# Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species

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**Summary**   We investigated the differential roles of physiological and morphological features on seedling survivorship along an experimental irradiance gradient in four dominant species of cool temperate-Mediterranean forests (*Quercus robur* L., *Quercus pyrenaica* Willd., *Pinus sylvestris* L. and *Pinus pinaster* Ait.). The lowest photochemical efficiency ($F_v/F_m$ in dark-adapted leaves) was reached in deep shade (1% of full sunlight) in all species except *Q. robur*, which had the lowest photochemical efficiency in both deep shade and 100% of full sunlight. Species differed significantly in their survival in 1% of full sunlight but exhibited similar survivorship in 6, 20 and 100% of full sunlight. Shade-tolerant oaks had lower leaf area ratios, shoot to root ratios, foliage allocation ratios and higher rates of allocation to structural biomass (stem plus thick roots) than shade-intolerant pines. Overall phenotypic plasticity for each species, estimated as the difference between the minimum and the maximum mean values of the ecophysiological variables studied at the various irradiances divided by the maximum mean value of those variables, was inversely correlated with shade tolerance. Observed morphology, allocation and plasticity conformed to a conservative resource-use strategy, although observed differences in specific leaf area, which was higher in shade-tolerant species, supported a carbon gain maximization strategy. Lack of a congruent suite of traits underlying shade tolerance in the studied species provides evidence of adaptation to multiple selective forces. Although the study was based on only four species, the importance of ecophysiological variables as determinants of interspecific differences in survival in limiting light was demonstrated.

*Keywords: allocation patterns, chlorophyll fluorescence, conservative strategy, shade tolerance, survival.*

## Introduction

Interspecific differences in juvenile plant responses to irradiance largely explain niche differentiation along successional and productivity gradients (Horn 1971, Bormann and Likens 1979, Shugart 1984, Glizenstein et al. 1986, Pacala et al. 1996). Thus, the analysis of functional traits underlying plant adaptations is critical for the development of an explanatory and predictive vegetation science.

Despite decades of research, no consensus has been reached on the various functional adaptations conferring shade tolerance, partly because of a poor understanding of ontogenetic effects (Niinemets 2006). Earlier hypotheses were based on the assumption that the ability to colonize low light environments was reached by maximizing the net rate of carbon gain in shady environments, following the analogous phenotypic responses exhibited by seedlings acclimated to shade (Givnish 1988). More recently, the ability to colonize low light environments has been linked to enhanced survival in low light rather than enhanced growth in low light, because shade-tolerant species do not necessarily grow faster than shade-intolerant species in the shade but rather they can survive better than shade-intolerant species under such conditions (Kitajima 1994, Kobe et al. 1995, Kobe and Coates 1997). These ideas support the notion that shade tolerance is associated with a conservative resource-use strategy (Veneklaas and Poorter 1998, Walters and Reich 1999). Morphological and physiological characteristics exhibited by shade-tolerant species are in many cases the opposite of those predicted by the net carbon gain maximization hypothesis because traits that maximize net carbon gain, which can be advantageous in high light, might decrease the probability of survival in shade (Kitajima 1994, Veneklaas and Poorter 1998, Walters and Reich 1999). The conflict between these views suggests the need for further studies in a variety of forest-types to examine interspecific variation in functional traits associated with the ability to colonize low light environments.

Our main objective was to investigate interspecific variation in functional traits along an experimental irradiance gradient in seedlings of four forest tree species (*Quercus robur* L., *Q. pyrenaica* Willd., *Pinus sylvestris* L. and *P. pinaster* Ait.) that dominate the overstory in the cool temperate-Mediterranean forest transition zone. The distribution of these species is associated with a productivity gradient, from cool temperate (*Q. robur*) to mesic or montane (*Q. pyrenaica* and *P. sylvestris*) to Mediterranean (*P. pinaster*) forests. Previous studies have shown that these species exhibit different regeneration mechanisms (evaluated as seedling survivorship) along an irradiance

