Performance of seedlings of Mediterranean woody species under experimental gradients of irradiance and water availability: trade-offs and evidence for niche differentiation

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Summary

• The aim of the study was to assess the potential importance for Mediterranean plants of trade-offs in the response to irradiance and water availability at the regeneration stage.
• Survival and growth patterns across an experimentally imposed irradiance gradient (1, 6, 20 and 100% sunlight) were studied in seedlings of eight Mediterranean woody species, together with the impact of a simulated summer drought.
• We found evidence of some of the trade-offs previously reported for non-Mediterranean plant communities, such as between survival in the shade and relative growth rate (RGR) at high light, but no evidence for others, such as between shade and drought tolerances. The impact of drought on survival and RGR was stronger in high light than in deep shade.
• The observed species-specific differences in performance provide a mechanistic basis for niche differentiation at the regeneration stage, contributing to possible explanations of species coexistence in Mediterranean ecosystems.

Key words: drought, plant strategies, plastic responses, regeneration niche, shade tolerance.

Introduction

Interspecific differences in seedling and juvenile survival and growth along resource gradients are key factors controlling plant community structure and dynamics (Grubb, 1977; Pacala & Tilman, 1994; Silvertown, 2004). A number of studies conducted in cool-temperate and tropical forests have suggested that survival and growth at such early stages are major bottlenecks in achieving canopy occupancy (Good & Good, 1972; Clark & Clark, 1992; Kobe, 1999). Specifically, it has been suggested that interspecific differences in seedling and juvenile performance involve a number of strategic trade-offs that restrict a species to optimal performance at a narrow range of the successional gradient, and thus these differences

are critically important in explaining community structure and dynamics (Pacala *et al.*, 1996; Kobe & Coates, 1997; Baraloto *et al.*, 2005).

Mediterranean forests experience a recurrent drought season during the summer, when temperature and radiation are at their maximums. The differential ability of species to cope with light and water limitations, particularly during the establishment stage, is considered a key trait governing community structure and dynamics (Pigott & Pigott, 1993; Espelta *et al.* 1995; Castro *et al.*, 2004; Gómez-Aparicio *et al.*, 2004; Zavala & Zea, 2004). Identification of seedling functional responses along resource gradients and implied trade-offs is thus critical to achieve a mechanistic understanding of vegetation dynamics in this region and to assess potential responses

of Mediterranean plant communities to expected global change scenarios (Lloret *et al.*, 2004; Körner *et al.*, 2005). However, the relevance of trade-offs at juvenile stages, and their consequences, for explaining niche differentiation has only been explored for a few species (Zavala *et al.*, 2000; Zavala & Zea, 2004). Multispecific comparative studies remain scarce (Marañón *et al.*, 2004) and are typically restricted to a small subset of species (e.g. Broncano *et al.* 1998; Rey Benayas *et al.*, 2005). In this study we target five shrub species (*Arbutus unedo*, *Pistacia lentiscus*, *Pistacia terebinthus*, *Quercus coccifera* and *Viburnum tinus*) and three tree species (*Quercus robur*, *Quercus faginea* and *Quercus ilex* ssp. *ballota*) that are typical components of Mediterranean plant communities (with the exception of *Q. robur*, which is a cool-temperate forest tree species whose southern distribution overlaps the distributions of many of the above-mentioned species). Depending on the disturbance regime and environmental conditions, these species can coexist locally in mixed forests and shrublands (whether as canopy-dominant or as understory species) or spatially segregate to form monospecific stands (particularly *Q. robur*, *Q. faginea* and *Q. ilex*).

In this study, we investigated the seedling responses of eight Mediterranean woody plant species along an experimental irradiance gradient and two contrasting water availability regimes. First, we tested for the existence of differential responses among species along these gradients that could support regeneration niche differentiation in a light–water availability phase space (*sensu* Grubb, 1977). Secondly, we examined whether seedling responses (survival and growth) conform to the resource (light and water) trade-offs described or hypothesized for other plant communities (see reviews by Sack & Grubb, 2002; Sack, 2004). Specifically, we tested: (a) a low-light survivorship vs high-light growth trade-off (e.g. Kitajima, 1994; Kobe *et al.* 1995; Pacala *et al.*, 1996; Kobe, 1999), (b) a low-light vs high-light growth trade-off (Boardman, 1977; Bazzaz, 1979; Agyeman *et al.*, 1999; Williams *et al.* 1999), and (c) a drought vs shade tolerance trade-off, expressed both at an interspecific (Smith & Huston, 1989) and at an intraspecific level, with acclimation or adaptation to shade leading to a poor performance under drought (Smith & Huston, 1989; Kubiske *et al.* 1996).

Materials and Methods

Experimental design and study site

The experimental setting was based on a factorial design with two factors: irradiance availability and species. The target species [*Arbutus unedo* L., *Pistacia lentiscus* L., *Pistacia terebinthus* L., *Quercus coccifera* L., *Quercus faginea* Lam., *Quercus ilex* ssp. *ballota* (Desf.) Samp., *Viburnum tinus* L. and *Quercus robur* L.] differ widely in seed size (by several orders of magnitude from *Quercus* species to *A. unedo*, *c.* 3 to 0.02 g, respectively; Catalán Bachiller, 1993) and also in leaf habit

