

Differential light responses of Mediterranean tree saplings: linking ecophysiology with regeneration niche in four co-occurring species

LORENA GÓMEZ-APARICIO^{1,2,4}, FERNANDO VALLADARES³ and REGINO ZAMORA¹

¹ Dpto. Ecología, Facultad de Ciencias, Universidad de Granada, Granada, E-1807, Spain

² Present address: Institute of Ecosystem Studies, 65 Sharon Turnpike, P.O. Box AB, Millbrook, New York 12545-0129, USA

³ Centro de Ciencias Medioambientales-CSIC, Serrano 115, Madrid, E-28006, Spain

⁴ Corresponding author (gomezl@ecostudies.org)

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Summary The ecophysiological mechanisms underlying plant–plant interactions and forest regeneration processes in Mediterranean ecosystems are poorly understood, and the experimental evidence for the role of light availability in these processes is particularly scant. We analyzed the effects of high and low irradiances on 31 ecological, morphological and physiological variables in saplings of four late-successional Mediterranean trees, two deciduous (*Acer opalus* subsp. *granatense* (Boiss.) Font Quer & Rothm. and *Quercus pyrenaica* Willd.) and two evergreen (*Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco and *Quercus ilex* L.), which coexist in mature montane forests. Species differed in both their capacity to withstand high radiation and in their shade tolerance. The two deciduous species were the least tolerant to high radiation, exhibiting both dynamic and chronic photoinhibition in full sunlight, with severe implications for gas exchange and photosynthetic performance. Excess light severely limited the survival of *A. opalus* subsp. *granatense*, even minor reductions of excessive radiation (from full sunlight to 80% sunlight) being crucial for sapling survival. Among species, *P. nigra* was the most tolerant of high irradiances but showed the poorest shade acclimation. Interspecific differences in the mechanisms of response to light provide a partial explanation of the differential regeneration patterns previously reported for these species, with shade-tolerant plants (i.e., deciduous broadleaf species) benefiting the most from associations with nurse plants. We conclude that light availability is an important environmental factor defining the regeneration niche of Mediterranean woody species.

Keywords: *Acer opalus* subsp. *granatense*, photoinhibition, photosynthetic light response, *Pinus nigra*, *Quercus ilex*, *Quercus pyrenaica*, shade tolerance.

Introduction

Light is one of the most heterogeneous factors affecting plants, varying by more than three orders of magnitude both in time and space (Pearcy 1999, Valladares 2003). Thus, it can easily change from being a limiting resource when scarce to being a

stress factor when excessive. Patterns of light heterogeneity and their effects on vegetation have been studied mainly in temperate and tropical forests where light is a limiting resource (Pacala et al. 1994, Chazdon et al. 1996, Nicotra et al. 1999). In these systems, succession is driven largely by interspecific differences in shade tolerance, slow-growing shade-tolerant trees replacing relatively shade-intolerant colonizers (Bazzaz 1979, Shugart 1984, Barnes et al. 1998). Light effects on vegetation processes have been less studied in Mediterranean-type ecosystems, where climatic and ecological characteristics make light a stress factor. The concurrence of high radiation and drought during the summer exacerbates the negative impact of drought on plant performance (Joffre et al. 1999), leading to photoinhibition and thereby further limiting carbon assimilation (Werner et al. 2002, Valladares et al. 2005). Moreover, both abiotic limitations on primary production and a long history of human-induced disturbances have led to a relatively low vegetation cover and large areas of bare soil exposed to full sunlight in most Mediterranean forest ecosystems (Groove and Rackham 2001). Therefore, regeneration and succession dynamics in these systems are largely determined not only by interspecific differences in shade tolerance, but also by the ability of seedlings and saplings to withstand high radiation.

In recent years, several studies conducted in arid and Mediterranean ecosystems have demonstrated that shade provided by pre-established vegetation is a requirement for woody species establishment (Hastwell and Facelli 2003, Maestre et al. 2003, Gómez-Aparicio et al. 2005b), suggesting that light conditions in open areas have a negative effect on survival of late-successional species. Although facilitation is the most frequent plant–plant interaction in stressful environments (Bertness and Callaway 1994, Callaway et al. 2002, Gómez-Aparicio et al. 2004), the effect of established vegetation on seedling and sapling performance is reported to be negative when the shade strongly limits carbon assimilation (Franco and Nobel 1989, Holmgren et al. 1997, Kitzberger et al. 2000, Forseth et al. 2001). The balance of the positive and negative effects of nurse plants under stressful abiotic conditions large-

ly depends on the shade and high-light tolerance of the seedlings and saplings, which in turn depends on a complex combination of species-specific morphological and physiological responses to light (Callaway 1992, Kitajima 1994, Valladares et al. 2002).

The main objective of our study was to determine the light responses of saplings of four late-successional Mediterranean trees, two deciduous (*Acer opalus* subsp. *granatense* (Boiss.) Font Quer & Rothm. and *Quercus pyrenaica* Willd.) and two evergreen (*Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco and *Quercus ilex* L.), which coexist in mature montane forests. The responses to light were evaluated for a wide array of variables, from survival and growth to morphological and physiological traits. Spatial patterns of regeneration of these species are relatively well known. Thus, several observational and experimental studies have reported patterns of recruitment associated with pioneer nurse shrubs for the four tree species (Gómez et al. 2003, Castro et al. 2004, Gómez-Aparicio et al. 2005a). However, the ecophysiological mechanisms underlying plant-plant interactions and forest regeneration in Mediterranean ecosystems are poorly understood, with only a few exceptions (e.g., Rodá et al. 1999). Gómez-Aparicio et al. (2004) showed that survival and growth of pines generally benefit less than broadleaf species from nurse shrubs. Among broadleaf species, deciduous species such as *A. opalus* subsp. *granatense* have been reported to benefit more from the association with nurse shrubs than more stress-tolerant species such as *Q. ilex* (Gómez-Aparicio et al. 2005c). We hypothesized that: (1) the four tree species in our study rank according to tolerance to high irradiance from the pine (most tolerant) to the deciduous species (least tolerant); and (2) tolerance to high irradiance is inversely related to shade tolerance (Reich et al. 2003).

Material and methods

Study site and species

The study site comprises 12 ha of natural forest located at 1650 m above sea level (a.s.l.) in La Cortijuela Botanical Garden (Sierra Nevada National Park, SE Spain). The experiment was performed in semi-natural conditions, providing the advantage of control over variables (e.g., light, water) without the artificiality of greenhouse experiments performed at an altitude, temperature or photoperiod differing from that experienced by the species in its natural habitat. The climate is continental Mediterranean, with cold winters and hot, dry summers. Mean minimum temperature in the coldest month (January) is -1.1 °C and mean maximum temperature in the hottest month (July) is 28.8 °C, with an annual mean of 11.5 °C. Rainfall occurs mainly in spring and fall, with an annual mean for 1990–2002 of 871 mm (data from a climate station located at the study site). Seedlings were raised from seeds collected from several trees per species growing in the surroundings of the Botanical Garden. *Quercus ilex* acorns were collected in fall 1998, stratified and sown in January 1999, and seedlings emerged in late May and June. Seeds of *A. opalus* subsp. *granatense*, *Q. pyrenaica* and *P. nigra* were

collected in fall 1999 and sown in December 1999, and seedlings emerged during spring 2000. Seeds were sown in cylindrical pots (0.3 l), which were watered to saturation and weeded periodically. In April 2001, 360 saplings per species were randomly chosen and transplanted individually to 2.5-l plastic pots containing a mixture of peat, vermiculite and top soil collected in the Botanical Garden (1:1:2, v/v).

