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ORIGINAL ARTICLE

Tertiary relict trees in a Mediterranean climate: abiotic constraints on the persistence of *Prunus lusitanica* at the eroding edge of its range

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ABSTRACT

Aim To investigate the ecophysiological traits allowing persistence of a subtropical relict tree (*Prunus lusitanica* L.) under a dry Mediterranean climate at the eroding edge of its range.

Location A glasshouse for the study under controlled conditions and two marginal populations located in riparian forests of central Spain and exposed to summer drought, in contrast to subtropical populations that grow in mountain cloud forests.

Methods Two experiments were conducted to assess tolerance to the abiotic conditions found in riparian habitats. In a glasshouse experiment, gas-exchange and light-use parameters were measured to evaluate seedling responses to a factorial combination of irradiance (60%, 10%, 2% and 0.5% full sun) and moisture (well watered control and drought stress). In a parallel field experiment, irradiance and soil moisture were measured as predictors of seedling survival at two sites in which half the seedlings were subjected to a threefold increase in summer precipitation by adding water every 10 days.

Results Soil moisture strongly determined survival both in the glasshouse and in the two field sites. In the field, periodic addition of water failed to increase survival. Water-use efficiency (WUE) increased with drought. Seedlings did not tolerate deep shade (2–0.5%) and their performance and survival were better when exposed to moderate (10%) or high (60%) irradiance. The effect of water stress on seedling performance was stronger at moderate irradiance, moderate at high irradiance and negligible at very low light. Seedling size (height and number of leaves) attained after 1 month of experimental treatments had a positive effect on survival at the end of the summer, hence greater survival was not achieved at the expense of vertical growth.

Main conclusions While studies in Macaronesia have shown that *P. lusitanica* occupies a wide range of moisture conditions along mountain slopes, it behaves as an obligate riparian species in dry peripheral populations. Intolerance to deep shade and tolerance to moderate and high irradiance allow the species to grow in small and moist gaps, or in treeless river sections. Despite the species' low range filling in marginal, drought-prone regions, long-term persistence might have been achieved through shifts to riparian habitats serving as local refuges.

Keywords
Abiotic constraints, climatic oscillations, drought tolerance, habitat shift, peripheral populations, rear edge, shade tolerance, Tertiary relicts.

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INTRODUCTION

Long-term climatic oscillations have been shown to result in deterministic patterns of extinction or range reduction in both Mediterranean and cool temperate woody floras (Herrera, 1992; Svenning, 2003). Considerable resilience in the face of cyclic climatic changes has been proposed as a necessary common feature for surviving species (Tzedakis *et al.*, 2002; Hampe & Petit, 2005). However, the functional attributes allowing their persistence are poorly understood (Valladares *et al.*, 2005a). The analysis of performance regarding relevant ecophysiological traits and their plasticity is a crucial first step for a mechanistic understanding of plant responses to climatic oscillations. These responses include acclimation of existing phenotypes to new conditions, genotypic selection and subsequent local adaptation, or establishment in distant refuge areas (Clark *et al.*, 2001; Santamaría *et al.*, 2003; Petit & Hampe, 2006). The ability to make phenotypic adjustments to the environment is critical because it determines whether adaptive or escape responses are necessary for the persistence of a given species.

Old lineages that evolved under climatic conditions differing markedly from those which they currently experience ('climatic relicts') provide an opportunity to explore the functional attributes involved in species persistence (Hampe & Petit, 2005). In the case of the extant Mediterranean flora, many old taxa evolved during the Tertiary period, well before the typical Mediterranean climatic regime was established (Suc, 1984; Herrera, 1992; Milne & Abbott, 2002). These taxa were part of lauroid forests growing under subtropical conditions that faced severe drought and increased warming as the Mediterranean climate became established (Palamarev, 1989; Milne & Abbott, 2002). As a result, these tree species invariably exhibit highly fragmented ranges and low population sizes in southern Europe, the eroding edge of their distribution (Hampe & Arroyo, 2002; Mejías *et al.*, 2002; Milne, 2004; Hampe & Petit, 2005; Calleja, 2006). In contrast to this scattered mainland distribution, these species are widespread in regions where temperature and precipitation oscillations have been modest, such as within the cloud forest of Macaronesia, resembling ancient subtropical scenarios (Fernández-Palacios & Arévalo, 1998).

Plant populations declining at the range limit of a given species are most likely to persist in regions providing local climatic refuges (Tzedakis *et al.*, 2002). According to this expectation, Mediterranean climatic relict trees are concentrated in riparian habitats where they have been able to escape from increasing water stress (Hampe & Arroyo, 2002; Mejías *et al.*, 2002; Calleja, 2006). Nevertheless, meeting water needs could also involve coping with a novel irradiance environment as compared with former habitats due to (1) decreased day length in terms of solar irradiance reaching the bottom of topographical depressions where springs occur, (2) differences in the disturbance regime and thus in the frequency and distribution of forest gaps, and (3) differences in the ratio of deciduous to evergreen tree species, which involve seasonal differences in the amount of irradiation. Thus, for small-scale habitat shifts to account for the current distribution of Tertiary relict tree species under Mediterranean conditions, irradiance-moisture trade-offs should be considered.

We followed this approach in a study of performance during early establishment of the Portuguese laurel cherry (*Prunus lusitanica* L.), a Tertiary relict tree, Iberian populations of which grow at the northern, eroding edge of the species' range (Calleja, 2006). In contrast to what has been observed in its subtropical habitats in Macaronesia (Fernández-Palacios & Arévalo, 1998), marginal populations grow in river belts, presumably because of down-slope habitat displacement (Calleja, 2006). Seedlings of this species are exposed to an irradiance–moisture mosaic in the understorey of these habitats, thereby facing functional conflicts in the use of light and water (Sack, 2004; Valladares *et al.*, 2005a). Previous studies have shown that shade can increase the detrimental effects of drought because more biomass needs to be allocated to aerial parts (Kubiske *et al.*, 1996), it can buffer water shortage because of reduced transpiration (Prider & Facelli, 2004), or it could have no effect at all on water status (Sack, 2004). At the opposite extreme of the irradiance gradient, seedlings can be subjected to stress due to excessive light, which represents a cost in terms of reduced carbon assimilation due to photoinhibition (Valladares *et al.*, 2005b).