(*Q. robur* and *P. terebinthus* are deciduous, *Q. faginea* is semideciduous, and *Q. ilex*, *Q. coccifera*, *P. lentiscus*, *A. unedo* and *V. tinus* are evergreen). Seedlings were grown outdoors from February until October 2002 at a commercial nursery (Viveros Barbol, Torremocha del Jarama, Madrid, Spain). The area is located at 40°50′ N, 3°29′ W and at 710 m above sea level. The climate is continental Mediterranean with hot and dry summers and cold winters. Mean maximum and minimum temperatures are 19.7 and 9.9°C, respectively. Most annual rainfall (372 mm) is received during the spring and fall (76–102 mm, respectively) and summer rainfall accounts for 47.1 mm (Instituto Nacional de Meteorología, 2002; values are means for the last 25 years). The soil substrate (pH 6.5) 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-K; Angiplant, La Rochelle Cedex, France) and 2 kg m^{-3} of Plantacote mix 4M fertilizer (15-10-15 N-P-K, Aglukon Spezialdünger GMBH & Co. KG, Dusseldorf, Germany).

Seeds were collected from characteristic Iberian localities in autumn 2001: *Q. robur* from south-east Galicia, *Q. faginea* from Torrelaguna, Madrid, *Q. ilex* ssp. *ballota* from Sierra Morena, Jaén, *Q. coccifera* from Cádiz, *P. lentiscus* from Valencia, *P. terebinthus* from northern Andalucía, *A. unedo* from Ávila and *V. tinus* from Moratalla, Murcia. The fact that the seeds were collected from a single location for each species might confound ecotypic with interspecific differences. However, the seeds were collected from populations under typical Mediterranean conditions, and ecotypic variations among populations of the studied species can be expected to be minor compared with interspecific differences. Seedlings were germinated from January to March 2002 and were transplanted to forest multipot (each pot 330 cm^3) containers from early spring to autumn, with each individual seedling occupying a single pot. Local air temperature and available photosynthetic photon flux density (PPFD) were recorded every 5 min throughout growing season with a data logger (HOBO model H08-006-04; Onset, Pocasset, MA, USA) and self-made external sensors that were cross-calibrated with a Li-Cor 190SA sensor (Li-Cor, Lincoln, NB, USA). Mean daily PPFD (400–700 nm) over the summer was 41 mol m^2 d^{-1}, which is referred to as full or 100% sunlight hereafter. Four irradiance intensities (1, 6, 20 and 100% sunlight) were established by using layers of neutral shade cloth supported by metal frames. This gradient spans the natural range of light availability found in Iberian forest understories, 20% sunlight being the most common shade under Mediterranean forest canopies and 6% sunlight being relatively frequent in humid and subhumid temperate forests (Gómez *et al.*, 2004; Valladares, 2004). Intensities of approximately 1% sunlight represent really dark understories, which have been reported for Mediterranean forests (Gratani, 1997) and are common in tropical and cool-temperate forests (Canham *et al.*, 1990; Frelich, 2002).

Water availability was also included in the design of the experiment to examine the impact of drought on the responses of seedlings at two of the four irradiance intensities (i.e. 6 and 100% sunlight). The 100% sunlight level in combination with water shortage was chosen to induce the strongest drought effect. The 6% sunlight level was chosen to test the impact of drought at a deep but still relatively common shade level. We chose not to use the 1% sunlight level not only because of its rarity in Mediterranean environments but also because, at extremely low irradiances, soil resource availability (water and nutrients) may only have a marginal impact in comparison with the impact of low irradiance, particularly on shoot growth (Canham *et al.*, 1996). Two watering levels (well-watered and water-stressed) were established. Half of the seedlings at each irradiance intensity were grown under well-watered conditions and the other half were grown under water-stressed conditions. The irrigation system consisted of watering by sprinklers. We monitored soil volumetric water content (SVC) six times in the growing season for a subsample of all the treatments (*n* = 40 for well-watered treatments and *n* = 22 for water-stressed treatments) and recorded the minimum value reached for each individual. Measurements were taken before watering to obtain minimum values between two watering events. SVC was estimated with a portable moisture measurement instrument based on the time domain reflectometry (TDR) method, TRIME-FM (Imko Micromodultechnik GMBH, Ettlingen, Germany), connected to a P2 probe. We compensated for increasing evapotranspiration under high irradiance by adjusting irrigation intensity and frequency for each irradiance intensity (Table 1) based on previous trials. Such a procedure mitigated against uncontrolled interactions between irradiance and water availability (e.g. lower water availability under high irradiance), although they could not be completely removed (Fig. 1). SVC values were above 10% for all the well-watered treatments and below 10% for all the water-stressed treatments. It was previously shown that effects of drought on seedling performance were only noticeable for values below 10% (Valladares *et al.*, 2005). On average, SVCs were 26.69 ± 0.47% and 7.27 ± 0.48% (mean ± 95% confidence interval) for well-watered and water-stressed

Table 1 Watering times and frequency for each irradiance × water combination

Irradiance (%)	Water treatment	
	Well-watered conditions	Water-stressed conditions
100	60 min every other day	30 min every other day
20	30 min every other day	
6	10 min every 3 d	5 min every 3–4 d
1	10 min every 3 d	

treatments, respectively. The mean SVCs corresponded to −0.007 and −2.164 MPa in terms of soil water potential for well-watered and water-stressed treatments, respectively (calculated using the filter-paper technique; Deka *et al.*, 1995). Air mean temperature (integrated over the entire month, days and nights included) during the experiment was similar (± 1°C) across different irradiance environments; for example, in the hottest month of the year (July), mean temperature over the day varied from 24.27 ± 0.12°C in 100% sunlight to 23.43 ± 0.10°C in 1% sunlight. Mean temperatures from 11:00 h to 16:00 h integrated over the whole of July varied from 29.99 ± 0.23°C to 32.70 ± 0.41°C for 1 and 100% sunlight, respectively. A total of 64–88 healthy seedlings per irradiance intensity and species were used for the experiment. Seedlings were arranged along four blocks randomly distributed within four shade frames corresponding to each irradiance intensity. Two extra blocks of 22 seedlings per species were included in the water-stressed treatments for the 6 and 100% sunlight levels.