Experimental design

Pots were randomly assigned to one of three light treatments (1) open or 100% full sunlight, representing the light environment of open interspaces between shrubs and trees in the mosaic of a Mediterranean forest; (2) 80% full sunlight, representing the mild shade cast by some shrub species in the area; and (3) 13% full sunlight. Thirteen percent of full sunlight, although higher than deep shade ($< 5\%$) traditionally used in shade-tolerance studies, was chosen because it represents the deepest shade generally found in the natural forests of the study zone (Valladares 2004). All pots were located outdoors, and irradiance was controlled with neutral shade cloth. Microclimatic conditions of each treatment were characterized by measuring air temperature (T) and photosynthetic photon flux (PPF) every minute for seven clear days in midsummer (Table 1). The PPF was recorded with Ha-li light sensors (EIC SL, Madrid, Spain) cross-calibrated with a LI-190SA quantum sensor (Li-cor, Lincoln, NE) connected to data loggers (HOBO 8, Onset). Saplings were randomly grouped into six blocks per treatment, each block containing 20 saplings per species that were moved periodically within the block to minimize possible effects of within-block light variability. There were no differences in seedling initial height between treatments or blocks for any species ($P > 0.05$ in all cases). Throughout the experiment, pots were watered to saturation at a variable frequency (never less than twice weekly) depending on the season and the light treatment, so that water was never a limiting factor. To check homogeneity of soil humidity between light treatments and blocks, volumetric soil water content was measured in July 2001 in 10 saplings per treatment and block combination ($n = 180$) with ThetaProbe sensors (Delta-T Devices, Cambridge, U.K.). There were no differences in soil water content between treatments or blocks (treatment: $P = 0.109$; block: $P = 0.158$).

The experiment started on June 1, 2001 and ended July 15, 2003. During this period, seedling survival was recorded twice per year, in September and March. Seedling height was measured every summer at the end of the growth period and accumulated relative height growth (RHG) over the entire study period was calculated as $RHG = (\ln H_2 - \ln H_1) / (t_2 - t_1)$, where H_2 and H_1 represent the final and initial height and $t_2 - t_1$ is the time that elapsed between measurements. When the experiment ended, 13 saplings per species and treatment ($n = 156$) were harvested. Because there were no live saplings in some combinations of species, treatment and block, the block was not considered as a factor at the time of harvest. Saplings were washed carefully to avoid losing fine roots and placed in plastic bags for transport to the laboratory.

Table 1. Environmental conditions in the three light treatments. Data are means \pm 1 SE of six sensors per treatment recording each minute during the midday period (1100–1500 h) for photosynthetic photon flux (PPF) and air temperature (T) or the whole day (for daily totals) on seven clear days in midsummer. Statistical differences between treatments were assessed by one-way ANOVA.

	Full sunlight	80% sunlight	13% sunlight	<i>P</i>
PPF ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Mean	1513.4 \pm 69.6	1210.9 \pm 107.7	180.2 \pm 27.7	< 0.0001
Maximum	2286.5 \pm 83.3	1916.5 \pm 156.3	397.0 \pm 42.4	
Minimum	535.5 \pm 27.6	312.6 \pm 38.5	61.7 \pm 7.3	
T ($^{\circ}\text{C}$)				
Mean	32.5 \pm 1.0	31.7 \pm 0.5	29.1 \pm 1.5	< 0.01
Maximum	40.0 \pm 2.4	39.2 \pm 1.8	34.0 \pm 2.2	
Minimum	21.1 \pm 1.5	21.3 \pm 0.7	18.3 \pm 1.8	
Daily PPF ($\text{mol m}^{-2} \text{day}^{-1}$)	45.50 \pm 2.14	36.74 \pm 4.94	5.34 \pm 0.78	< 0.0001

Leaf and plant morphology

Harvested plants were divided into stems, roots and leaves. Total stem and root length was measured with a digital caliper to the nearest 0.1 mm. Leaf areas of each broadleaf sapling were measured on scanned images of the leaves with a computer imaging system (Medidor de objetos 1999–2000 R. Ordiales Plaza®, Almería, Spain). To calculate leaf areas in the pines, we measured length and diameter of three needles per sapling and calculated their projected area. Each needle was kept in an individual paper bag and, with the rest of the plant material, dried at 45 $^{\circ}\text{C}$ for one week for dry mass determination. Specific leaf area (SLA) for each pine sapling was calculated from dry mass and projected area of the three individual needles. Total leaf area per pine sapling was calculated from total needle mass and SLA. Specific leaf area, root:shoot ratio, leaf mass ratio (LMR) and leaf area ratio (LAR) were calculated for all species.

Leaf pigment and nutrient content

In June 2003, leaf chlorophyll was estimated in situ with a SPAD chlorophyll meter (SPAD-520, Minolta, Osaka, Japan). Measurements were made on 72 saplings (four saplings per light treatment and block) of each broadleaf species (SPAD is not designed for use with conifers). Values of SPAD were transformed to mg m^{-2} of total chlorophyll (Chl a+b) based on species-specific calibration curves. The curves were constructed by regressing SPAD values against values of total chlorophyll content (mg m^{-2}) determined spectrophotometrically in dimethyl sulfoxide (DMSO) extracts of the same leaf portions, following the protocols of Barnes et al. (1992). In addition, Chl a, Chl b and total carotenoids were determined in DMSO extracts of leaves collected from six saplings per species and treatment, and ratios of Chl a:Chl b and Carotenoids:Chl calculated. Total nitrogen (N) was measured in leaves of three harvested saplings per species and treatment with a Fisons-Carlo Erba EA 1108CHNS-0 (Thermo Finnigan, San Jose, CA) element analyzer. Total chlorophyll, carotenoids and N contents were expressed on an area and a dry mass basis.

Chlorophyll fluorescence

In vivo Chl a fluorescence of current-year, fully developed leaves at the top of the crown layer was measured for four saplings per species and treatment randomly selected among the six blocks per treatment ($n = 48$) for three days representative of the beginning (June 28), middle (August 12) and end (October 4) of summer 2001. Measurements were made with a portable modulated fluorometer (model FMS2, Hansatech, Kings Lynne, U.K.), equipped with a leaf-clip holder to monitor incident solar radiation (PPF_{leaf}) and leaf temperature (T_{leaf}). Minimal (F_o) and maximal (F_m) fluorescence were measured at predawn and 1 h after sunset, and the maximum photochemical efficiency of photosystem II (PSII) in darkness (F_v/F_m) was calculated. Minimal (F_o') and maximal fluorescence in the light (F_m') were measured at 2.5-h intervals during the day, and the photochemical efficiency of the open reaction centers of PSII (F_v'/F_m') was calculated according to Genty et al. (1989). Predawn F_v/F_m was also measured in four saplings per species, light treatment and block ($n = 288$) in July 2002, to estimate the physiological state of saplings in the second summer of the experiment. Just before harvest in July 2003, a final measurement was made of predawn F_v/F_m , midday F_v'/F_m' , and midday non-photochemical quenching ($\text{qN} = (F_m - F_m') / (F_m - F_o')$; Buschman 1995) in four saplings per species, light treatment and block ($n = 288$). To assess induction of and subsequent recovery from photoinhibition, 72 seedlings were transferred from 13% sunlight to full sunlight for 2 h at midday on a clear day in July 2002. We measured F_v/F_m in each seedling just before exposure to sunlight and after recovery for 30 and 90 min in the shade.