Within the above framework, we hypothesized that: (1) in Mediterranean populations, *P. lusitanica* should show lower performance under drier conditions; (2) seedlings should be able to increase their water-use efficiency (WUE) under drought and this efficiency should result in better survival; (3) in order to perform well in shady riparian habitats, seedlings should either retain tolerance to deep shade or, alternatively, they should show better performance in forest gaps by tolerating high irradiances, as found in Macaronesian habitats (Arévalo & Fernández-Palacios, 1998); and (4) water stress should decrease seedlings' ability to cope with extreme irradiances, either deep shade (Kubiske *et al.*, 1996) or full sunlight (Valladares *et al.*, 2005b). Non-independent variation of irradiance and moisture under field conditions makes the independent analysis of both effects and their interaction difficult, especially if target plants are not found along the whole continuum of irradiance and moisture, as is often the case. For this reason, to test the above hypotheses we conducted two separate experiments. First, we conducted a glasshouse experiment in which we could expose seedlings to the desired multiple combinations of irradiance and moisture, including the most stressing ones. In a parallel field experiment we analysed seedling responses to the natural variation of abiotic conditions and, in addition, we simulated a scenario of increased precipitation under a range of light conditions. The glasshouse experiment was used to determine the tolerance limits to irradiance and moisture, as well as to test for the existence of interactive effects of both factors. The field experiment was intended to confirm the ecophysiological responses and to determine the strength of water limitation of seedling recruitment under natural conditions. By analysing

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the tolerance to extreme and interacting abiotic conditions, we aimed to elucidate the strength of abiotic constraints and the mechanisms allowing persistence in Mediterranean habitats and, consequently, affecting population stability at the eroding edge of the range of *P. lusitanica*.

METHODS

Study species

The Portuguese laurel cherry (*P. lusitanica*) is a perennial, lauroid tree up to 15 m tall. Although fossil evidence is lacking, several indirect pieces of evidence support the subtropical origin and the relict status of *P. lusitanica* (Calleja, 2006). First, this species belongs to a group of perennial, lauroid cherries, which mainly radiated in tropical regions of Asia (Kalkman, 1965). Second, many fossil records have been found in southern Europe for most of the other relict species currently co-existing with *P. lusitanica* in laurel forests (Barrón & Peyrot, 2006). Third, available molecular phylogenies of *Prunus* consistently group together the perennial lauroid cherries bearing raceme inflorescences. This group includes *P. lusitanica* and *Prunus laurocerasus*, both species being probably the result of the well known disjunction of the European Tertiary flora (Aubréville, 1976; Bortiri *et al.*, 2006). At present this cherry occurs in Macaronesian mountain cloud forests, where populations grow on slopes under favourable subtropical conditions (Arévalo & Fernández-Palacios, 1998). The species' range in Europe is restricted to the Iberian Peninsula, where it grows in riparian habitats (Calleja, 2006).

Glasshouse study: plant material and indoor environment

An indoor experiment was conducted to determine seedling tolerance to different irradiances and moisture conditions, as well as the interaction of both factors. Seedlings were grown from fruits collected under five individual trees in a population growing close to Castañar de Ibor (Cáceres Province, central-western Spain). On 10 December 2004, fruits were transported to a glasshouse, where pulp-free seeds were individually sown in plastic standard containers 100 cm^3 in volume and 15 cm deep. The soil substrate (pH 6.5) consisted of a 3 : 1 volume mixture of peat and washed river sand. Seedlings that emerged up to 15 May 2005 were kept at conditions similar to those observed in the field by means of a centralized cooling and humidifier system. Mean daily temperature was 28 ± 5°C and relative humidity was on average 27 ± 11%. Photosynthetically active radiation (PAR) was measured with four quantum sensors cross-calibrated with a LiCor 190SA sensor (LiCor, Lincoln, NE, USA). Readings were recorded every 5 min with a Hobo H08-006-04 data logger (Onset, Pocasset, MA, USA). Mean daily total PAR available for non-shaded plants was 23.7 ± 5.8 mol m^{-2} day^{-1} (n = 115 days for the whole experimental period). This value corresponded to 60% of the maximum full sunlight outdoors, and was similar to those usually found within the understorey of our natural populations.

Glasshouse study: experimental treatments

We used a fully factorial design with irradiance and water supply as factors and several estimates of seedling performance as response variables. Seedlings were arranged in four irradiance levels, each one exposed to either continuous watering or gradual drought. Thirty to 40 seedlings were used in the four light treatments under well watered conditions, and 60–70 seedlings in the four light treatments under drought conditions. As a higher rate of mortality was expected in the latter, more stressing conditions, allocation of more seedlings ensured a minimum sample size when measures of ecophysiological variables were performed later. Light treatments were established by using different layers of neutral shade cloth supported by metal frames. For 60%, 10%, 2% and 0.5% irradiances, mean values of total daily PAR (mol m^{-2} day^{-1}) were 23.7 ± 5.8, 5.5 ± 1.2, 0.67 ± 0.62 and 0.15 ± 0.32, respectively. This irradiance gradient spanned the natural range of light availability found in Iberian forest understoreys (Valladares & Guzmán, 2006). The 0.5% irradiance level was used to evaluate seedling responses to the very dark conditions that can be found in certain temperate and many tropical forests (Niinemets & Valladares, 2006). Seedlings were exposed to these light environments from 20 May to the end of the experiment on 15 September.