Survival and growth measurements

We carried out a total of seven mortality censuses during the experiment. Censuses were performed on 24 June (only in the well-watered set of seedlings), 28 August, 5 September, 15 September, 23 September, 30 September, and 5 October for both well-watered and water-stressed seedlings. We labelled as dead individuals those that had lost all their aerial structures, did not have any photosynthetically active leaves (i.e. green
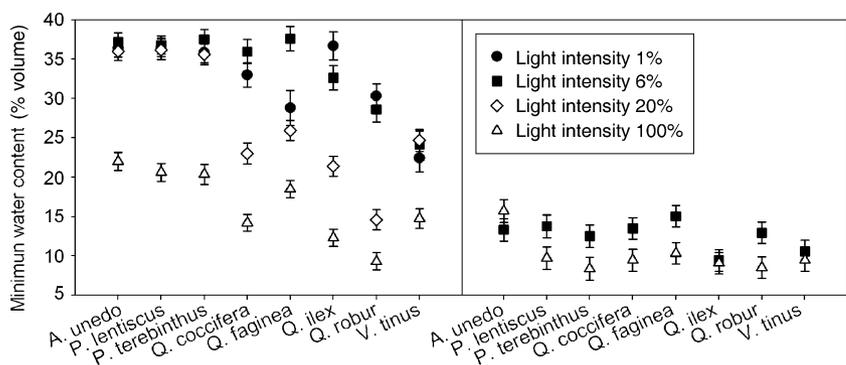

Fig. 1 Minimum water content for each species, irradiance and water combination (left panel, well-watered treatment; right panel, well-stressed treatment). Vertical bars denote 0.95 confidence intervals. Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.

and bendy leaves) and exhibited loss of stem flexibility in the upper third portion of the plant. Two rain events were registered on 6 and 14 October which produced 10.9 and 1.24 mm, respectively (data provided by the staff of the nursery). No resprout was observed, however, after these early autumn rain events. Seedlings were sprayed with a fungicide solution (50% Carbendazyme, Fungicida Polivalente; COMPO Agricultura SL, Barcelona, Spain) twice during the experiment in order to control fungal infections. None of the mortality events showed signs of a fungal infection-mediated death.

We evaluated seedling growth responses both as relative growth rate in biomass (hereafter RGR) and as relative diameter and height growth to account for plastic adjustments in crown architecture. We recorded seedling height (length of the main stem) and collar diameter for each individual twice in the growing season (i.e. early summer and early autumn). Stem diameter was measured at the base of the main stem with a calliper (accuracy 0.01 mm). We estimated relative height and diameter growth as $(\ln G_2 - \ln G_1)/(t_2 - t_1)$, G_1 and G_2 being the height or diameter in time 1 and 2 (t_1 and t_2, respectively). This was calculated for the whole set of individual observations. A first sample of five seedlings per species (with the exception of *V. tinus*, for which these data are missing) was harvested, dried to constant mass and weighed before placing the seedlings into the treatments. Another sample of 12–20 seedlings per treatment (light, species and water combination) was harvested at the end of the experiment (mid-October). RGR was calculated as $(\ln W_2 - \ln W_1)/(t_2 - t_1)$, W_1 and W_2 being the dry masses in time 1 and 2 (t_1 and t_2, respectively). Cotyledon masses were excluded. Absolute growth in terms of biomass, diameter and height was also calculated.

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 irradiance, species and water combination. The χ^2 test was used to test significant survival differences (for multiple simultaneous comparisons). Cox's F-test was used afterwards to test for differences in survival functions among species for each irradiance × water combination and among irradiance or water levels for each species. RGR analysis was performed with the software CLASSICAL PLANT GROWTH ANALYSIS version 1.1 (Hunt *et al.*, 2002). Differences in RGR among treatments were tested for significance using the 95% confidence limits provided by the software. Differences across factors (species, irradiance and water) in absolute growth (biomass, height and diameter) and relative height and diameter growth were analysed by analysis of variance (ANOVA). Fisher's least significant difference (LSD) test was used for *post hoc* analysis. Before ANOVA, data were checked for normality and homogeneity of variances, and were log-transformed to correct deviations from these assumptions when needed (Zar, 1999). Spearman rank correlations

were used to test for specific relations among key variables. All the statistical analysis was performed using STATISTICA version 6.0 (Statsoft Inc., Tulsa, OK, USA).