Gas-exchange responses

Photosynthetic light response curves were constructed for 3–4 plants per species and treatment during July 2003 by measuring net assimilation rates (A) at varying PPFs with a portable open gas exchange system (CIRAS 2, PP-System). Temperature was set at 27 ± 2 $^{\circ}\text{C}$ and CO_2 at 370 ± 20 ppm. We plotted A against incident PPF and the curve was fitted using the Photosyn Assistant software version 1.1.1. (Richard Par-

sons, Dundee, U.K.). The photosynthetic response of the leaves to PPF was modeled by a rectangular hyperbola (quadratic equation presented by Chartier and Prioul 1976), where the initial slope is the apparent quantum efficiency (Φ), the light compensation point (Γ) and apparent respiration (R) were estimated from axis intercepts, and the photosynthetic capacity (A_{\max}) is the upper asymptote, which is reached at the light saturation point (P_s). An additional parameter (convexity, Θ) was calculated to describe the progressive rate of bending between the linear gradient and the maximum value. Photosynthetic and respiration rates were expressed both on a leaf area basis and a dry mass basis.

Data analyses

Sapling survival data were subjected to analysis of variance (ANOVA) based on Generalized Logit Models (Proc CATMOD) in the SAS 2000 software package (SAS Institute, Cary, NC), with species, treatment and block nested into treatment as factors. Height was analyzed for each species by a mixed, repeated-measures ANOVA (Proc GLM, SAS), where treatment was a within-group fixed factor and year was a between-group random factor. Differences between species and treatments for the different groups of variables were analyzed by ANOVA (Proc GLM, SAS), with the exception of the number of shoots, which was analyzed by Generalized Linear Models (GLZ, Statsoft v.6.0, Tulsa, OK) because of its Poisson distribution. Both species and treatment were considered fixed factors. Block was introduced as a third random factor in fluorescence analyses. For morphological variables, plant dry mass at the time of harvest was introduced as a covariate to neutralize possible ontogenetic effects in the comparisons. Because we repeated the same model for several plant traits, we used the fixed Bonferroni correction to avoid experiment-wise type I error, adjusting α to 0.05 in the statistical tests, which resulted in different P -values. When necessary, dependent variables were log-transformed to achieve requirements of normality and homocedasticity (Zar 1996).

Results

Survival and growth

Sapling survival varied significantly between species ($P < 0.0001$), being much lower for *A. opalus* subsp. *granatense* (63.7%) than for the other three species (> 92%). Survival varied significantly between treatments ($P < 0.0001$), but only for *A. opalus* subsp. *granatense* ($P < 0.0001$, Species \times Treatment interaction), which showed almost three times lower survival in full sunlight (30%) than in 80 and 13% sunlight (77.5% and 83.5% survival, respectively). There were no differences in survival between blocks ($P = 0.106$). Height differed significantly between treatments for all species ($P = 0.013$ for *A. opalus* subsp. *granatense*; $P = 0.049$ for *Q. pyrenaica*; $P = 0.006$ for *Q. ilex*; and $P = 0.013$ for *P. nigra*), the tallest plants appearing in 13% sunlight and the shortest in full sunlight (Figure 1). Between-treatment differences were not constant over

time, resulting in significant Year \times Treatment interaction terms ($P = 0.009$ for *A. opalus* subsp. *granatense*; $P = 0.0004$ for *Q. pyrenaica*; $P = 0.029$ for *Q. ilex*; and $P = 0.037$ for *P. nigra*). The greatest effects of light treatments on growth appeared (in the case of *P. nigra*) or intensified (in the case of the other species) during the last year. *Pinus nigra* had the highest RHG in all treatments, and the two *Quercus* species had the lowest RHG values (Figure 1).

Leaf and plant morphology

Sapling dry mass decreased with decreasing irradiance (Table 2 and Figure 2), but the decrease—a 59% reduction in dry mass from full sunlight to 13% sunlight—was significant only for *P. nigra* (significant treatment \times species interaction term). Root:shoot ratio increased gradually with increasing light availability, whereas leaf area showed the opposite pattern (Figure 2). The remaining morphological variables varied only between 13% sunlight and the other two treatments. Thus, saplings in 13% sunlight had fewer shoots and invested more biomass in leaves (higher LMR), had higher SLA and, consequently, higher leaf area per unit plant mass (higher LAR) than saplings in full or 80% sunlight (Figure 2). The light treatment did not significantly affect total root length. However, the treatment \times species interaction term was significant (Table 2), because root length of *Q. pyrenaica*, *Q. ilex* and *P. nigra* saplings did not vary between treatments, whereas root length of *A. opalus* subsp. *granatense* increased from 58.3 ± 8.1 cm in full sunlight (length similar to that of *Q. pyrenaica* and *Q. ilex*)

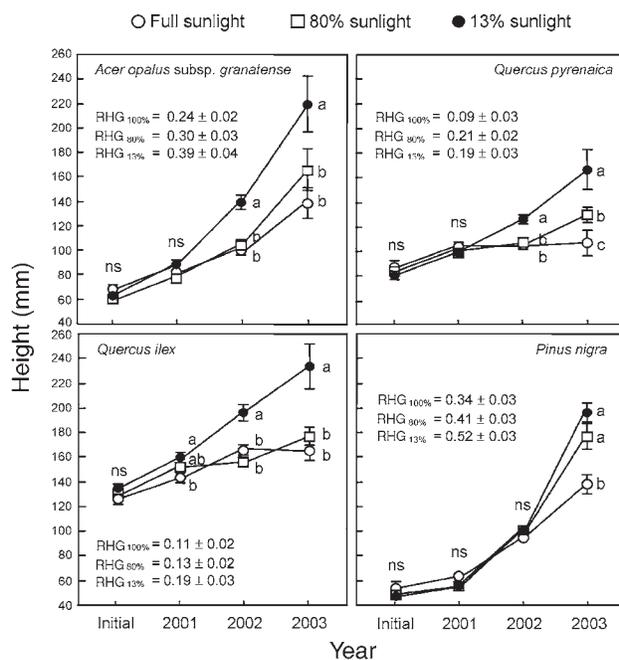


Figure 1. Height (mean \pm 1 SE) of saplings in each year and treatment. Within a year, different letters indicate significant differences between treatments at $\alpha < 0.05$ according to the Bonferroni/Dunn test. Relative height growth (RHG, mean \pm 1 SE) indicates the rate of growth (year^{-1}) over the entire study period.