On 5 June, two watering levels were established and maintained for 100 days: a 'well watered' (control) treatment with a constant water supply, and a 'drought' treatment aimed at simulating the gradual drought experienced by seedlings in the field. Watering was provided manually, controlling the amount of water given to each individual seedling with a precision of ≤ 1 cm^3. We compensated for increasing evapotranspiration under high irradiance by adjusting the irrigation regime for each irradiance level. Soil water content (SWC) was not affected by irradiance level either in the well watered or in the gradual drought treatment (Fig. 1). Water content showed a very narrow range of variation (35–40%) in well watered seedlings. Water content in the simulated drought experienced a decrease of 82% throughout the experiment, fitting a negative exponential function [SWC = 4.95 + exp(4.43–0.11 × date), R^2 = 0.98].

Glasshouse study: measurements of seedling performance

We carried out 10 mortality censuses during the experimental period; two before and eight after the drought treatment began on 5 June. Seedlings were considered dead if they had no photosynthetically active leaves and exhibited loss of stem flexibility. On 5 July, after 1 month's exposure to experimental treatments, we evaluated seedling growth responses both as absolute and relative growth rates in stem height and number of leaves. Relative values were calculated as

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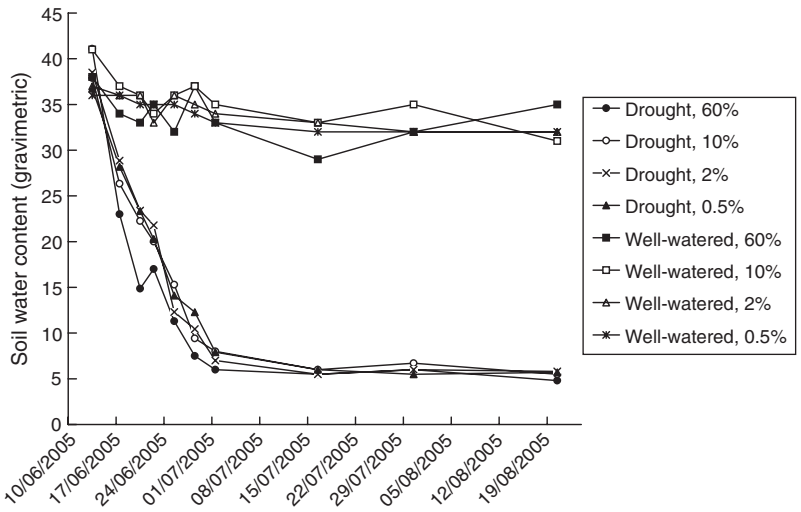

Figure 1 Time course of gravimetric soil moisture through the experimental period in well watered (four upper lines) and drought (four lower lines) irrigation treatments in the indoor environment.

Table 1 Summary statistics (mean ± 1 SD) for performance-related variables at all irradiance–moisture combinations used in glasshouse experiments with *Prunus lusitanica* seedlings.

		Irradiance treatment			
Variable	Moisture treatment	60% sunlight	10% sunlight	2% sunlight	0.5% sunlight
A_{max}	Well watered[a]	6.62 ± 2.05[a]	7.91 ± 1.16[a]	1.61 ± 0.71[b]	1.58 ± 0.11[b]
	Drought[b]	3.44 ± 0.37[a]	2.98 ± 0.29[a]	1.62 ± 0.51[b]	0.62 ± 0.24[b]
R_d	Well watered[a]	−1.75 ± 0.74[a]	−1.06 ± 0.66[b]	−0.29 ± 0.09[c]	−0.74 ± 0.43[b]
	Drought[b]	−0.69 ± 0.24[a]	−0.67 ± 0.19[a]	−0.14 ± 0.15[ab]	−0.09 ± 0.10[b]
Φ	Well watered[a]	0.06 ± 0.01[a]	0.06 ± 0.01[a]	0.04 ± 0.02[a]	0.05 ± 0.03[a]
	Drought[b]	0.05 ± 0.02[a]	0.09 ± 0.04[a]	0.03 ± 0.01[a]	0.49 ± 0.38[b]
Γ	Well watered[a]	31.83 ± 6.16[a]	21.00 ± 13.73[b]	5.17 ± 4.42[b]	15.72 ± 6.49[b]
	Drought[a]	17.60 ± 5.14[a]	8.65 ± 1.52[a]	12.89 ± 11.16[a]	49.66 ± 17.30[b]
P_s	Well watered[a]	156.67 ± 19.08[a]	145.67 ± 25.97[a]	72.83 ± 33.21[b]	69.03 ± 32.61[b]
	Drought[b]	106.93 ± 16.22[a]	52.37 ± 16.93[b]	113.07 ± 13.59[a]	153.19 ± 115.51[a]
g	Well watered[a]	0.06 ± 0.01[a]	0.09 ± 0.02[b]	0.02 ± 0.01[c]	0.01 ± 0.00[c]
	Drought[b]	0.02 ± 0.01[a]	0.02 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[b]
WUE	Well watered[a]	109.03 ± 10.22[a]	96.48 ± 14.60[a]	64.71 ± 6.25[b]	86.91 ± 20.99[ab]
	Drought[b]	144.36 ± 30.47[a]	118.59 ± 10.56[b]	60.18 ± 10.36[c]	94.70 ± 30.62[b]
F_v/F_n	Well watered[a]	0.75 ± 0.04[a]	0.81 ± 0.04[a]	0.74 ± 0.05[b]	0.66 ± 0.09[c]
	Drought[b]	0.72 ± 0.12[a]	0.80 ± 0.04[a]	0.69 ± 0.09[b]	0.64 ± 0.06[b]
qN	Well watered[a]	0.91 ± 0.02[a]	0.84 ± 0.02[b]	0.88 ± 0.04[b]	0.92 ± 0.02[a]
	Drought[a]	0.92 ± 0.02[a]	0.85 ± 0.02[b]	0.88 ± 0.04[b]	0.95 ± 0.02[a]
AETR	Well watered[a]	52.77 ± 4.95[a]	50.26 ± 4.60[a]	16.48 ± 2.13[b]	12.09 ± 1.99[b]
	Drought[b]	51.72 ± 7.06[a]	26.61 ± 3.29[b]	16.50 ± 4.85[c]	8.34 ± 1.72[d]