gradient, with oak species being more shade-tolerant than pine species (Sánchez-Gómez et al. 2006). In general, oaks can establish under the canopy of pine stands, and tend to exclude pines later in succession (Lookingbill and Zavala 2000). Pines, however, are likely to persist in frequently disturbed stands, or under stressful (i.e., xeric) conditions (Zavala and Zea 2004). Oaks and pines have different ways of energy capture owing to differences in crown architecture, foliage physiognomy (needles versus broad leaves) and leaf habit (i.e., evergreen pines versus deciduous oaks) all of which can contribute to the differences in their ability to colonize low light environments (Walters and Reich 1999). In this study we focus on the seedling stage because it is the stage at which selective pressures are highest (Reich et al. 2003). For this purpose, seedlings of the four species were grown under controlled conditions in a factorial experiment with irradiance ranging from 1 to 100% of full sunlight. Mortality was monitored from early spring to autumn to account for temporal variation in mortality rates. We examined interspecific variation in key morphological and physiological variables related to either a conservative resource-use strategy or a maximization of net carbon gain strategy (see Table 1). Phenotypic plasticity for leaf- and whole-plant-level traits was included. Phenotypic plasticity can be expected to influence seedling responses along an irradiance gradient because the ability to change the phenotype in response to the environment is a mechanism used by plants to optimize resource acquisition (West-Eberhard 1989, Sultan 1992). The specific hypotheses tested were: (1) species specific differences in shade tolerance and foliage physiognomy during the first year of growth (estimated as survival in 1% sunlight) are correlated with key morphological and physiological variables; (2) shade tolerance in these species is linked to a reduced phenotypic plasticity in response to light; and (3) the extent of plasticity differs among variables, as suggested in previous studies (Valladares et al. 2000*a*, 2000*b*).

## Materials and methods

### Experimental design and study site

The experimental setting was based on a factorial design with irradiance and species as the factors. Seedling of four tree species (*Q. robur*, *Q. pyrenaica*, *P. sylvestris* and *P. pinaster*) were grown outdoors from February until November at a commercial nursery (Viveros Barbol, Torremocha del Jarama, Madrid, Spain) located at 40°50′ N, 3°29′ W and 710 m a.s.l. The climate is continental Mediterranean with hot and dry summers and cold winters. Mean maximum and minimum temperatures are 19 and 9.5 °C, respectively. Most annual rainfall (350–500 mm) is received during spring and fall (250–350 mm) (Instituto-Nacional-de-Meteorología 2001). Soil substrate (pH 6.5) consists of a 3:1 (v/v) mixture of peat Vriezenveen PP1 (Potgrond Vriezenveen B.V., Westerhaar, the Netherlands) and washed river sand. We also added 3 kg m$^{-3}$ of Guanumus Angibaud fertilizer (3,35,2 N,P,K, Angiplant, La Rochelle Cedex, France) and 2 kg m$^{-3}$ of Plantacote mix 4 M fertilizer (15,10,15 N,P,K, Aglukon Spezialdünger GMBH,

Dusseldorf, Germany).

Seeds and acorns were collected from Spanish localities in 2000: *Q. robur* from Galicia, *Q. pyrenaica* from Sierra de Guadarrama, Madrid and *P. sylvestris* and *P. pinaster* from Sierra de Gredos, Ávila. Seedlings were germinated in February and March 2001 under similar environmental conditions and were then transplanted to forest multi-pot (330 cm$^3$ per pot) containers and grown from early spring to autumn. Local air temperature and available photosynthetic photon flux (PPF) were registered every 5 min during the growing season with a data logger (HOBO model H08-006-04, Onset, Pocasset, MA) and external sensors cross-calibrated with a Li-Cor 190SA sensor (Li-Cor, Lincoln, NE). Mean daily PPF over the summer was 42 mol m$^{-2}$ day$^{-1}$. Four irradiance treatments (1, 6, 20 and 100% of full sunlight) were established by using layers of neutral shade cloth supported by metal frames. This gradient spans the natural range of light availability found in Iberian forest understories which can be extremely heterogeneous (Gómez et al. 2004, Valladares 2004). An irradiance of about 20% of full sunlight is commonly found and irradiances of 6% of full sunlight are frequent in humid and sub-humid temperate forests (Canham et al. 1990, Frelich 2002). Irradiances around 1% of full sunlight are representative of the most light-limited end of the natural gradient in Mediterranean forests (Gratani 1997). Air mean temperature during the experiment was similar (± 1 °C, standard error indicated) across irradiance environments. For example, in the hottest month of the year (July), mean (± standard error) temperature varied from 25.66 ± 0.17 °C in the 100% irradiance treatment to 24.43 ± 0.14 °C in the 6% irradiance treatment. At the start of the study, there were 44 seedlings per irradiance treatment and species. Seedlings were arranged along three blocks randomly distributed within each irradiance treatment. Blocks were included in the experimental design to test for homogeneity of irradiance in each light treatment.