Table 2. Summary of analyses of variance of differences in leaf and plant morphology between treatments and species. All models are General Linear Models (F -values), except for shoot number (SN), which was analyzed with Generalized Linear Models (L-R χ^2 values). Dry mass was introduced as a covariable. Significance levels: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$. After Bonferroni correction, differences were considered significant at $P < 0.007$. Abbreviations: RL = root length; R:S = root:shoot ratio; LA = leaf area; LMR = leaf mass ratio; LAR = leaf area ratio; and SLA = specific leaf area. Symbol ‡ = The Generalized Linear Models does not offer L-R χ^2 values for the whole model.

Factors	Dry mass	RL	R:S	LA	SN	LMR	LAR	SLA
Treatment	14.99****	1.99	13.23****	31.14****	11.36	4.93**	47.37****	150.84****
Species	42.09****	41.75****	130.65****	15.02****	124.07****	176.51****	14.62****	424.51****
Treatment \times species	4.01**	3.33**	1.29	1.76	13.88	1.02	0.39	3.78**
Dry mass		0.68	0.08	254.58****	49.34****	1.37	2.69	0.13
Model	15.24****	12.95****	42.24****	40.51****	‡	63.34****	16.30****	213.95****
R^2	0.51	0.53	0.79	0.78	‡	0.85	0.59	0.95

to 98.9 ± 8.5 cm and 108.4 ± 11.1 cm in 80 and 13% sunlight, respectively (length similar to *P. nigra*; Figure 2). All morphological variables differed significantly among species (Table 2). *Pinus nigra* had the highest leaf area and LMR, whereas the two deciduous species had the lowest values (Figure 2). In contrast, the two deciduous presented the highest SLA, LAR and root:shoot ratios. Shoot number was highest in *Q. pyrenaica* and lowest in *A. opalus* subsp. *granatense*.

Leaf pigment and nutrient concentrations

All leaf chemistry variables differed significantly between treatments and species (Table 3). The effect of light on Chl a+b concentration varied depending on the species, resulting in a significant treatment \times species interaction term (Table 3). Thus, the amount of Chl a+b per unit area was higher in 13% sunlight than in 80% and full sunlight for the three broadleaf species, although significantly higher only for *A. opalus* subsp. *granatense*. Mass-based Chl a+b concentration showed the same pattern of decrease with light in the three broadleaf species, the decrease being much greater in *Acer* (Figure 3). *Pinus nigra* saplings, on the contrary, had similar chlorophyll concentrations in all light treatments on an area and a dry mass basis. Area- and mass-based carotenoid concentrations, Carotenoids:Chl ratio and Chl a:Chl b ratio increased with increasing light in all saplings. Leaves of all species had more N on an area basis in full and 80% sunlight than in 13% sunlight, whereas the reverse was true when N was expressed on a dry mass basis. The response of instantaneous N-use efficiency (NUE; photosynthetic capacity divided by leaf N) to light varied depending on the species (significant treatment \times species interaction term; Table 3). The two deciduous species had higher NUE in 13% sunlight than in the other light treatments, whereas the two evergreens had similar NUE in all treatments (Figure 3). The N:Chl ratio was lower in 13% sunlight than in the other treatments (Figure 3). Among species, *Q. ilex* had the highest area-based concentrations of Chl a+b, carotenoids and N and the lowest mass-based concentrations of Chl a+b, carotenoids and N, whereas *A. opalus* subsp. *granatense* presented the opposite pattern (lowest area-based concentrations of Chl a+b, carotenoids and N, but the highest mass-based concentrations; Figure 3). The Chl a:Chl b ratio was highest in *A. opalus*

subsp. *granatense* and the Carotenoids:Chl ratio was lower in *P. nigra* than in the three broadleaf species. The N:Chl ratio was lower in *A. opalus* subsp. *granatense* than in any other species (Figure 3).

Chlorophyll fluorescence

The F_v/F_m ratio differed significantly between treatments and species throughout the study period (Table 4). The three broadleaf species exhibited photoinhibition (values lower than the theoretical optimum 0.8, Demmig-Adams and Björkman 1987) in full sunlight in all three years and also in 80% sunlight in 2003. However, F_v/F_m never fell below 0.7, and measurements made in 2001 showed that differences between treatments disappeared at the end of the summer for all species (Figure 4). *Pinus nigra* had the highest F_v/F_m values in the three years, consistently above 0.8. Among broadleaf species, there were differences only between *Q. ilex* and the two deciduous species in 2001, but this difference disappeared the following years. During summer 2001, F_v/F_m was lowest in June.

Daily curves of F_v'/F_m' showed a major decrease during midday, coinciding with the time of highest irradiance of the day (Figure 4). *Pinus nigra* showed higher values of F_v'/F_m' than the three broadleaf species, as well as a smaller decrease in F_v'/F_m' in the high irradiance treatments (significant treatment \times species interaction term; Table 4). Among months, the smallest decrease in F_v'/F_m' occurred in August reflecting the lower PPFs in that month (Figure 4). Midday F_v'/F_m' also differed significantly between species and treatments in 2003, corroborating patterns found in 2001 (Table 4). The qN response to light was the reverse of that of F_v'/F_m' , with the highest values at the highest PPF. Among species, qN values were highest for *A. opalus* subsp. *granatense* (0.86 ± 0.02) and lowest for *P. nigra* (0.77 ± 0.02), whereas *Q. pyrenaica* and *Q. ilex* presented intermediate values (0.82 ± 0.02 and 0.82 ± 0.04 , respectively).

Transfer of saplings from 13% sunlight to the open caused an immediate decrease in F_v/F_m , which was greatest in *A. opalus* subsp. *granatense* and smallest in *P. nigra*, the two *Quercus* having intermediate values (Figure 5). Only in *P. nigra* did the F_v/F_m values return to around 0.8 following a 2-h recovery period in the shade.