Values with the same superscript letter in comparisons within moisture and irradiance treatments are statistically indistinguishable (Scheffé *post hoc* tests). Abbreviations and units: A_{max}, maximum photosynthetic rate, μmol CO_2 m^{-2} s^{-1}; R_d, dark respiration rate, μmol CO_2 m^{-2} s^{-1}; Φ, quantum yield, μmol CO_2 μmol^{-1} quanta; Γ, light-compensation point, μmol quanta m^{-2} s^{-1}; P_s, light-saturation point, μmol quanta m^{-2} s^{-1}; g, stomatal conductance, mol H_2O m^{-2} s^{-1}; WUE, water-use efficiency, μmol CO_2 mol H_2O; F_v/F_n, photochemical efficiency; qN, non-photochemical quenching; AETR, apparent electron-transport rate, μmol quanta m^{-2} s^{-1}.

$(\ln G_2 - \ln G_1)/(t_2 - t_1)$, G_1 and G_2 being the height or number of leaves at time 1 (5 June) and time 2 (5 July) (t_1 and t_2, respectively).

Also after 1 month of experimental drought, measures of physiological performance related to carbon gain and light and water use were taken under both moisture treatments (Table 1). Five to eight randomly selected seedlings were used for each light–water combination. From light curves of gas-exchange values, we computed maximum photosynthetic rate (A_{max}, μmol CO_2 m^{-2} s^{-1}), dark respiration rate (R_d, μmol CO_2 m^{-2} s^{-1}), quantum yield (Φ, μmol CO_2 μmol^{-1} quanta), light compensation point (Γ, μmol quanta m^{-2} s^{-1}), light saturation point (P_s, μmol quanta m^{-2} s^{-1}), stomatal conductance (g, mol H_2O m^{-2} s^{-1}), and water-use efficiency (WUE, μmol CO_2 mol H_2O). Measurements were made with a LiCor 6400. Finally, we measured chlorophyll

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fluorescence on fully expanded leaves with a pulse-modulated fluorometer (FMS2, Hansatech Instruments, Norfolk, UK). With this procedure we derived measures of photochemical efficiency (F_v/F_n), non-photochemical quenching (*qN*), and apparent electron-transport rate (AETR, μmol quanta m^{-2} s^{-1}), which are indicative of the capacity for efficient photon use under combined water and light stress.

Field study: site description

We carried out a complementary field experiment to evaluate the effect of natural levels of soil moisture and irradiance on seedling establishment. In addition, we artificially increased soil moisture to evaluate the strength of water limitation and the likely recruitment dynamics under more humid conditions. The experiment was conducted from May to September 2005 in two populations close to Navatrasierra (Cáceres Province, central-western Spain). The use of two populations allowed us to describe the ecophysiological responses of seedlings under contrasting abiotic scenarios. In the study area, the climate is Mediterranean subhumid, with mean annual precipitation ranging from 800 to 1120 mm in the four closest weather stations. Rains are concentrated in spring and autumn, with only 54 mm falling in the 3.2 month drought period. Mean annual temperature is 14.4°C. In the area, *P. lusitanica* stands grow along stream banks, usually at a distance < 5 m from the water. Soils are of a siliceous nature, acid, with schists as the main parent rock. They are deep, with abundant quartzite clasts. The organic horizon is deep and large amounts of litter accumulate on the soil surface.

The first study site (referred to as Mesto; 39°35′30″ N, 5°19′31″ W) was located at 780 m elevation. The second site (referred to as Hoya) was located at an elevation of 740 m (39°34′26″ N, 5°17′18″ W). The Mesto population (1195 individuals) is located within a mixed forest stand dominated by *P. lusitanica*, with *Alnus glutinosa*, *Castanea sativa*, *Fraxinus angustifolia* and *Quercus ilex*, and *Quercus faginea* as co-existing species. In the Hoya site (722 individuals), *A. glutinosa* dominates the riparian forest, with *P. lusitanica* and oak species as secondary components. In each site, we established one study plot that was fenced to avoid interference by browsing mammals. Both study plots encompassed a similar range of irradiance values (mean 0.19, range 0.06–0.35 in Hoya; mean 0.18, range 0.08–0.30 in Mesto; $F = 1.846$, d.f. = 1, 198, $P = 0.175$). However, differences in soil moisture were indicative of a dry environment in Hoya (mean 20.35, range 2.10–55.10) and a humid environment in Mesto (mean 31.31, range 2.20–81.61; $F = 13.432$, d.f. = 1, $P = 0.0003$).

Field study: experimental design

Within the exclosure made in each site, we selected 20 plots *c.* 1 m^2 in area on 15 May 2005. Five seedlings > 30 cm apart from each other were marked in each plot. Half the plots in each site were given a water-addition treatment, while the other half were left as control plots. Water addition consisted of periodically applying 10 L m^{-2} water taken from the nearby stream on each plot. Irrigation was applied every 10 days from 15 June to 30 September, spanning the whole drought period. The amount of water per seedling (2 L per date) was chosen to mimic the natural conditions experienced in the absence of a pronounced summer drought. On the basis of average summer precipitation (54 L m^{-2}) in the area, we calculated that watered plots experienced a 2.9-fold increase in the amount of above-ground water received throughout the summer period. Soil moisture was monitored with time domain reflectometry (TDR; Tektronic model 1502 C, Beaverton, OR, USA) with the probe inserted at 15 cm depth. One hour after watering, irrigation increased soil moisture in watered plants (22.8 ± 9.5%, $n = 20$) compared with control plants (14.9 ± 8.1%, $n = 20$; $F = 9.314$, d.f. = 38, $P = 0.004$ for the treatment effect in a two-way ANOVA including site as a factor). The magnitude of this difference decreased gradually and the difference was not significant 10 days after watering (18.5 ± 8.6% and 14.9 ± 8.1% in watered and control plots, respectively; $n = 20$, $F = 2.265$, d.f. = 1, 38, $P = 0.141$).

