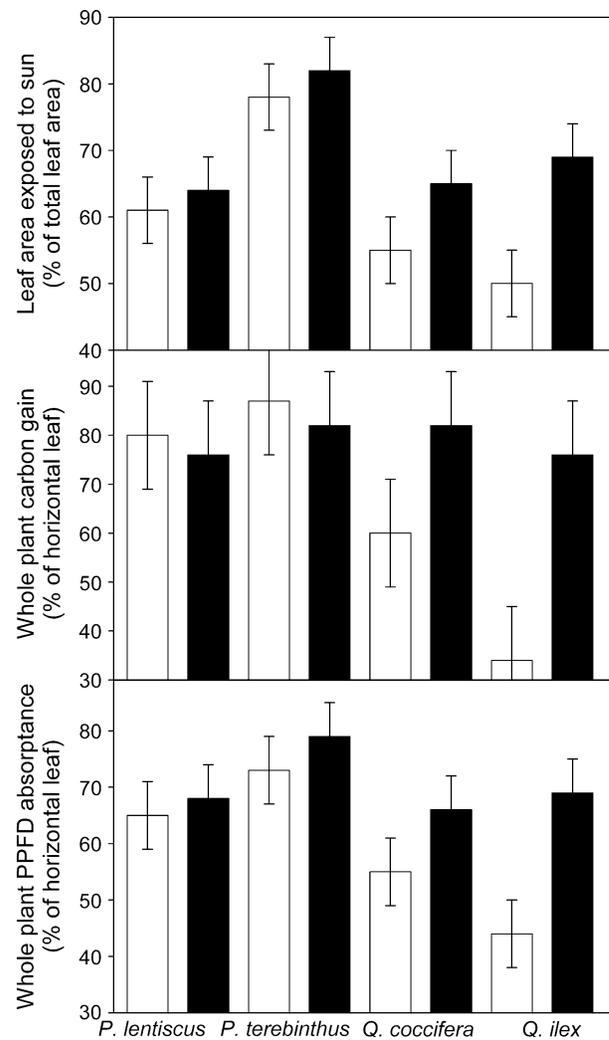

Fig. 2. The relationship between photochemical efficiency (F_v/F_m) and soil water content (expressed both as gravimetric water and as soil water potential) at 50% mortality of saplings of each species. Open symbols, sun phenotypes; closed symbols, shade phenotypes. Error bars indicate 95% confidence interval after ANOVA.

Fig. 3. Perfomances of the crown architectures of sun and shade-grown saplings of *Pistacia* and *Quercus* species based on simulations with Y-Plant for a clear summer day. The white and black bars are for sun and shade-grown saplings, respectively. (Top) Percentage of the leaf area exposed to direct solar radiation. (Middle) Simulated whole plant daily carbon gain per unit leaf area expressed as a percentage of the value for a horizontal leaf. (Bottom) Fraction of incident PPFD absorbed by the plant expressed as a percentage of the value for a horizontal leaf. Error bars indicate the 95% confidence intervals after ANOVA

while significant phenotypic differences were observed in the *Quercus* saplings. By comparison with shade phenotypes, *Quercus* sun phenotypes absorbed less PPFD, had lower carbon gain, and had lower fractions of their leaf area exposed to direct sunlight. (Fig. 3). The contrasting architectures resulted in differing diurnal exposures to light. (Fig. 4). Comparisons of simulations under a clear summer day showed that the oak sun-phenotype architecture effectively reduced absorptance of high PPFD (>1600 µmol m^{-2} s^{-1}) during midday relative to the shade phenotype. Shaded leaves within the sun phenotype architecture received only about one-third of the PPFD received by shaded leaves in the shade phenotype. This difference was a result of the more open architecture of the shade phenotype, which allowed greater penetration of diffuse skylight.