Results

Seedling survivorship

We found interspecific differences in seedling survival at all irradiance intensities (Fig. 2). At the intraspecific level, irradiance had the greatest impact at 1% sunlight. In contrast, there were no statistically significant differences in survival within species among the irradiance intensities of 6, 20 and 100% sunlight. In deep shade (1% sunlight), *A. unedo* and *P. terebinthus* experienced the lowest survival rates while *Quercus* species experienced the highest survival rates (*Q. robur* and *Q. coccifera* were the most shade tolerant and *Q. faginea* and *Q. ilex* the least shade tolerant of the *Quercus* species). Relative to the other species, *V. tinus* and *P. lentiscus* showed intermediate survival rates in deep shade (Fig. 2). Under high-light conditions, *Quercus* species still experienced the highest survival rates. *A. unedo* had the same survival rate as *Q. ilex* and *Q. faginea*. *P. terebinthus* had the lowest survival rate in 100% sunlight while *V. tinus* and *P. lentiscus* had intermediate survival rates (similar to those found under deep shade). *P. terebinthus* exhibited consistently low survival rates across the irradiance gradient, which can be interpreted in terms of poor acclimation of this species to the experimental conditions in the nursery. Drought had a strong impact on the responses of the species to irradiance, and species survival rankings differed greatly among water-stress treatments. For example, at 100% sunlight, *Quercus* species experienced the second lowest survival rates after *P. terebinthus*. In contrast, *A. unedo* and *V. tinus*, which had low survival rates in deep shade, experienced the highest survival rates in the water-stress treatment. At 6% sunlight, *Q. robur* experienced a remarkably low survival rate. The survival rate for *Q. ilex* was much higher than that for *Q. robur*, but it was still lower than those of the other species, which were statistically indistinguishable (Fig. 2). In general, the impact of drought on survival was higher in 100% sunlight than in 6% sunlight (Fig. 2). We did not find a significant correlation (Spearman's $R = -0.311$; $P = 0.453$) between shade and drought tolerance in terms of survival (1% sunlight under well-watered conditions and 100% sunlight under water-stressed conditions, respectively).

Absolute and relative seedling growth

As light decreased, so did the absolute biomass growth of the species (Table 2). For *Q. ilex*, *Q. coccifera*, *P. terebinthus* and *A. unedo*, maximum absolute biomass growth was achieved at the maximum light intensity (100% sunlight). The other species did not show higher absolute biomass growth in 100% sunlight than in 20% sunlight. The species ranking in absolute biomass growth was *Q. robur* > *Q. ilex* > *Q. coccifera* > *Q. faginea*
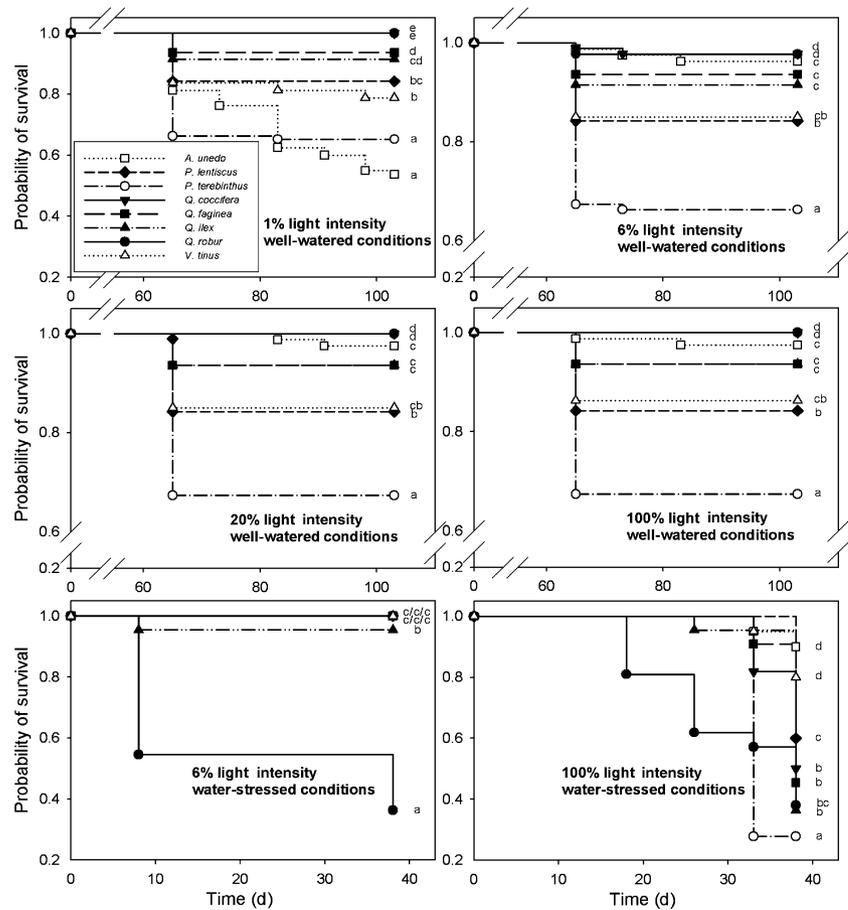
Fig. 2 Cumulative survival proportion over time for each species and irradiance × water availability combination studied. Analysis was performed with the Kaplan–Meier product limit. The letter codes indicate homogeneous groups (χ² test).