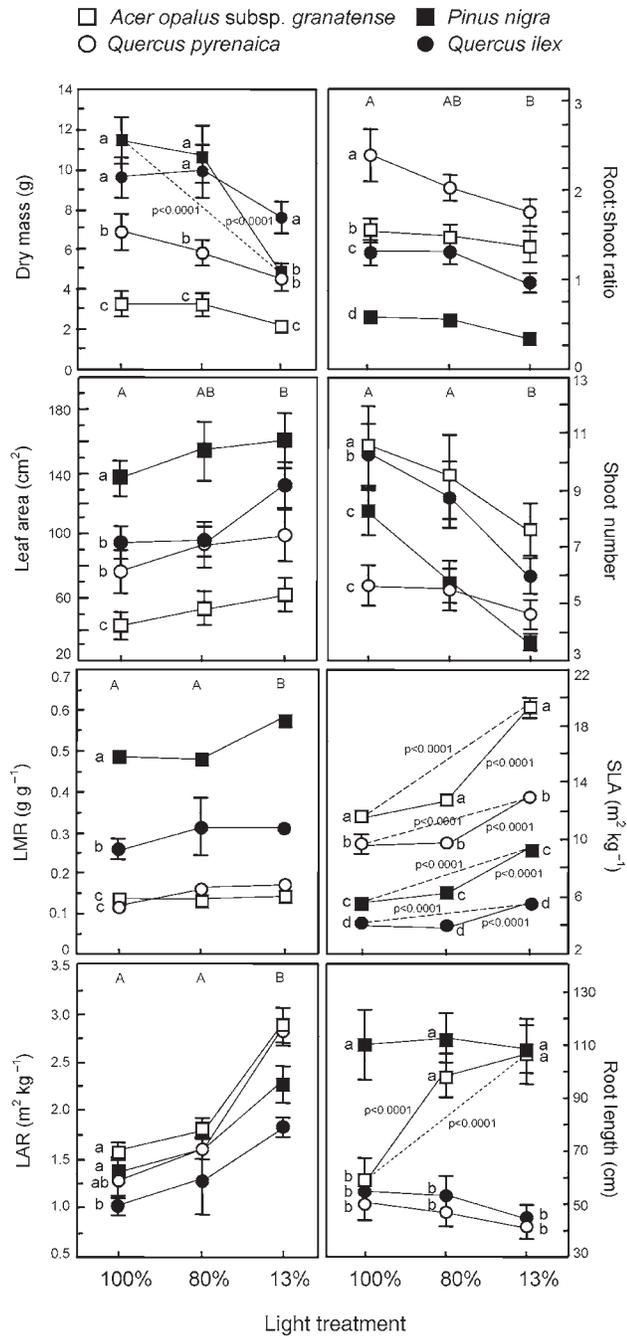


Figure 2. Differences between treatments and species in leaf and plant morphology (mean ± 1 SE). Uppercase letters indicate significant differences between treatments and lowercase letters indicate significant differences between species at $\alpha < 0.05$ according to the Bonferroni/Dunn test. The P -values indicate between-treatment significant differences for each species for the variables with a significant treatment \times species interaction term (see Table 2). Abbreviations: LMR = leaf mass ratio; LAR = leaf area ratio; and SLA = specific leaf area.

Gas-exchange responses

Among the gas exchange variables measured, only photosynthetic capacity per unit mass ($A_{max, mass}$), R_{area} and Γ differed significantly between light treatments (Table 5). Although

Table 3. Summary of F -values from an analysis of variance of differences in leaf chemistry between treatments and species. Significance levels: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$. After Bonferroni correction, differences were considered significant at $P < 0.005$. Abbreviations: Chl = chlorophyll; N = nitrogen; and NUE = photosynthetic capacity divided by leaf N. Chlorophyll and N were expressed on an area and on a dry mass basis.

Factors	Chl a+b _{area}	Chl a+b _{mass}	Carotenoids _{area}	Carotenoids _{mass}	Chl a:Chl b	Carotenoids:Chl	N _{area}	N _{mass}	NUE	N:Chl
Treatment	33.78****	38.51****	7.82***	4.20**	4.08**	16.91****	5.29**	6.16**	11.41***	8.13**
Species	122.26****	18.79****	16.66****	30.83****	42.66****	8.51****	20.78****	32.43****	32.38****	7.52****
Treatment \times species	9.59****	9.01****	1.82	1.19	0.80	1.35	1.17	0.16	9.39****	0.38
Model	42.73****	18.36****	8.35****	10.71****	12.75****	5.60****	7.34****	10.53****	15.92****	3.99**
R^2	0.71	0.51	0.51	0.58	0.63	0.40	0.66	0.74	0.82	0.48

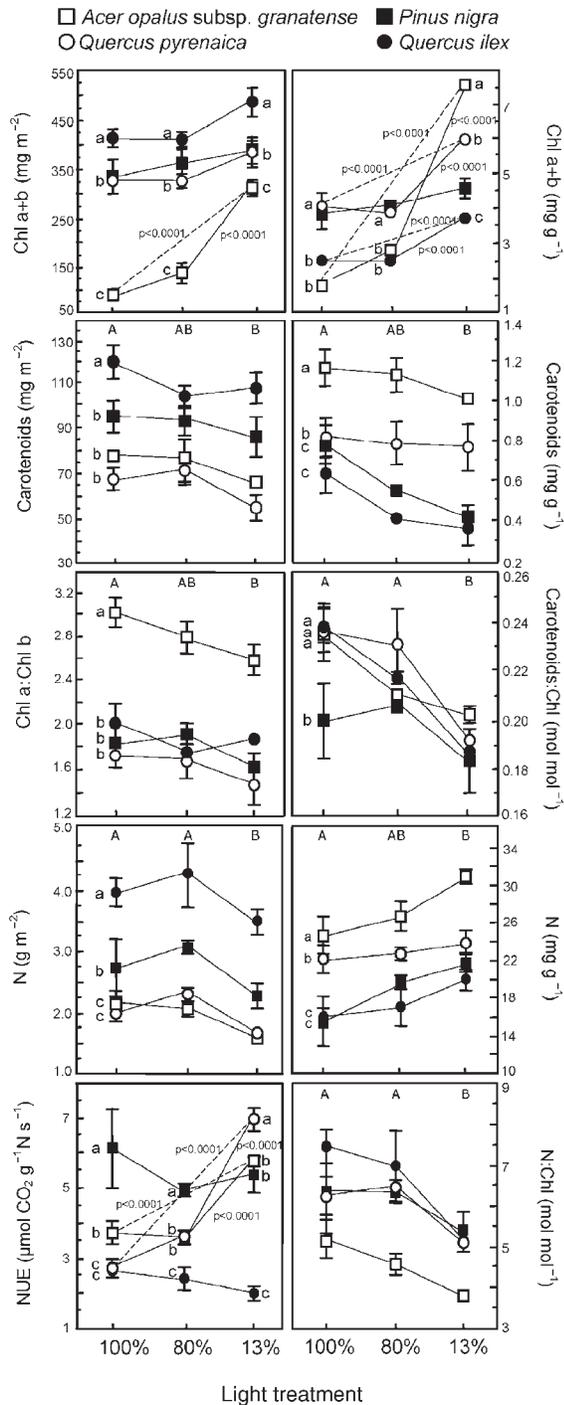


Figure 3. Differences between treatments and species in leaf pigment and nutrient content (mean \pm 1 SE). Uppercase letters indicate significant differences between treatments, whereas lowercase letters indicate significant differences between species at $\alpha < 0.05$ according to Bonferroni/Dunn test. The *P*-values indicate between-treatment significant differences for each species for the variables with a significant treatment \times species interaction term (see Table 3). Statistical analyses for Chl a+b were conducted with SPAD data and so statistical between-treatment differences are not presented for *P. nigra*. However, analysis performed on data obtained by chemical assay (not shown) indicated no between treatment differences for chlorophyll concentrations of *P. nigra* needles, and chlorophyll values equivalent to those of *Q. pyrenaica*.

photosynthetic capacity per unit area ($A_{max,area}$) did not differ between light treatments, there was a significant treatment \times species interaction effect on both $A_{max,area}$ and $A_{max,mass}$. Thus, chronic photoinhibition in *A. opalus* subsp. *granatense* and *Q. pyrenaica* resulted in decreased $A_{max,area}$ and $A_{max,mass}$ in saplings in full and 80% sunlight, whereas *Q. ilex* and *P. nigra* showed highest values in full sunlight (Table 6 and Figure 6). The Γ also showed a significant treatment \times species interaction, being significantly lower in 13% sunlight than in 80% and full sunlight for all species except *P. nigra* (Table 6). In all species, R_{area} was higher in full and 80% sunlight than in 13% sunlight. Between species, $A_{max,area}$, $A_{max,mass}$ and R_{mass} differed significantly (Table 5). *Quercus ilex* presented the lowest values of the three variables, whereas *P. nigra* presented the highest $A_{max,area}$ and *A. opalus* subsp. *granatense* had the highest $A_{max,mass}$ and R_{mass} .