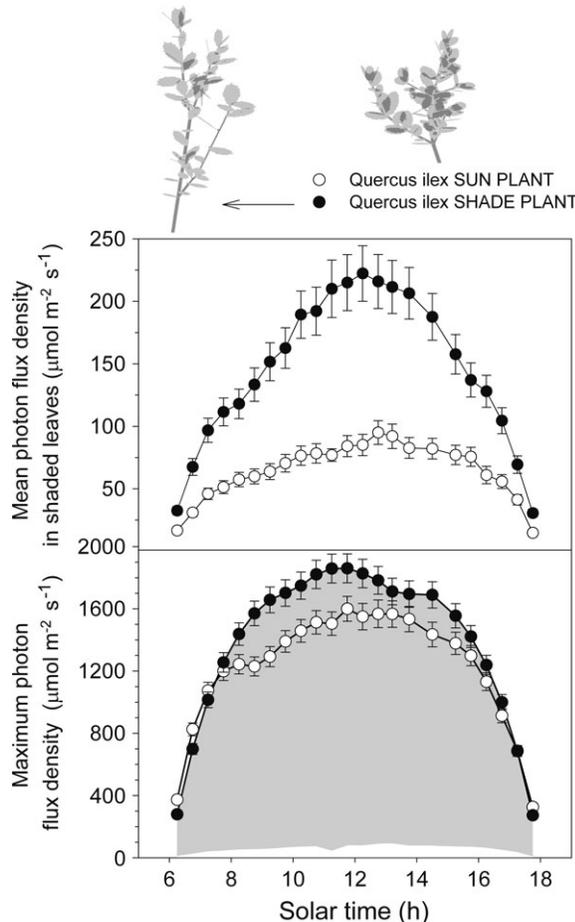Leaf-level photosynthetic and transpiration rates were higher in sun than in shade phenotypes of both *Quercus* species, but were not significantly different between sun and shade phenotypes in the two *Pistacia* species (Fig. 5). Transpiration was significantly reduced by drought in all species, but significant differences were apparent between *Pistacia* and *Quercus*. (Fig. 5). Whole plant transpiration was reduced by 40% in the two *Pistacia* species and by 90% in the two *Quercus* species. Whole plant water-use efficiency of saplings experiencing drought (soil water potential −1 to −4.5 MPa) was significantly higher in shade than in sun phenotypes in both *Quercus* species, but no phenotypic differences were evident for the *Pistacia* species (Fig. 5).

Photochemical efficiency (F_v/F_m) decreased with increasing weighted PPFD dose over the last 6 h in all species and phenotypes. Significant species×phenotype interactions were evident, particularly when the effect of drought was included (Fig. 6). The slopes did not differ between sun and shade phenotypes in *Pistacia*, whereas for the *Quercus* species, they were steeper in shade than in sun phenotypes. Extreme drought (soil water potential <−4.5 MPa) reduced F_v/F_m even at very low weighted PPFD dose in the two *Pistacia* species, but not in the two *Quercus* species. Extreme drought also exerted a stronger effect in sun than in shade phenotypes of *Pistacia* (Fig. 6). Carbon gain was reduced by *c.* 3% when the effects of weighted PPFD dose on F_v/F_m were estimated for the whole crown with Y-Plant in saplings experiencing drought (soil water potential −1 to −4.5 MPa), with the exception of sun phenotypes of *Quercus coccifera*, which were almost unaffected by photoinhibition (Fig. 7). Carbon gain was significantly more reduced by photoinhibition in shaded foliage than in foliage exposed to high light. The impact of photoinhibition on carbon gain under high light exhibited a non-linear relationship with survival time and drought tolerance. Plants exhibiting only minor reductions of carbon gain under high light were those surviving longer and to drier soils during the simulated drought (Fig. 8).