### Survival, morphological and physiological measurements

We made five mortality censuses during the experiment. Survival was estimated as the difference between the first and the last censuses at each irradiance. Individuals that had lost all their aerial structure, had no photosynthetically active leaves (i.e., green, flexible leaves) or had a brittle stem (easily broken by finger pressure) at the upper third, were recorded as dead. Over the entire monitoring interval, our mortality criteria were robust as we observed no resprouting in any individuals classified as dead. Seedlings were sprayed with a fungicide solution (50% Carbendazyme, Fungicida Polivalente, COMPO Agricultura SL, Barcelona) twice during the experiment to control fungal infections. None of the mortality events showed signs of a fungal-infection-mediated death.

At the beginning of October, we recorded seedling height (length of the main stem) and collar diameter (± 0.01 mm). Seedling slenderness index was calculated as the ratio of height and collar diameter (Table 1). In vivo chlorophyll fluorescence signals of five plants per species and treatment were measured in September. Measurements were taken around

Table 1. Morphological and physiological variables studied and the rationale for choosing them. Definitions are given where appropriate. Abbreviations: SLA = specific leaf area; LAR = leaf area ratio; and DM = dry mass.

| Variables | Definition | Rationale |
|---|---|---|
| SLA ($m^2 kg^{-1}$) | Leaf area displayed per unit of leaf biomass | Allometric variable related to light capture at the leaf level and stress tolerance |
| LAR ($m^2 kg^{-1}$) | Leaf area displayed per unit of whole plant biomass | Allometric variable related to light capture at the whole plant level |
| Slenderness index ($cm\ mm^{-1}$) | Seedling height divided by collar diameter | Allometric response involved in shade avoidance |
| Shoot/root ratio | Biomass allocated to shoot divided by biomass allocated to root | Allometric response involved in light capture enhancement at the whole plant level |
| Total leaf area ($cm^2$) | – | Growth variable and estimator of allocation to light capture |
| Total biomass (g) | – | Estimator of absolute growth and performance |
| Height (cm) | – | Growth variable also related to shade avoidance |
| Collar diameter (mm) | – | Growth variable and estimator of performance |
| $F_v/F_m$ | Photochemical efficiency | Overall indicator of physiological stress |
| Carotenoids/chlorophylls ratio | Total carotenoids divided by total chlorophylls per unit of leaf biomass | Capacity to dissipate excessive radiation (relative photoprotection) |
| Chlorophyll a/b ratio | Total chlorophyll a divided by total chlorophyll b per unit of leaf biomass | Balance between key photosynthetic pigments involved in light capture |
| Total chlorophylls ($\mu mol\ g_{DM}^{-1}$) | – | Variable related to light capture at the leaf level |
| Total carotenoids ($\mu mol\ g_{DM}^{-1}$) | – | Overall photoprotection and pigments involved in light capture in the shade |

0800–1000 h with a field fluorescence monitoring system (FMS2, Hansatech Instruments Ltd., Pentney, Norfolk, U.K.) equipped with a leaf-clip holder that monitored incident photosynthetic photon flux (PPF) and leaf temperature. Measurements were made on fully developed leaves from the upper third of the crown. Maximal photochemical efficiency of PSII ($F_v/F_m$) was calculated as:

$$F_v/F_m = \frac{F_m/F_0}{F_m} \qquad (1)$$

where $F_m$ and $F_0$ are the maximum and the minimum fluorescence, respectively, measured in leaves after 30 min of dark adaptation. Non-photochemical quenching (NPQ) was calculated as:

$$NPQ = \frac{F_m}{F'_m} - 1 \qquad (2)$$

where $F'_m$ is the maximum fluorescence of the light-adapted sample.