Discussion

Interspecific differences in tolerance to high light

We found that excess light can severely limit survival of some species. Whereas mortality for *Q. pyrenaica*, *Q. ilex* and *P. nigra* was low in all treatments (around 10%), mortality of *A. opalus* subsp. *granatense* in full sunlight reached 70%, almost three times higher than in any of the shade treatments. Moreover, the finding that the 80% sunlight treatment reduced mortality of *A. opalus* subsp. *granatense* to values similar to those in 13% sunlight indicates that even minor reductions in solar radiation can be crucial for sapling survival of certain tree species.

Although not translated into differential survival, we identified between-species differences in the capacity to withstand excess radiation. High irradiance increased the carotenoid concentrations of the species studied, in agreement with the well-known protective role of these pigments (Young 1991, Demmig-Adams and Adams 1992, Havaux et al. 1998), and raised their Carotenoids:Chl ratios, enhancing the photoprotective action per unit chlorophyll (Kyparissis et al. 2000). Additionally, the three broadleaf species, and especially *A. opalus* subsp. *granatense*, decreased their chlorophyll concentrations in response to high irradiances thereby reducing radiation absorbance and avoiding photooxidation (Kyparissis et al. 1995). Nevertheless, these three species suffered chronic photoinhibition in the full sunlight treatment throughout the study period. Moreover, photoinhibition increased in the last year of the study, especially for the two deciduous species, with negative effects on gas exchange. Thus, whereas *P. nigra* and *Q. ilex* had the highest A_{max} in full sunlight, *A. opalus* subsp. *granatense* and *Q. pyrenaica* had the highest A_{max} and Φ in the shade (13% sunlight). Both A_{max} and Φ diminished significantly with decreasing F_v/F_m ($P = 0.012$, $r^2 = 0.43$ for $A_{max,area}$; $P = 0.008$, $r^2 = 0.52$ for $A_{max,mass}$; $P = 0.009$, $r^2 = 0.47$ for Φ ; $n = 12$ in all cases), resulting in chronic photoinhibition that reduced the capacity of deciduous species to maximize photosynthetic utilization of light under both saturating and non-saturating conditions (Osmond 1994).

Table 4. Summary of F -values from an analysis of variance of differences in photochemical efficiency of photosystem II in darkness (F_v/F_m) and with the open reaction centers (F_v'/F_m') and in non-photochemical quenching (qN) between treatments and species. For F_v'/F_m' , the mean of the three central values of the daily curves (measures at 1130, 1400 and 1630 h) was used as the dependent variable. In 2001, month (June, August, October) was introduced as a third factor. Block was considered when the sample size allowed. Significance levels: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$. After Bonferroni correction, differences were considered significant at $P < 0.025$ for 2001; $P < 0.05$ for 2002; and $P < 0.017$ for 2003.

Year	Factors	F_v/F_m	F_v'/F_m'	qN
2001	Treatment	115.32****	118.72****	
	Species	30.71****	30.32****	
	Month	21.97****	19.63****	
	Treatment × species	7.53****	7.15****	
	Treatment × month	3.05*	2.38	
	Species × month	1.87	1.58	
	Model	15.44****	17.94****	
2002	Treatment	6.14**		
	Species	23.79****		
	Block [Treatment]	1.40		
	Treatment × species	0.98		
	Species × block [Treatment]	0.65		
	Model	7.98****		
2003	Treatment	27.97****	89.97****	82.05****
	Species	10.49****	16.64****	3.00*
	Block [Treatment]	0.98	5.08	1.05
	Treatment × species	0.45	1.23	0.99
	Species × block [Treatment]	0.36	1.10	1.01
	Model	10.21****	24.87****	18.07****

Pinus nigra exhibited no photoinhibition in any year of study, corroborating our finding that it was the only study species whose shade-adapted seedlings recovered their F_v/F_m shortly after a sudden exposure to full sunlight. Moreover, it had the highest midday F_v'/F_m' , minimizing the risk of photoinhibition and reflecting its high photosynthetic capacity in full sunlight (Öquist et al. 1993). In contrast, shade-adapted saplings of *A. opalus* subsp. *granatense* suffered the slowest recovery in F_v/F_m after a sudden exposure to high light, and had the highest values of qN, implying a reduced capacity for photon use in photosynthesis under conditions of high irradiance. Overall, our findings suggest that the evergreen species are less sensitive to high irradiances than the deciduous species and that, among the study species, *P. nigra* and *A. opalus* subsp. *granatense* are the most and least tolerant to high irradiance, respectively.

Shade tolerance and interspecific differences in the responses to shade

The shade treatment (13% sunlight) reproduced natural deep shade of many Mediterranean forests (Valladares 2004). This shade treatment was insufficient to limit carbon gain critically and to increase mortality because all species showed high survival. Thus, survival in 13% sunlight was not a good indicator of interspecific differences in shade tolerance. However, there was a range of responses to shade in many morphological and physiological traits that can be associated with either shade-avoidance or shade-tolerance syndromes (sensu Henry and

Aarssen 1997). Shaded saplings of the four species showed strong vertical growth (shade avoidance) at the expense of reduced underground biomass (lower root:shoot ratio). However, the shade-tolerance response involved several leaf-level modifications having the effect of maximizing carbon assimilation at low irradiances, such as reductions in light compensation point and Chl a:Chl b ratio, and increasing foliar area and SLA. The reduction in light compensation point was associated with increased allocation of leaf N to chlorophyll, which occurs in low irradiances and maximizes PPF absorption (Terashima and Evans 1988). Saplings in the shade not only modified their total chlorophyll concentration, but also decreased their Chl a:Chl b ratio, implying increased chlorophyll allocation to the construction of light-harvesting complexes that are richer in Chl b than the reaction centers (Osunkoya et al. 1994). Moreover, by increasing leaf area and SLA, chlorophylls were distributed over the highest possible area, further enhancing photosynthetic performance in low irradiance (Björkman 1981).