Field study: measurements of irradiance, soil moisture and seedling survival

A year-round integrated measurement of irradiance was obtained by taking hemispherical photographs over each seedling with a fish-eye camera. Images were treated with HEMIVIEW ver. 2.1 canopy analysis software (1999, Delta-T Devices, Cambridge, UK). The resulting values of direct site factor (direct radiation) were then used as descriptors of the light environment at each seedling point. Measurements of soil moisture were taken for each seedling with a Thetaprobe Sensor (Delta-T Devices) that registered values of water volumetric percentage in the uppermost soil layer (10 cm). All measurements were taken on 15 and 16 June 2005, just before the onset of the irrigation treatment. Initial moisture values could be taken as good estimators of the water regime throughout the summer period, as starting values were highly correlated with those obtained in the midsummer period (19 July, $r = 0.741$, $P << 0.001$ in the Hoya site; $r = 0.902$, $P << 0.001$ in the Mesto site; $n = 20$ readings per site). Post-summer seedling survival was checked on 30 September.

Data analysis

For the glasshouse study, we used two-way ANOVA to test for significance of irradiance and water effects and their interactions on performance of seedlings. Multiple logistic regressions were used to explore patterns of association between performance, growth, and survival variables. The Kaplan–Meier product–limit method was used to estimate the survival functions from the survival times of seedlings. To test for effects of treatments on survival functions, Gehan–Wilcoxon (GW) or χ^2 statistics were used for comparing two or multiple samples, respectively. For analysing the effects of water

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addition and site on the survival response of seedlings in the field, we used generalized linear models with the logit link function and survival binomial response. Irradiance and soil moisture were used as continuous predictors. All statistical analyses were performed with the software STATISTICA ver. 6.0 (StatSoft Inc., 2003).

RESULTS

Seedling performance under controlled conditions

After 1 month of gradual reduction in water availability, seedlings showed significantly lower stomatal conductance, electron-transport rate, photochemical efficiency and reduced photosynthetic rates (Tables 1 and 2). This reduction of photosynthetic rates was low relative to that experienced by stomatal conductance, resulting in increased WUE under dry conditions. Drought reduced stomatal conductance, electron-transport rate and photosynthesis, and these effects were explained primarily by differences at moderate or high irradiances (Tables 1 and 2).

Seedling performance was significantly affected by irradiance treatments (Tables 1 and 2). Stomatal conductance and photosynthetic rate were very low in shade and in deep shade, but moderately high at moderate and high irradiances. The same was true for photochemical efficiency and apparent electron-transport rate (Table 1). The light compensation point and photosynthesis saturation point showed expected reductions at decreased irradiances in well watered seedlings (Tables 1 and 2). Overall, these results clearly indicate no shade tolerance and better performance at intermediate irradiances.

Seedling growth and survival under controlled conditions

Absolute growth increment was higher at moderate and high irradiances, again indicating a low shade tolerance (Fig. 2). The number of leaves was significantly affected by irradiance, while the effect on seedling height did not reach statistical significance ($F = 87.993$, d.f. = 3, 174, $P < 0.001$; $F = 2.349$, d.f. = 3, 174, $P = 0.074$, respectively). The effect of the water regime was not significant after 30 days' drought, but there were irradiance–moisture interactions caused by drought sensitivity only at 10% irradiance ($F = 4.137$, d.f. = 1, 176, $P = 0.007$; $F = 3.723$, d.f. = 1, 176, $P = 0.012$ for height and number of leaves, respectively; Fig. 2). Results were almost identical when relative growth rates were used in the analyses (data not shown).

Post-summer seedling survival in the glasshouse was dramatically affected by soil water content, in such a way that survival was close to 0% in drought stressed plants (Fig. 3). In well watered seedlings, survival was 88% under moderate irradiance, 48% under high irradiance, 20% in shade and 0% in deep shade. Differences in survival functions for the eight treatments were highly significant ($\chi^2 = 3.164$, d.f. = 8, $P = 0.002$). Post-summer survival curves can be classified into four main treatment combinations with a decreasing survival rank: moderate irradiance-well watered, high irradiance-well watered, shade-well watered, with the remaining treatments rendering very low survival rates (Fig. 3). These results demonstrate that, even when continuous watering was supplied, survival was not enhanced by shade.

One-month-old seedlings surviving in September were larger than dead ones when measured on 5 July, indicating that greater growth was associated with a higher probability of survival ($F = 4.611$, d.f. = 1, 180, $P = 0.033$; $F = 19.514$, d.f. = 1,180, $P = 0.00002$ for height and number of leaves, respectively; all treatments pooled). The increment in the number of leaves achieved by seedlings after 1 month of experimental treatments (from 5 June to 5 July) was a very good predictor of post-summer survival, whereas the effect of the increment in seedling height was only marginally significant (multiple logistic regression: Wald statistic = 12.242, d.f. = 1, $P = 0.0004$ for number of leaves; Wald

Table 2 Results from two-way ANOVA testing for the effects of irradiance and soil moisture on physiological responses of *Prunus lusitanica* seedlings in the glasshouse experiment.