Table 2 Absolute biomass growth for each species and treatment

	Absolute biomass growth (g)																
	Treatment																
	1% ww			6% ww			6% ws			20% ww			100% ww			100% ws		
Species	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Arbutus unedo	0.66	0.12	c(c)	1.08	0.16	c(d)	1.03	0.13	c(d)	2.62	0.44	b(d)	4.09	0.79	a(c)	1.37	0.09	c(ef)
Pistacia lentiscus	0.31	0.05	d(c)	1.03	0.19	c(d)	1.06	0.15	c(d)	3.11	0.40	a(cd)	3.63	0.38	a(c)	1.84	0.16	b(de)
Pistacia terebinthus	0.75	0.17	d(c)	0.86	0.19	d(d)	1.04	0.12	d(d)	2.45	0.38	b(d)	4.41	0.51	a(c)	1.95	0.69	bc(cde)
Quercus coccifera	2.16	0.30	c(b)	2.24	0.32	c(c)	1.92	0.33	c(c)	3.96	0.52	b(c)	5.91	0.64	a(ab)	2.61	0.24	c(c)
Quercus faginea	1.74	0.31	b(b)	2.48	0.27	b(c)	2.29	0.16	b(c)	3.96	0.41	a(c)	4.61	0.43	a(bc)	2.27	0.10	b(cd)
Quercus ilex	3.37	0.21	c(a)	4.57	0.37	c(b)	4.20	0.20	c(a)	5.77	0.52	b(b)	7.28	0.33	a(a)	4.11	0.43	c(b)
Quercus robur	3.57	0.33	c(a)	5.48	0.45	b(a)	3.05	0.20	c(b)	7.80	0.56	a(a)	6.83	0.64	a(a)	4.98	0.34	b(a)
Viburnum tinus	0.56	0.07	b(c)	0.52	0.09	b(d)	0.50	0.06	b(d)	0.87	0.12	ab(e)	1.01	0.25	a(d)	0.68	0.11	ab(f)

All irradiance intensity × water supply combinations are given. The first letter code indicates homogeneous groups [analysis of variance (ANOVA); Fisher's test, $P = 0.05$] along the irradiance × water availability gradient, with the same letter within a row denoting no significant difference. The letter code in parentheses indicates homogeneous groups (ANOVA; Fisher's test, $P = 0.05$) across species, with the same letter within a column denoting no significant difference.
SE, standard error; ww, well-watered treatment; ws, water-stressed treatment.

> (*P. lentiscus* = *A. unedo* = *P. terebinthus*) > *V. tinus*. This ranking did not change substantially along the light gradient (but see Table 2 for details). *Q. robur* had higher total dry mass than *Q. ilex* in 6 and 20% sunlight but not at the two extreme irradiance intensities (i.e 1 and 100% sunlight), where these two species did not differ. *V. tinus* exhibited lower total dry mass than *P. lentiscus*, *P. terebinthus* and *A. unedo* in 20 and 100% sunlight but not in 1 and 6% sunlight. In 6% sunlight, drought decreased total dry mass for *Q. robur*. However, drought decreased the total dry mass for all the species but *V. tinus* in 100% sunlight (Table 2). Absolute diameter growth and absolute height growth were highly correlated with absolute biomass growth in the experiment (Spearman's R = 0.960; P = 0.001; Spearman's R = 0.830; P = 0.001, respectively).

Along the irradiance gradient, *Quercus* species showed significantly lower relative height and diameter growth rates than the other species (Fig. 3). Maximum relative height growth rates tended to occur at 20% sunlight, while maximum relative diameter growth rates occurred at either 6 or 100% sunlight for all the species (Fig. 3). *Quercus* species were indistinguishable from each other in relative height growth but did differ significantly in relative diameter growth. At low light intensities (i.e. 1 and 6% sunlight), *Q. coccifera* was the *Quercus* species with the highest relative diameter growth rates. At 100% sunlight, *Q. ilex* became the species with the highest relative diameter growth rate (significantly higher than that of *Q. faginea*). *V. tinus* had intermediate relative height and diameter growth rates (between those of the *Quercus* species and the other species). At low and intermediate light intensities, *V. tinus* exhibited relative growth rates (in height and diameter) similar to those of *Pistacia* species and *A. unedo*, but at the highest

irradiance intensity it experienced growth rates closer to those of *Quercus* species. *Pistacia* species and *A. unedo* showed higher RGR mean values than those of *Quercus* species along the irradiance gradient, but differences were not statistically significant (Fig. 3).

Drought had a small impact on relative height growth (data not shown) but a major impact on relative diameter growth. The effect of drought on relative diameter growth was stronger in 6% than in 100% sunlight. At 100% sunlight, drought decreased relative diameter growth in *Q. robur* and *A. unedo*, but exerted no effect in the other species. However, in 6% sunlight, drought decreased relative diameter growth for all the species except *Q. robur*, *Q. faginea* and *P. terebinthus* (Fig. 4).