Because of trade-offs at the leaf level in N allocation to chlorophyll and Rubisco, increasing N allocation to chlorophyll in shade is associated with less investment in Rubisco, which in turn implies lower NUE (Walters and Reich 1996). However, the deciduous broadleaf species (*A. opalus* subsp. *granatense* and *Q. pyrenaica*) showed both lower N:Chl ratios and higher NUE in 13% sunlight as a result of increases in total N concentration, total chlorophyll concentration and A_{max} in the shade. This suggests that these species perform well under low light

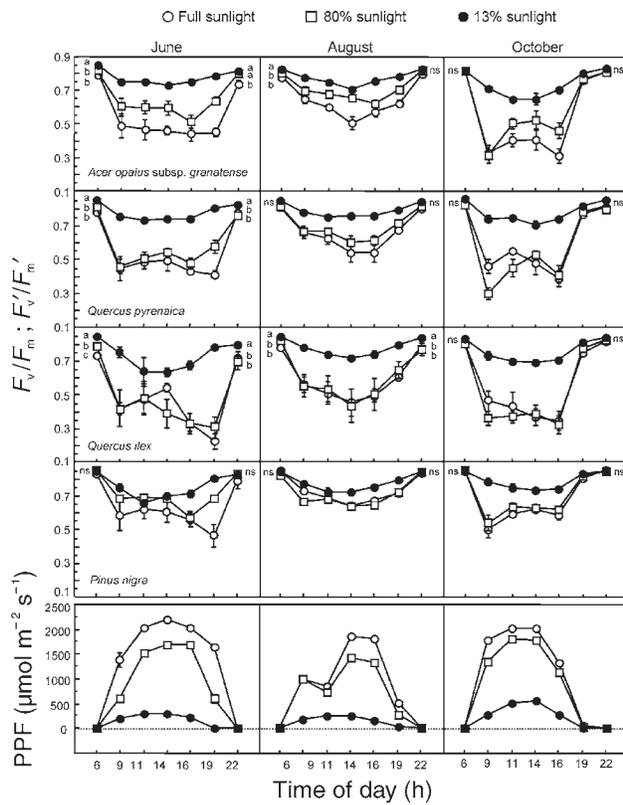


Figure 4: Diurnal course of photochemical efficiency of PSII in the dark (F_v/F_m , measured predawn (0600 h) and after-sunset (2200 h)) and the light (F_v'/F_m' , measured between 0900 and 2100 h), and photosynthetic photon flux (PPF) intercepted by single leaves at three times representative of the beginning (end of June), middle (mid-August) and end (early October) of the summer 2001. Different letters indicate significant differences in F_v/F_m between treatments at $\alpha < 0.05$ according to the Bonferroni/Dunn test. Values are means and bars indicate ± 1 SE. Abbreviation: ns = not significant.

conditions. By contrast, *P. nigra*, which was the most high light tolerant species, showed the poorest shade acclimation, suggesting a trade-off between sun and shade adaptation (Reich et al. 2003). In the shade, *P. nigra* registered the greatest decrease in root:shoot ratio (42% versus 10–20% in broad-leaf species), together with the smallest reductions in light

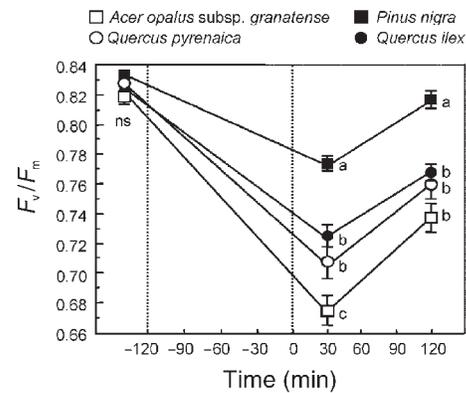


Figure 5: Kinetics of recovery from photoinhibition in saplings grown in 13% sunlight after 2 h of exposure to full sunlight ($n = 18$ per species). The period of exposure to full sunlight is indicated with dotted lines. Different letters indicate significant differences in photochemical efficiency of photosystem II (F_v/F_m) between species at $\alpha < 0.05$ according to the Bonferroni/Dunn test. Values are means and bars indicate ± 1 SE. Abbreviation: ns = not significant.

compensation point and dark respiration rates. As a result, dry mass of *P. nigra* saplings in 13% sunlight was only 41% of that in full sunlight compared with 70% in the other species. These findings are consistent with the view that pines are primarily light-demanding species (Ceballos and Ruíz de la Torre 1979, Nikolov and Helmisaari 1992, Keeley and Zedler 1998).

Although all species reduced their root biomass with decreasing light availability, only *A. opalus* subsp. *granatense* also modified total root length in response to the light treatments. The root length of maple saplings in 80% and 13% sunlight (99.7 ± 8.4 cm and 108.3 ± 10.1 cm, respectively) was almost twice than in the full sunlight treatment (58.2 ± 8.1 cm). This result could have important implications for the natural regeneration of the species in Mediterranean environments, where the combination of shade and drought can severely affect plant performance (Valladares and Pearcy 2002). Under dry, shade conditions a smaller allocation of resources to roots would limit water absorption. However, the construction of longer roots with a higher area–biomass relationship could counteract the smaller investment in root biomass, resulting in high water absorption efficiency in the shade. There-

Table 5. Summary of F -values from an analysis of variance of differences in gas exchange responses between treatments and species. Significance levels: **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$; and * $P < 0.05$. After Bonferroni correction, differences were considered significant at $P < 0.006$. Abbreviations: $A_{\max,area}$ = photosynthetic capacity per unit area; $A_{\max,mass}$ = photosynthetic capacity per unit mass; Φ = apparent quantum efficiency; Θ = convexity; R_{area} = respiration per unit area; R_{mass} = respiration per unit mass; P_S = light saturation point; and Γ = light compensation point.

Factors	$A_{\max,area}$	$A_{\max,mass}$	Φ	Θ	R_{area}	R_{mass}	P_S	Γ
Treatment	0.15	31.86****	1.52	1.50	4.98**	0.38	3.35*	25.07****
Species	11.06****	50.39****	4.47*	2.45	0.92	17.52****	3.46*	1.68
Treatment \times species	4.89**	9.81****	1.51	0.51	1.39	1.54	0.36	4.98**
Model	6.04***	24.81****	2.38*	1.31	1.77	5.69***	1.84	5.88****
R^2	0.61	0.88	0.29	0.09	0.19	0.59	0.21	0.60

Table 6. Summary of F -values from an analysis of variance of differences in gas exchange responses between treatments ($n = 3\text{--}4$ saplings). For the variables that showed a significant treatment \times species interaction (see Table 5), differences (Bonferroni/Dunn test, $\alpha < 0.05$) between treatments within a species are indicated with different letters. Abbreviations: $A_{\max, \text{area}}$ = photosynthetic capacity per unit area ($\mu\text{mol CO}_2/\text{m}^2 \text{ s}$); $A_{\max, \text{mass}}$ = photosynthetic capacity per unit mass ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$); R_{area} = respiration per unit area ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); R_{mass} = respiration per unit mass ($\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$); Φ = apparent quantum efficiency; Θ = convexity; P_S = light saturation point; and Γ = light compensation point.