	Irradiance		Moisture		Irradiance × moisture	
Variable	F (d.f. = 3, 174)	P-value	F (d.f. = 1, 176)	P-value	F (d.f. = 3, 170)	P-value
A_{max}	39.415	< 0.001	37.264	< 0.001	8.298	< 0.001
R_d	1.169	0.336	0.837	0.367	1.093	0.366
Φ	6.864	0.001	7.663	0.009	6.709	0.001
Γ	12.287	< 0.001	2.004	0.166	13.013	< 0.001
P_s	1.510	0.230	0.043	0.836	7.525	0.001
g	41.607	< 0.001	80.738	< 0.001	27.844	< 0.001
WUE	21.365	< 0.001	6.607	0.015	2.129	0.115
F_v/F_n	43.327	< 0.001	6.775	0.014	1.767	0.173
qN	24.368	< 0.001	0.604	0.442	0.662	0.581
AETR	214.662	< 0.001	29.015	< 0.001	18.594	< 0.001

Levels of both treatments were 60, 10, 2 and 0.5% sunlight for irradiance and well watered control and gradual drought for soil moisture. See Table 1 for abbreviations and units. Thirty to 40 seedlings were used in the four light treatments under well watered conditions; 60–70 seedlings in the four light treatments under drought conditions.

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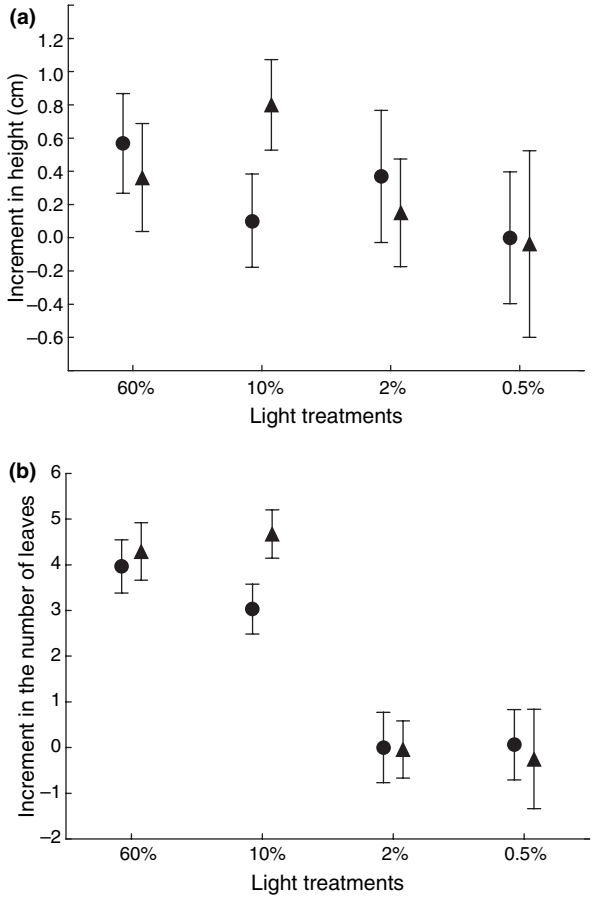

Figure 2 Mean and 95% confidence intervals for the absolute increment in height (a) and number of leaves (b) for the two irrigation treatments along the irradiance gradient. Squares, drought treatment; triangles, well watered treatment. Thirty to 40 seedlings were used in the four light treatments under well watered conditions; 60–70 seedlings in the four light treatments under drought conditions.

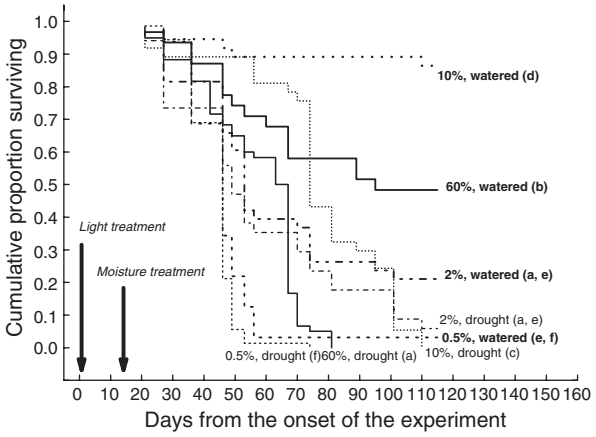

Figure 3 Kaplan–Meier curves denoting temporal changes in the cumulative proportion of seedlings surviving from the onset of the experiment on 20 May to 15 September 2005. Different line patterns are used for each of the eight irradiance–water combinations, thicker lines denote well watered (control) treatments. Treatments sharing the same letter in parentheses are statistically undistinguishable in *post hoc* comparisons (Gehan–Wilcoxon test). Arrows indicate the onset of light and moisture treatments.

statistic = 2.117, d.f. = 1, $P = 0.090$ for height; $n = 182$ seedlings alive on 5 July).

When mean values for each of the eight treatment combinations were used, significant ($P < 0.05$) positive correlations existed between previous seedling growth, stomatal conductance ($r_s = 0.732$), photosynthetic rate ($r_s = 0.871$), WUE ($r_s = 0.754$) and photochemical efficiency ($r_s = 0.892$). The correlations between these variables and percentage survival of seedlings were significant in the case of stomatal conductance ($r_s = 0.961$) and photosynthetic rate ($r_s = 0.890$), but not for WUE ($r_s = -0.035$) and photochemical efficiency ($r_s = 0.581$). Overall, these results showed that seedlings reaching large sizes had better photosynthetic performance and survival probability.