RGR mean values in 100% sunlight and well-watered conditions were consistently higher than the RGR mean values in 100% sunlight under water-stressed conditions for all the species. However, this not was the case in 6% sunlight (Fig. 5), where the limited light suppressed growth so strongly (*Quercus* species did not experience significantly positive RGR values in 6% sunlight) that no effect of drought could be detected. A significant negative relationship between survival in deep shade and growth in high light was found in our set of species. Thus, species with high growth in 100% sunlight experienced low survival rates in shade and *vice versa*. This relationship was found both for RGR and for relative diameter growth, but not for relative height growth, probably because of the confounding influence of elongation in the shade (Fig. 6). We also found a negative relationship between relative diameter growth at low light and relative diameter growth at high light, but a positive relationship between low-light RGR and high-light RGR (Fig. 7). No relationship was found between
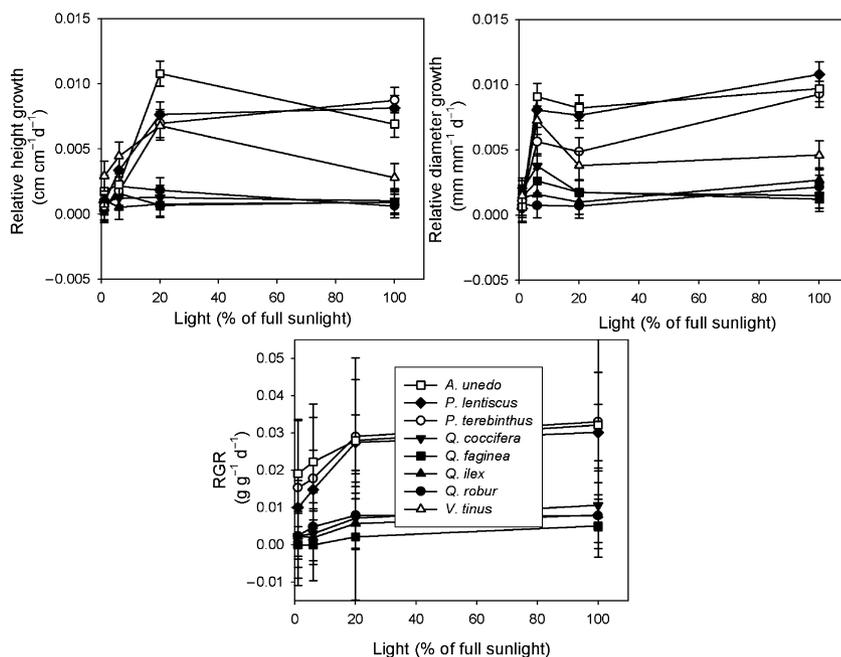

Fig. 3 Relative height growth, relative diameter growth and relative growth rate (RGR) along the irradiance gradient for each species. Vertical bars denote 95% confidence intervals. Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.

Fig. 4 Drought impact on relative height and diameter growth for each species in 100 and 6% sunlight. Vertical bars denote the standard error. * denotes significant differences between water treatments (analysis of variance; Fisher's test, $P < 0.05$). Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.
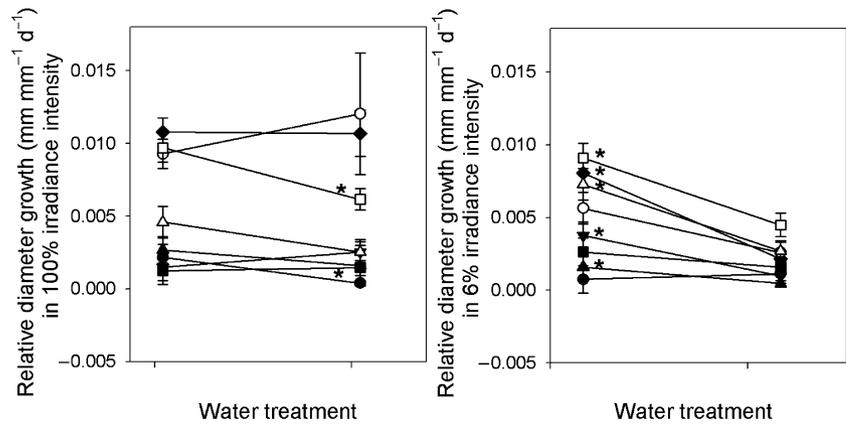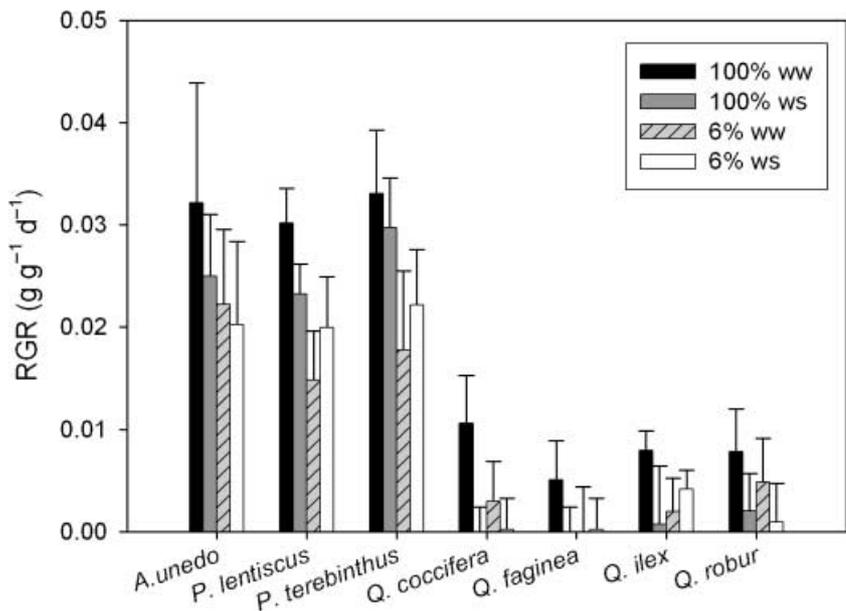
Fig. 5 Mean values within species for relative growth rate (RGR) for the four combinations of irradiance and water availability studied. Vertical bars denote standard errors ($n = 12–20$). ww, well-watered conditions; ws, water-stressed conditions. Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.

relative height growth in low light and relative height growth in high light (data not shown).

Discussion

Seedling responses and trade-offs along the irradiance gradient

Our results have shown important species-specific differences in survival and growth along the irradiance gradient imposed in the experiment. In agreement with previous studies conducted with tropical species (Bloor, 2003), the limiting effects of irradiance were only apparent under very deep shade (1% sunlight), suggesting that Mediterranean species generally exhibit a degree of shade tolerance during the early establishment stages. However, there is increasing evidence that both light and water can be limiting for woody seedling establishment

in heterogeneous Mediterranean environments (Hastwell & Facelli, 2003; Maestre *et al.*, 2003a).