Discussion

Drought not only reduced carbon gain and transpiration but also exerted a strong, direct influence on survival of saplings of the four woody species studied. However, the influence of photoinhibition on performance and survival was less evident (Fig. 9). Simulations with Y-Plant indicate that photoinhibition under high light and drought would reduce whole plant carbon gain by only 3%. However, resistance to photoinhibition was significantly correlated with survival time and tolerance to a drying soil. The estimations of the impact of photoinhibition on whole crown carbon gain agree with previous assessments that showed reductions of *c.* 5% in a shrubland of *Quercus coccifera* (Werner *et al.*, 2001) and in individual plants of the Californian schlerophyll *Heteromeles arbutifolia* (Valladares and Pearcy, 1999). They also agree with other studies pointing to a minor impact of photoinhibition on
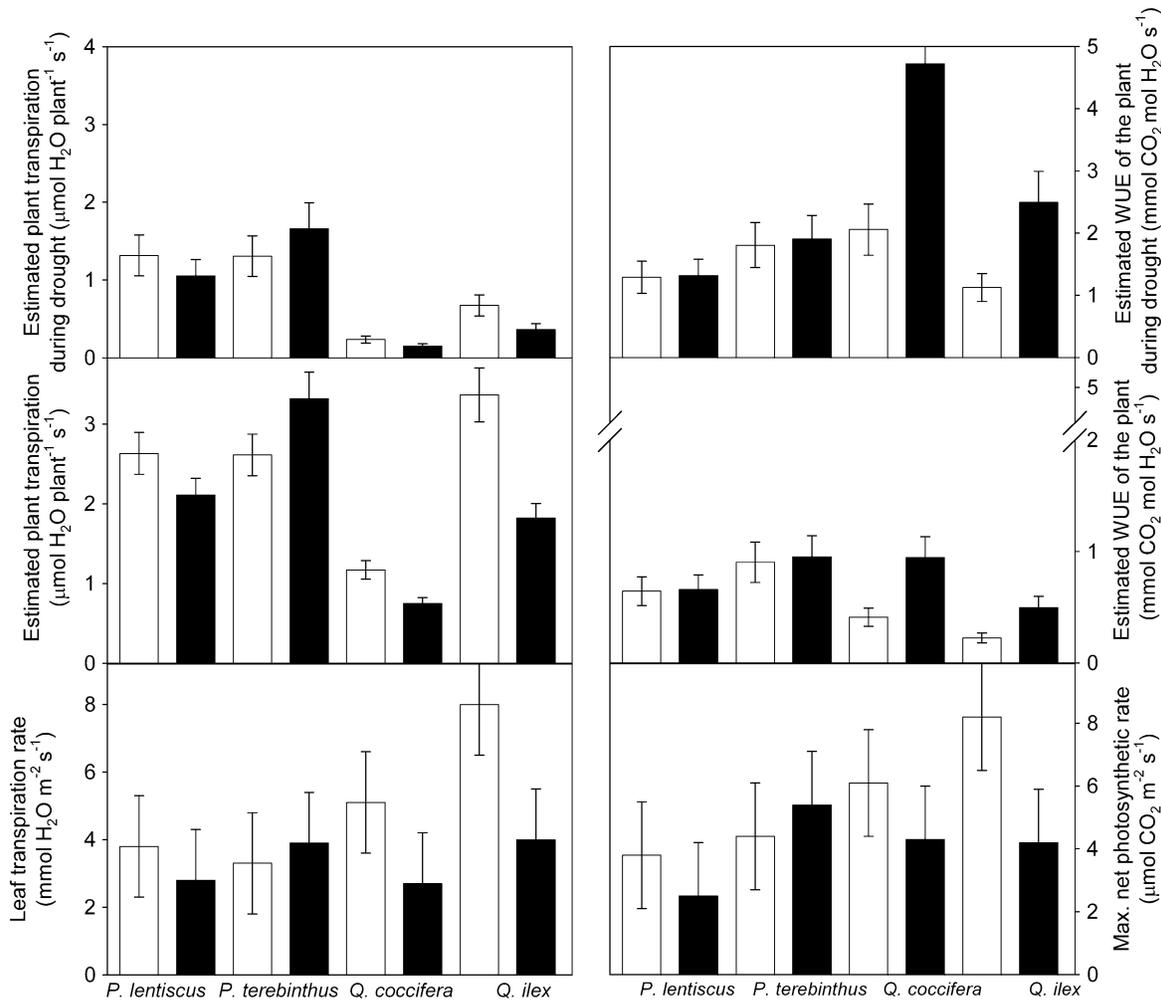
Fig. 4. The diurnal course of absorption of PPFD by sun (open symbols) and shade (closed symbols) phenotypes of *Quercus ilex.* The top panel shows the PPFD absorbed by shaded leaves or the shaded parts of leaves. The bottom panel shows the maximum PPFDs absorbed by the sunlit leaves or sunlit parts of leaves. The shaded area gives the range of variation of absorbed PPFDs for the shade phenotype. Each data point is the mean ±SE for 20 leaves of one individual plant.