Variable	Light treatment (%)	Species			
		<i>Acer opalus</i> subsp. <i>granatense</i>	<i>Quercus pyrenaica</i>	<i>Quercus ilex</i>	<i>Pinus nigra</i>
$A_{\max, \text{area}}$	100	7.08 \pm 0.55 a	5.91 \pm 0.35 a	10.33 \pm 2.81 a	16.13 \pm 2.81 a
	80	8.38 \pm 0.17 ab	7.73 \pm 0.42 a	9.14 \pm 1.46 a	15.19 \pm 2.77 a
	13	9.17 \pm 0.38 b	11.6 \pm 0.56 b	5.12 \pm 1.34 a	12.05 \pm 1.15 a
$A_{\max, \text{mass}}$	100	85.66 \pm 6.68 a	62.74 \pm 3.68 a	41.99 \pm 11.41 a	115.76 \pm 11.05 a
	80	95.91 \pm 1.96 a	75.29 \pm 4.08 a	36.55 \pm 5.83 a	97.46 \pm 17.78 a
	13	178.99 \pm 7.38 b	151.89 \pm 7.32 b	29.23 \pm 8.77 a	91.91 \pm 11.88 a
R_{area}	100	-1.77 \pm 0.21	-1.03 \pm 0.05	-2.83 \pm 1.30	-1.49 \pm 0.18
	80	-1.43 \pm 0.10	-1.43 \pm 0.16	-1.52 \pm 0.22	-1.62 \pm 0.23
	13	-0.91 \pm 0.09	-0.79 \pm 0.03	-0.74 \pm 0.32	-1.10 \pm 0.08
R_{mass}	100	-20.32 \pm 2.38	-12.97 \pm 0.09	-11.51 \pm 5.30	-8.47 \pm 1.02
	80	-17.35 \pm 1.22	-13.91 \pm 1.51	-6.07 \pm 0.89	-10.39 \pm 1.48
	13	-17.95 \pm 1.77	-10.17 \pm 0.41	-4.24 \pm 1.82	-10.62 \pm 0.82
Φ	100	0.04 \pm 0.004	0.02 \pm 0.00	0.06 \pm 0.03	0.06 \pm 0.01
	80	0.04 \pm 0.005	0.03 \pm 0.00	0.04 \pm 0.00	0.06 \pm 0.01
	13	0.06 \pm 0.001	0.05 \pm 0.00	0.05 \pm 0.01	0.06 \pm 0.02
Θ	100	0.79 \pm 0.13	0.88 \pm 0.06	0.75 \pm 0.09	0.44 \pm 0.22
	80	0.94 \pm 0.02	0.93 \pm 0.04	0.88 \pm 0.03	0.72 \pm 0.14
	13	0.93 \pm 0.004	0.86 \pm 0.02	0.64 \pm 0.30	0.75 \pm 0.20
P_S	100	249.33 \pm 22.56	304.00 \pm 16.09	234.33 \pm 60.67	320.33 \pm 40.31
	80	236.75 \pm 50.64	262.67 \pm 24.97	241.33 \pm 50.66	307.00 \pm 17.24
	13	177.67 \pm 7.86	270.33 \pm 9.59	136.07 \pm 41.86	244.00 \pm 67.00
Γ	100	43.17 \pm 5.12 a	43.73 \pm 0.24 a	40.33 \pm 3.81 a	39.67 \pm 1.74 a
	80	37.97 \pm 3.63 a	40.73 \pm 4.89 a	34.07 \pm 4.29 a	44.53 \pm 1.42 a
	13	16.2 \pm 1.73 b	21.27 \pm 0.58 b	17.86 \pm 4.23 b	30.65 \pm 8.85 a

fore, the effect of shade on the morphology of *A. opalus* subsp. *granatense* appears to involve the optimization of both light capture (by an increased leaf surface area) and water capture (by an increased root surface area per biomass invested).

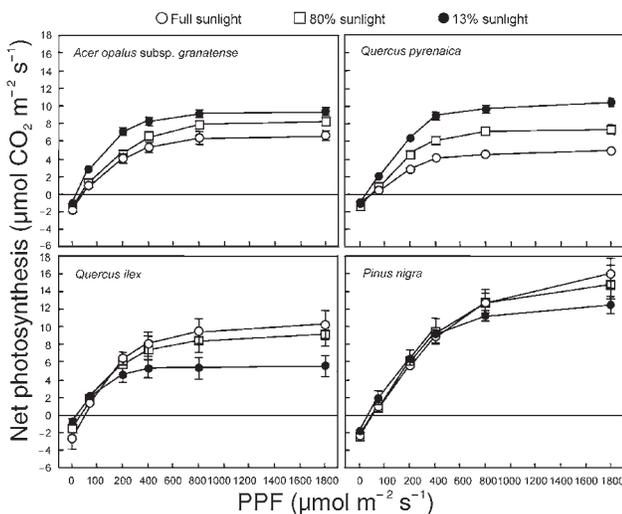


Figure 6. Photosynthetic curves obtained from measurements made in July 2003. Values are mean \pm 1 SE for 3–4 saplings per species and treatment. Abbreviation: PPF = photosynthetic photon flux.

Linking ecophysiology with regeneration niche

The interspecific differences in light responses that we observed provide new insights into the regeneration processes and plant–plant interactions under natural conditions. As hypothesized, the deciduous species were the least tolerant to high radiation, undergoing both dynamic and chronic photo-inhibition in full sunlight, with serious implications for gas exchange and photosynthetic performance. Moreover, the high light intolerance of *A. opalus* subsp. *granatense*, which was manifested not only by poor performance, but also by poor survival under high irradiances, agrees well with the report of greater dependence on nurse relationships for establishment of *A. opalus* subsp. *granatense* compared with *Q. pyrenaica* (Gómez-Aparicio et al. 2005c). Among evergreen species, *P. nigra* showed the greatest capacity to tolerate high irradiances, in agreement with the weak positive effect that nurse shrubs exert on the recruitment of this species under natural conditions (Gómez-Aparicio et al. 2004).

Natural regeneration in understory shaded microhabitats is likely related to the capacity of each species to perform under low-light conditions by means of complex combinations of physiological and morphological responses (Canham 1988, Givnish 1988). Such responses imply strategies of both shade-avoidance and shade-tolerance. Although it has been suggested that these strategies represent alternative, mutually ex-

clusive adaptations to light limitations (Henry and Aarsen 1997), our study species showed a combination of features from each strategy. By using a mixed strategy, sapling growth of all species (except *P. nigra*) was only moderately reduced even in 13% sunlight which is comparable with the natural deep shade found in many managed Mediterranean forests that are characterized by an open structure and the dominance of successional shrubs. Consequently, light-mediated limitation on biomass production under woody canopies, which is the main negative effect of low irradiances reported for nurse relationships in arid and Mediterranean systems (Callaway et al. 1996, Kitzberger et al. 2000, Maestre et al. 2001), seems to be of minor importance for many Mediterranean tree species. Moreover, these light-mediated growth limitations are negligible when compared with the increased survival and reduced photoinhibition (Long et al. 1994, Valladares et al. 2005) resulting from moderate shade.

The interspecific differences in the mechanisms of response to light identified in this study, where variability in other abiotic factors such as water or nutrient variability was eliminated, were entirely in accordance with the differential regeneration patterns previously reported for these four important tree species of Mediterranean mountain forests (Gómez-Aparicio et al. 2004, 2005a, 2005c). Thus, we conclude that light availability is a relevant environmental factor defining the regeneration niche of Mediterranean woody species.

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