The link found between survival and growth was consistent with a trade-off between survival in low light and growth in high light (Kitajima, 1994; Kobe *et al.*, 1995; Pacala *et al.*, 1996; Kobe, 1999). This trade-off, however, was found with respect to relative diameter growth and RGR but not with respect to relative height growth, probably because of the confounding effect of elongation. Elongation in response to shade is a well-documented shade avoidance strategy that results in biomechanically weak phenotypes with poor light interception efficiency (Pearcy *et al.*, 2005).

Our results provide contradictory evidence regarding the existence of low- vs high-light growth trade-offs (Boardman, 1977; Bazzaz, 1979; Agyeman, 1999; Williams *et al.*, 1999) with the direction of the trade-off changing depending on the growth estimate considered. For example, we found a negative
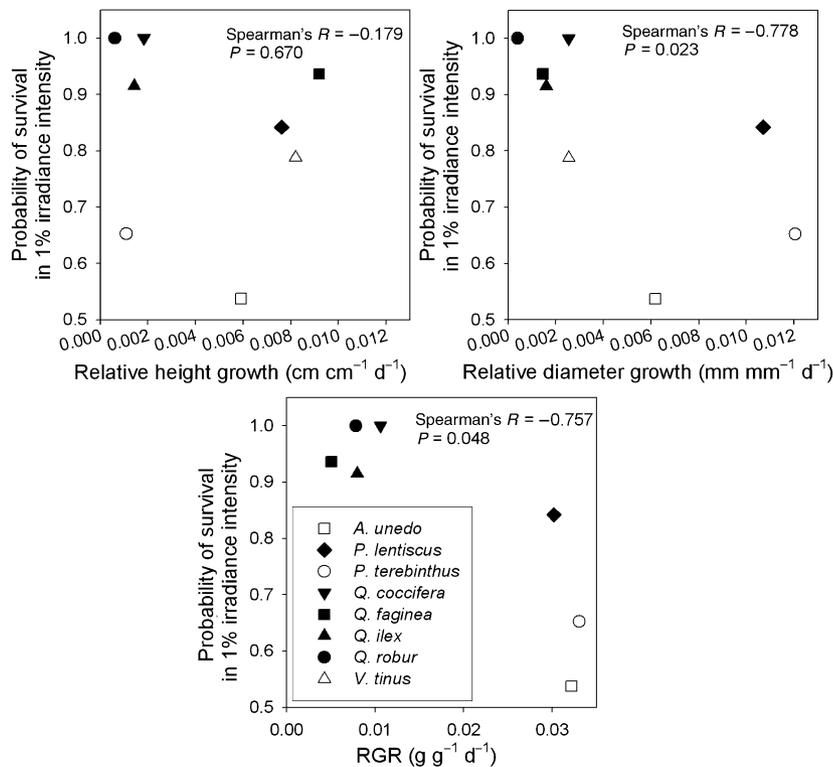
Fig. 6 Survival in deep shade vs relative height growth, relative diameter growth and relative growth rate (RGR) in high-light conditions. The values of Spearman's *R* and significance are only shown for statistically significant correlations. Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.
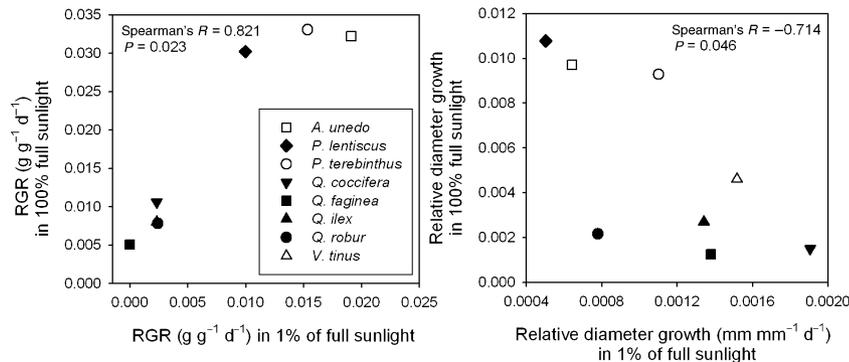

Fig. 7 Low-light growth vs high-light growth for relative growth rate (RGR) and relative diameter growth. Spearman's *R* and significance are shown. Genera are as follows: *A.*, *Arbutus*; *P.*, *Pistacia*; *Q.*, *Quercus*; *V.*, *Viburnum*.

relationship between relative diameter growth in low light vs relative diameter growth in high light, in agreement with some studies (Boardman, 1977; Bazzaz, 1979; Agyeman, 1999; Williams *et al.*, 1999), a positive relationship between whole-plant growth in low light vs whole-plant growth in high light, in agreement with others (Loach, 1970; Ramos & Grace, 1990; Chazdon, 1992; Kitajima, 1994; Grubb *et al.*, 1996; Bloor & Grubb, 2003), and no significant relationship between relative height growth in high light vs relative height growth in low light (data not shown). Thus, the results obtained for RGR, which is relatively independent of allocation, partially support the idea that shade-intolerant species grow faster than shade-tolerant species both in deep shade and under high light, as suggested by Kitajima (1994) and Bloor & Grubb (2003).