Fig. 5. Mean leaf transpiration rate and net photosynthetic rate (both measured on 5–8 leaves with the gas-exchange equipment) and whole plant transpiration and water-use efficiency (*WUE*, both estimated with Y-Plant) in 2-year-old saplings of four different woody species. Whole plant values are derived from simulations for a clear summer day. Open bars, sun phenotypes; closed bars, shade phenotypes. Error bars indicate 95% confidence interval after ANOVA.

growth and phenology of Mediterranean plants under field conditions (Oliveira and Peñuelas, 2002). The combined effect of drought and high light in Mediterranean-type sclerophylls only marginally affects the productivity of these plants, which are in general slow-growing stress tolerators. Photoinhibition seems to be part of a suite of stresses with only minor direct effects on the performance of Mediterranean-climate woody plants. However, if climatic severity extends over long periods of time, long-term carbon balance in Mediterranean woody plants can be significantly and adversely affected even by a minor reduction in carbon gain such as that due to photoinhibition. Under drought and high temperatures respiratory carbon losses have been shown to be large in Mediterranean oaks, causing them to become carbon sources rather than sinks as aridity increases (Reichstein *et al.*, 2002).

The impact of photoinhibition on plant performance significantly differed between species and phenotypes, with particularly negligible effects in sun phenotypes of *Quercus*

coccifera. These plants exhibited an efficient morphological photoprotection provided by their crown architecture that minimized excessive light. Sun phenotypes of *Quercus coccifera* in the drying cycle were able to survive longer and to lower soil water potentials than any other species-phenotype. The interplay between leaf-level physiology, drought tolerance, and crown architecture allows for a range of impacts of photoinhibition on the performance of Mediterranean ecosystem plants (Fig. 9), but, in general, they all exhibit an efficient structural and physiological protection against excessive irradiance (Valladares and Pugnaire, 1999; Martinez-Ferri *et al.*, 2000; Oliveira and Peñuelas, 2002). However, there is a cost to this efficient photoprotection in sun phenotypes; shaded foliage received very low PPFDs most of the day, significantly limiting whole-plant carbon gain. Photosynthesis of shaded foliage is very important in crowns of overall low-leaf-area indexes characteristic of Mediterranean shrubs (Caldwell *et al.*, 1986; Tenhunen *et al.*, 1987). Conversely, enhanced light
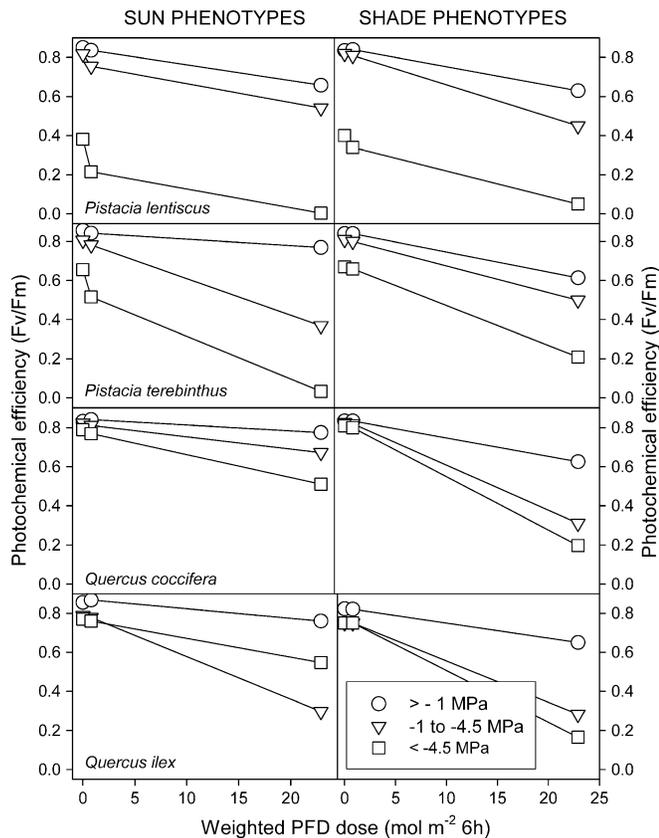
Fig. 6. Photochemical efficiency (F_v/F_m) versus weighted PPFD dose over the previous 6 h in leaves of sun and shade phenotypes of the four species studied under three levels of water stress (circles, no stress; triangles, moderate stress −1 to −4.5 MPa; squares, extreme stress <−4.5 MPa).