Seedling survival under natural conditions

The generalized linear model including irradiance and initial soil water content as continuous predictors, and site and irrigation treatment as factors, revealed a non-significant effect of water addition on post-summer seedling survival (Table 3). However, initial (natural) soil moisture, irradiance and site significantly affected seedling fate (dead vs. alive). Moisture values were greater for surviving seedlings, and the difference was consistent among sites ($F = 0.890$, $P = 0.346$ for the site × fate interaction in a two-way ANCOVA with irradiance as a covariate and fate and site as factors; Fig. 4). Irradiance had also a highly significant effect on survival, with surviving seedlings growing under lower irradiances. However, mean values for surviving control plants did not indicate shade tolerance (15.9 ± 1.5% in Hoya; 16.7 ± 2.2% in Mesto; Fig. 4). The effect of fate on irradiance depended on site and was mainly due to differences in the Hoya site ($F = 7.935$, $P = 0.005$ for the site × fate interaction; Fig. 4).

DISCUSSION

Seedling dependence on soil moisture

A gradual reduction in water availability, mimicking a summer drought, negatively affected CO_2 assimilation rates, reduced growth, and eventually caused almost complete mortality of seedlings in the glasshouse. A similar effect was found in the field study, which showed that soil moisture was, by far, the best predictor of post-summer seedling survival. Periodic water addition failed to increase survival, further indicating that a continuous water supply, like that provided naturally in stream banks and supplied in the glasshouse, is essential for seedling survival. All these results support the hypothesized dependence of *P. lusitanica* on a continuous water supply, which, in drought-prone Mediterranean ecosystems, makes this species a strictly riparian one (Calleja,

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Table 3 Results of generalized linear modelling (logit link function) testing for the effects of site and irrigation treatment on post-summer survival (binomial response) of *Prunus lusitanica* seedlings.

	d.f.	Wald statistic	*P*-value
Intercept	1	0.257	0.612
Soil moisture	1	9.920	0.002
Irradiance	1	7.687	0.006
Site	1	5.976	0.015
Irrigation treatment	1	1.196	0.273
Site × irrigation treatment	1	0.008	0.928

Soil moisture and irradiance were included as continuous predictors. Calculations are based on a set of 200 tagged seedlings (100 per site).

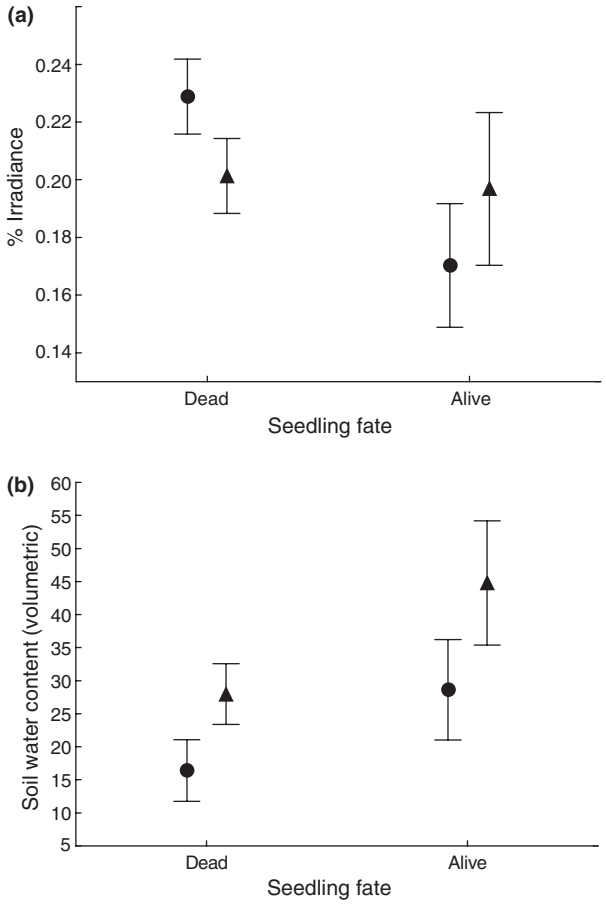

Figure 4 Means and 95% confidence intervals of irradiance (a) and soil moisture (b) values for dead and living seedlings after the summer period at the Hoya (dry) and Mesto (humid) field sites. (Hoya, $n = 71$ living and 26 dead seedlings; Mesto, $n = 71$ living and 17 dead seedlings). Circles, Hoya; triangles, Mesto.

2006). Previous studies on seedling survival of Tertiary relict tree species growing in Mediterranean sites have shown similarly dramatic effects of drought (Hampe & Arroyo, 2002; Mejías *et al.*, 2002; Valladares *et al.*, 2005a). In these environments, the high need for soil moisture can be met only by a local shift to riparian habitats, which consequently act as local climatic refuges.

Coping with summer drought

The most significant physiological response of seedlings elicited by reduced water supply was an increase in WUE. This response resulted from a decrease in transpiration and water expenditure, while keeping moderate carbon assimilation rates. This result was consistent with our hypothesis 2, and with most previous results concerning plant acclimation to water limitation (Ehleringer, 1993; Hanba *et al.*, 2002). Furthermore, values of WUE measured for *P. lusitanica* were similar to, or even higher than, those reported for deciduous Mediterranean oaks (Quero *et al.*, 2006), which was unexpected in view of the subtropical origin of the laurel cherry compared with drought-adapted oaks. For a subtropical tree, high WUE in the seedling stage would help to withstand windows of suboptimal conditions in riparian habitats. In fact, field measurements of soil moisture in both study sites showed that the frequency distribution of the values of soil moisture beneath experimental seedlings was strongly right skewed (see Methods, Field study), in such a way that most seedlings faced some degree of water shortage due to microsite-specific moisture conditions. Thus the conservative use of water can be considered as a trait enhancing successful establishment even in riparian habitats, due to the unpredictability of water supply in a predominantly dry Mediterranean climate.

In contrast to our expectations, WUE showed a high correlation with seedling growth, but a weak association with survival across treatments, a result differing from most of the remaining performance-related variables. One possible interpretation is that seedlings were able to keep stomatal conductance and water loss far from lethal values, as suggested by the above comparison with oaks.