The trade-off between survivorship in the shade and growth in high light has been associated with two main general plant strategies, the acquisitive vs the conservative strategy (Diaz *et al.*, 2004). Large seeds have been associated with the conservative strategy (Castro-Diez & Montserrat-Marti, 2003; Diaz *et al.*, 2004), and in general with a stress-tolerance strategy (Hewitt, 1998; Reich *et al.*, 2003). Seed size influences interspecific responses to light and water availability at the seedling stage, with species having larger seed size performing better under resource-limiting conditions than species having smaller seed size (Osunkoya *et al.*, 1994; Coomes & Grubb, 2003). For example, larger seeds benefit seedlings in conditions of shading (Hewitt, 1998; Bond *et al.* 1999) and nutrient limitation (Jurado & Westoby, 1992; Seiwa, 2000). In agreement with this idea, we found a significant relationship between

Table 3 Spearman correlations between survival or growth and log seed biomass for the most extreme irradiance intensities (1 and 100% sunlight)

Correlation	1% sunlight		100% sunlight	
	Spearman's R	P-value	Spearman's R	P-value
Survival vs log seed biomass (mg)	0.922	0.001*	0.602	0.114
RGR vs log seed biomass (mg)	−0.714	0.070	−0.714	0.070
Relative height growth vs log seed biomass (mg)	−0.500	0.207	−0.809	0.015*
Relative diameter growth vs log seed biomass (mg)	−0.547	0.160	−0.881	0.003*
Absolute biomass growth vs log seed biomass (mg)	0.810	0.015*	0.690	0.058
Absolute height growth vs log seed biomass (mg)	0.762	0.020*	0.714	0.046*
Absolute diameter growth vs log seed biomass (mg)	0.786	0.021*	0.595	0.119

*Significant correlations at $P = 0.05$.
RGR, relative growth rate.

growth and seed size and between survival and seed size in our species. Seed size was positively related to survival and absolute growth in both deep shade and high light, but it was negatively related to relative growth (Table 3). The effect of seed size on survival changed meaningfully with light intensity, with large seed size enhancing survival significantly at low light but not significantly at high light. Thus, seed size had a critical effect on species-specific differences in seedling performance along the irradiance gradient. The large range of seed sizes in the present study may have increased the relative magnitude of this effect. Correlations and constraints identified at early stages may well weaken during subsequent growth; for example, the linkage of RGR with seed size can dissipate, leading to species cross-overs in RGR (Sack & Grubb, 2001; Kitajima & Bolker, 2003; Baraloto *et al.*, 2005). Studies on seedlings have obvious limitations. Although germination and seedling establishment are major demographic bottlenecks in Mediterranean ecosystems, studies of responses in saplings, juveniles and adults are required to accurately evaluate the relevance of these trade-offs for community structure and dynamics.

Seedling responses to combined shade and drought

Various hypotheses have been proposed to explain the effects of drought on seedling performance along irradiance gradients (for a review, see Sack & Grubb, 2002; Sack, 2004). The trade-off hypothesis states that drought will be increasingly harmful under shadier conditions because of conflicts between the simultaneous demands for allocation of resources to cope with above-ground and below-ground limitations (Smith & Huston, 1989; Kubiske *et al.*, 1996). The facilitation hypothesis suggests that, under drought, the negative effects of light limitation can be offset by its benefits for plant water status (Callaway, 1995; Holmgren, 2000; Flores & Jurado, 2003; Prider & Facelli, 2004). Finally, it has been suggested that drought has a fixed proportional impact along the irradiance gradient (Sack & Grubb, 2002; Sack, 2004).

On the whole, we found that the impact of drought on both growth and survival was higher in high light than under shade, as found by Sack (2004) and in agreement with the facilitation hypothesis. Indeed, shade has been hypothesized to have a positive effect under drought (e.g. Holmgren, 2000), as part of a facilitative effect by which the negative effects of irradiance suppression are offset by its benefits for plant water status (Callaway, 1995; Canham *et al.*, 1996). This facilitative effect of shade under dry conditions has been found to be a mechanism involved in establishment and regeneration processes in Mediterranean environments (e.g. Maestre *et al.*, 2003b). The impact of drought under high-light conditions is presumably greater as a result of tissue desiccation, which together with high temperature and photo-oxidative stress (Valladares, 2001) has a direct impact on seedling survival and growth. Nevertheless, the different results found here with another growth estimator (relative diameter growth) highlight the importance of using several performance estimators when testing trophic trade-offs.

Finally, the interspecific trade-off hypothesis states that shade-tolerant species are more vulnerable to drought than light-demanding species (Smith & Huston, 1989). The existence of such a trade-off was not detectable in our experiment and we did not find a significant relationship between drought and shade tolerance. In fact, species such as *P. terebinthus* showed low relative tolerance of both shade and drought. Other experiments (Sack, 2004) have shown that shade and drought tolerances vary independently among species, indicating a high potential for species niche differentiation along combined irradiance and water availability gradients.

Although large seeds have been linked to drought tolerance (Leishman & Westoby, 1994; Seiwa *et al.*, 2002), in this study the species with the largest seeds (*Quercus*) were found to experience the highest mortality rates under drought. It has been suggested that there is a whole suite of traits linked to both shade and drought tolerance (Caspersen & Kobe, 2001; Sack *et al.*, 2003), but also that no one narrowly defined functional type is associated with the combined tolerance

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of shade and drought (Sack *et al.*, 2003). Seed reserves can supply all resources but water (Kitajima, 2002) and despite seed size could indirectly underlie certain resistance to drought by producing big seedlings that dry out slower than small seedlings (Coomes & Grubb, 2003). It is quite likely that factors other than seed size are responsible for drought tolerance during the establishment phase.

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