Fig. 7. Reduction of estimated carbon gain over the day in 2-year-old saplings of four different woody species for the whole plant and for shaded foliage. Estimations were obtained with the computer crown simulation model, Y-Plant, for plants under drought (between −1 and −4.5 MPa of soil water potential) and are expressed as a percentage of the values obtained with the same plant without any reduction in photochemical efficiency. Open bars, sun phenotypes; closed bars, shade phenotypes. Error bars indicate 95% confidence interval after ANOVA.

capture of shade phenotypes increased the photosynthesis of shaded leaves, but also increased the impact of photoinhibition on highly exposed foliage. Thus, for shade phenotypes, the impact of photoinhibition on leaves exposed to direct sunlight was compensated at least in part by the relatively high photosynthetic rates of the self-shaded leaves. As shown for a tussock grass and a leguminous shrub from xeric Mediterranean locations (Valladares and Pugnaire, 1999), there is a trade-off between harvest of enough light for photosynthesis and avoidance of excessive irradiance. These results show that, in three of the four species studied, shade phenotypes do not necessarily experience greater high-light stress than their sun counterparts. Seedling survival can greatly benefit from shade under arid conditions (Rousset and Lepart, 1999; Flores and Jurado, 2003). The results show that the shade phenotype that develops in the first year does not necessarily confer a disadvantage if the seedling is exposed to high light in the second year. The results therefore support the notion that nurse plants can exert beneficial effects on late successional woody plants in Mediterranean ecosystems, facilitating their establishment and subsequent survival. These results support the notion that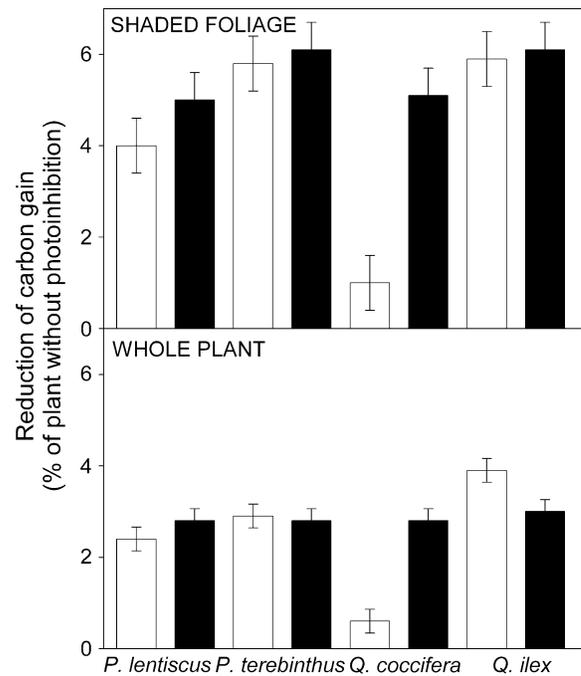 afforestation and reforestation projects should use shrubs as nurse plants to facilitate early establishment of target plants in Mediterranean ecosystems (Gómez-Aparicio *et al.*, 2004).