Tolerance to extreme irradiances and the occupancy of riparian habitats

In general, species originating and still surviving in dense subtropical forests can be expected to exhibit shade tolerance. However, 'shade specialists' are rare, and shade tolerance can vary across species' distribution range, as shown for the Tertiary relict tree *Ilex aquifolium* (Valladares *et al.*, 2005a; Niinemets & Valladares, 2006). Our measurements of well watered seedlings showed that shade did not improve seedling performance and, in fact, seedlings performed much better when exposed to moderate or even high irradiances, which are the rule in Mediterranean understoreys (Valladares & Guzmán, 2006). Furthermore, shading of seedlings had important negative effects for fitness, as it resulted in significantly lower survival rates compared with those obtained under moderate or high irradiance. These results, together with the similarity of the preferred irradiance values of *P. lusitanica* (10–20%) with those of eumediterranean oak species (Quero *et al.*, 2006; Sánchez-Guzmán *et al.*, 2006), lead us to conclude that the

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species is not suited to grow and survive in dense subtropical forests, as also suggested by a previous study (Fernández-Palacios & Arévalo, 1998).

Prunus lusitanica behaves as a gap-maker and gap-colonizer species in subtropical forests, where its abundance declines in closed mature stands (Fernández-Palacios & Arévalo, 1998). As hypothesized, in dry Mediterranean environments, close dependence on a high water supply (which was evidenced in our two experiments), coupled with shade intolerance, would force *P. lusitanica* to be an efficient colonizer of small gaps, which are very frequent in riparian forests due to the severe impact of water flow on tree-root systems (Calleja, 2006). In fact, the spatial distribution of irradiance within the riparian forests studied showed that half of the experimental seedlings were exposed to light intensities (20–40% direct radiation) that are typical of relatively large gaps, particularly in open forests. In addition, the reduction in seedling performance when exposed to high irradiance was clearly lower than that caused by shade, suggesting the existence of some mechanisms buffering the effects of high light, such as dynamic photoinhibition or increased carotenoid content, which have been found in submediterranean tree species (Gómez-Aparicio *et al.*, 2006).

Overall, our results indicate that (1) *P. lusitanica* is well suited to grow in a wide range of light environments (excluding extremely dark ones) along riparian habitats acting as local climatic refuges, and (2) its optimum irradiance range does not suggest shade tolerance. These features support previous qualitative views that included the species in a 'palaeomediterranean' subset of the Tertiary dendroflora (Palamarev, 1989; Blanco *et al.*, 1997), for which shade tolerance would not be a requisite for population persistence. This can be invoked as one of the reasons why *P. lusitanica* was able successfully to face climate changes leading to a Mediterranean regime, while other co-existing Tertiary genera such as *Persea*, *Ocotea* or *Picconia* went extinct in southern continental Europe. Apart from tolerance to high light and a conservative use of water, *P. lusitanica* should have been able to cope with decreased temperatures through the Quaternary. In fact, according to palaeobotanical reconstructions, the species was associated with cold-resistant temperate genera such as *Taxus* and *Ilex* (Pignatti, 1978).

Interactive effects of irradiance and moisture

As light capture and water absorption cannot be maximized simultaneously, water stress should affect the response to irradiance (Smith & Huston, 1989; Abrams & Mostoller, 1995; Niinemets & Valladares, 2006). The effect of water stress on seedling performance and growth was strong at moderate (optimum) irradiances and intermediate at high irradiance. At the opposite extreme, the effect of water shortage was negligible at very low light, due to the extremely poor performance in deep shade. Our results only partially support the trade-off hypothesis of higher impact of drought in the shade (Kubiske *et al.*, 1996), as the predicted effect was found for only one of the extremes of the irradiance gradient (60% and 10%) and not for the opposite extreme (2% and 0.5%). On the other hand, the size of aerial parts attained by seedlings had a highly positive effect on seedling survival, and hence greater survival was not achieved at the expense of aerial growth, as implied by the trade-off hypothesis. We conclude that drought modified most light responses of seedlings, but in a complex, nonlinear way (see also Quero *et al.*, 2006).

Neither did we find supporting evidence for a higher impact of drought on seedling performance at extremely high irradiances (Valladares *et al.*, 2005b). Virtually all variables estimating seedling performance suffered greater change with drought at intermediate than at high irradiance (Table 1). The only trait experiencing greater change with drought at high irradiance was WUE, a response characteristic of light-demanding trees (Lipscomb & Nilsen, 1999; Hanba *et al.*, 2002). As shown by our field data, dry microsites tend to be associated with high irradiance, hence buffering of the negative effects of drought at high irradiances would reinforce the ability of *P. lusitanica* to establish in open sites. Thus, high-irradiance, well watered microsites can be better exploited by seedlings than shaded ones, despite high light being suboptimal when compared with moderate irradiance.

CONCLUSION

This study shows how seedlings respond to abiotic constraints and their tolerance to extreme and interacting conditions. We showed that Mediterranean populations of *P. lusitanica* are characterized by a high moisture requirement during summer, an inability to cope with severe drought, a lack of tolerance to deep shade, and a tolerance of high irradiance. Thus, in order to persist in drought-prone environments, *P. lusitanica* has to rely on moist sites under a wide range of light conditions other than deep shade. These results lead us to propose a mechanistic explanation for the stability of the rear edge of the range by reaching refuges in riparian habitats. Furthermore, close dependence on these habitats can help to explain the low range filling of the species in the Iberian Peninsula, where 128 widely spaced populations occur. Finally, we suggest that buffered range modification through habitat shift at the very local scale could be a widespread phenomenon, the importance of which is likely to increase under current and predicted decreases in precipitation in the Mediterranean region (IPCC, 2001).

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