While multiple stress interactions, such as those between drought and high light, have been widely investigated (see Niinemets and Valladares, 2004, and references therein), only a few studies provide quantitative knowledge of their real impact on the performance of Mediterranean plants (Gamon and Pearcy, 1990; Méthy *et al.*, 1996; Valladares and Pearcy, 1997). These results show that high-light stress exacerbated drought-stress effects only in shade phenotypes of *Quercus coccifera* and in sun phenotypes of *Q. ilex*. In other species–phenotypes there was no significant interaction. Consequently, general conclusions cannot be drawn regarding interactive effects of drought and high-light stress since it depends on the species–phenotype combination. In addition, other factors above the level of physiological effects need to be considered as shown by (i) high photoinhibition but relatively long survival of shade versus sun phenotypes (this study's results), and (ii) higher susceptibility to drought (cavitation at high water potential), but higher survival in the field of *Pistacia lentiscus* versus *Quercus coccifera* (Vilagrosa *et al.*, 2003).

For the species studied there were two contrasting responses to the drought cycle. The two water-spending
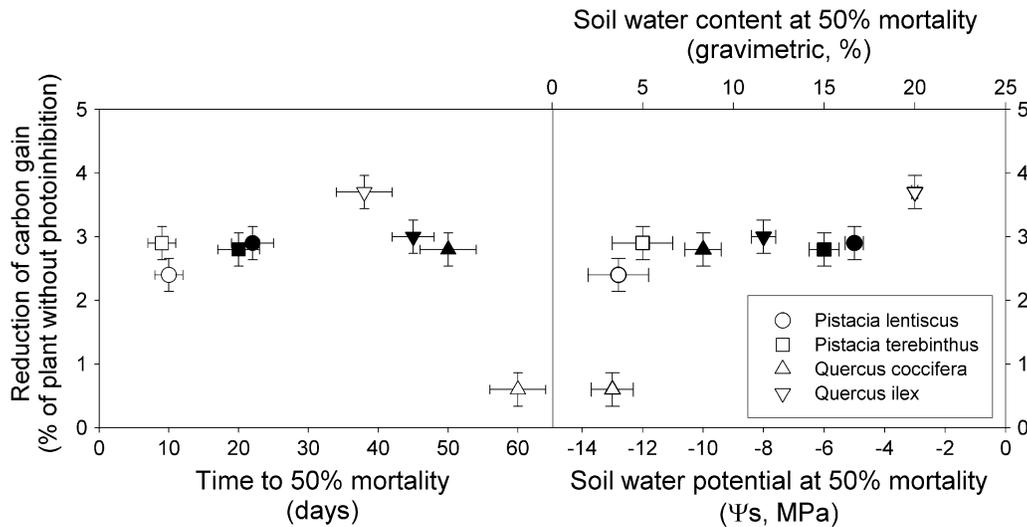
Fig. 8. Reduction of estimated carbon gain over the day versus time elapsed till 50% mortality and versus soil water content (expressed both as gravimetric water and as soil water potential) at 50% mortality in 2-year-old saplings of four different woody species. Estimations were obtained with the computer crown simulation model, Y-Plant, for plants under drought (between -1 and -4.5 MPa of soil water potential) and are expressed as a percentage of the values obtained with the same plant without any reduction in photochemical efficiency. Open symbols, sun phenotypes; closed symbols, shade phenotypes. Error bars indicate 95% confidence interval after ANOVA.
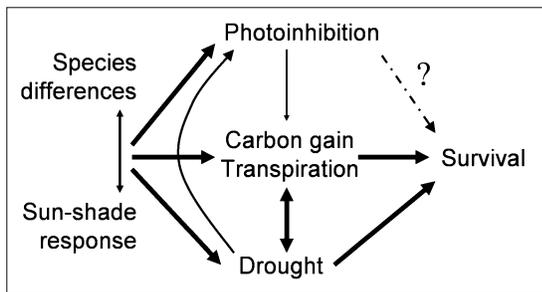

Fig. 9. Relationships among the different factors and processes influencing carbon gain and survival of the saplings of the four Mediterranean woody species studied. Thickness of the arrow indicates estimated relative influence.

Pistacia species reduced plant transpiration by only 40%, thereby depleting soil water more quickly. Death of 50% of the saplings occurred in 2–3 weeks. By contrast, the two water-saving *Quercus* species reduced plant transpiration by 90% and survived for 5–7 weeks before 50% sapling mortality occurred. The differences in survival were not simply due to differences in transpiration and hence the rate of depletion of soil water at the beginning, but rather to the pattern of water use and depletion during the drought. They probably reflect different stomatal responses and water use envelopes (Sperry *et al.*, 2002).

The contrasts in water relations and drought responses of 2-year-old saplings of *Pistacia* versus *Quercus* observed in this study are in agreement with those from 1-year old-seedlings (Vilagrosa *et al.*, 2003), where xylem cavitation occurred at significantly higher leaf water potential in water-spending than in water-saving seedlings (-3 MPa versus -5 MPa, respectively). Leaf water potentials as low

as -5 MPa are infrequent in adult Mediterranean trees, but juveniles that do not have access to the water table can experience water potentials of -5 MPa or lower (Méthy *et al.*, 1996; Terradas, 2001). Leaves and shoots of *Quercus ilex* are known to tolerate leaf water potentials as low as -10 MPa (Larcher, 1960). Resistance to implosion of xylem conduits under high tensions is important and is conferred by the narrow lumens and thick walls in the xylem of Mediterranean evergreen oaks, but these properties also result in a high cost for tissue construction (Hacke *et al.*, 2001). By contrast, *Pistacia* sp. are more vulnerable to cavitation and xylem implosion, but rely on low costs of wood construction to produce new branches and leaves once water is again available (Vilagrosa *et al.*, 2003). The decreased transpiration observed in water-savers allows for extended survival during drought periods, which may be adaptive in allowing sapling survival until autumn rainfalls. However, *Pistacia*'s dense and shallow root system facilitates xylem refilling after less predictable rain events, also promoting plant survival (Flexas *et al.*, 2001; Vilagrosa *et al.*, 2003). Thus, *Quercus* juveniles could survive chronic and sustained droughts coupled with seasonal precipitation better than *Pistacia*, while *Pistacia* could better utilize occasional unpredictable precipitation events to survive droughts.

The two *Pistacia* species were shown to be less plastic in response to light and water availabilities than the two *Quercus* species for a number of traits. However, *Pistacia* and *Quercus* species coexist in Mediterranean shrublands despite their contrasting response to drought and heat (Filella *et al.*, 1998; Maestre *et al.*, 2003). This suggests that phenotypic plasticity is not necessarily adaptive in

Mediterranean ecosystems, as argued in previous studies (Valladares *et al.*, 2000, 2002*a*), since species not only with contrasting life cycles or ecophysiological features but also with contrasting plasticities co-occur in these habitats. Plasticity is a target of natural selection and can evolve under changing environmental conditions (Pigliucci, 2001). In fact, different populations of Mediterranean oaks exhibit different levels of phenotypic plasticity (Balaguer *et al.*, 2001; Gratani *et al.*, 2003). Fragmentation of Mediterranean forests and shrublands, may favour increased phenotypic plasticity over local adaptation (Sultan and Spencer, 2002). Whether this increased plasticity will enhance performance and survival of saplings in a global change scenario or make them more vulnerable to unpredictable environmental fluctuations remains uncertain.

Species of contrasting functional traits and plasticities co-occur in Mediterranean ecosystems. High drought tolerance coupled with conservative water use and relatively high levels of plastic response to environment (a strategy observed in *Quercus* sp. saplings) seems to render good results under current climatic conditions (chronic drought alternated with seasonal rainfall), while the opposite syndrome (observed in *Pistacia* saplings) seems to enhance performance under future scenarios characterized by extreme aridity and more erratic rainfalls. The present study shows evidence for functional advantages of sun phenotypes under high light and drought (i.e. avoidance of excessive light), but these advantages did not translate into higher survival. The real adaptive value of plasticity of Mediterranean plants in a global change scenario deserves more attention.

Acknowledgements

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