Five leaf samples per species and treatment were taken for determination of chlorophylls and carotenoids. Leaf samples (0.05 g) were incubated in 3 ml of dimethyl sulfoxide at 65 °C for 2 h in the dark. Absorbance of the extracts was measured spectrophotometrically at 663.0, 646.8, 480.0, 435.0 and 415.0 nm and concentrations ($\mu mol\ g_{DM}^{-1}$) of chlorophylls and carotenoids calculated according to Wellburn (1994). Total chlorophyll concentration, chlorophyll a/b ratio, total carotenoid concentration and ratio of carotenoids to chlorophylls were calculated. All seedlings were harvested in mid October and separated into leaves, stems, structural roots (= 2 mm) and fine roots (< 2 mm). Dry mass of each fraction was determined after a minimum of 3 days at 65 ± 2 °C, and the following morphological and structural variables calculated: specific leaf area (SLA, $m^2 kg^{-1}$), leaf area ratio (LAR, $m^2 kg^{-1}$), total leaf area ($cm^2$), total biomass (g), shoot/root ratio, percentage of total biomass allocated to foliar biomass (% foliar biomass), percentage of total biomass allocated to structural biomass (i.e., stems and structural roots, % structural biomass) and percentage of total biomass allocated to fine roots (% fine roots biomass).

*Data analysis*

For each species, cumulative survival in 1% of full sunlight was obtained by Kaplan-Meier analysis. Chi-square test was used to test significant differences in survival. For the other variables, one-way analysis of variance (ANOVA) was used to test for statistical differences between light treatments and species, and the Fisher LSD-test was used for post hoc analysis. Before ANOVA, data were checked for normality and homogeneity of variances, and were log-transformed when nec-

essary to correct deviations from these assumptions (Zar 1999). We performed an analysis of variance (ANOVA) to test for the potential significance of blocks that would reveal heterogeneity in irradiance within a light treatment. Linear correlations were analyzed between survival in a specific light environment and likely predictive variables. Spearman rank correlations were used, because the variables chosen for the correlation analysis did not fit the assumptions of normality and homogeneity of variances. All the statistical analysis was performed with STATISTICA v.6.0 (Statsoft, Tulsa, OK). An index of phenotypic plasticity for selected variables was calculated for each species as the difference between the minimum and the maximum mean values among the different irradiance treatments divided by the maximum mean value. Morphological plasticity was calculated considering only those variables that are standardized for size (i.e., SLA, LAR, slenderness and shoot/root ratio). Physiological plasticity was calculated considering all the physiological variables studied. This phenotypic plasticity index has been used in previous studies (Valladares et al. 2000a, Valladares et al. 2000b, Balaguer et al. 2001), and has the advantage that changes in variables expressed in different units and with contrasting ranges can be compared because it scales from 0 to 1.

## Results

### Survival and morphological variables

The block effect on the response variables studied was not significant ($F = 0.410$, $P = 0.664$). Species differed significantly (Chi-square = 77.07, $P < 0.001$; Figure 1) in survival in 1% of full sunlight, but there were no significant differences between species in the other light treatments. Oaks had higher SLA than pines in all the treatments. In 100 and 20% of full sunlight, the species fully segregated with *Q. robur* showing the
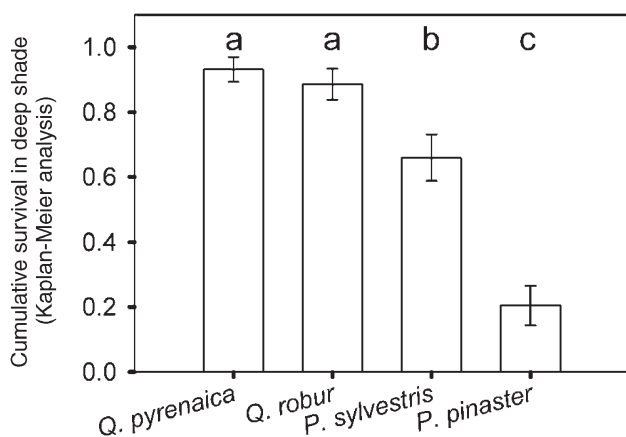


Figure 1. Cumulative survival of seedlings of the four species in deep shade (i.e., 1% of full sunlight). Mean and standard errors derived from Kaplan-Meier analysis are shown. The letter codes indicate homogeneous groups (Cox's *F*-test, $\alpha = 0.05$).

highest SLA, followed in decreasing order by *Q. pyrenaica*, *P. sylvestris* and *P. pinaster* (Table 2). The species did not fully segregate in the 6 and 1% of full sunlight treatments, although *Q. robur* again had the highest SLA followed by *Q. pyrenaica* with both pines exhibiting the lowest SLA (Table 2). Differences between pines and oaks in mean SLA values were lower under shady conditions than under high irradiances (Table 2). Despite *Q. pyrenaica*'s high SLA, this species had the lowest LAR in most of the treatments, especially in 1% of full sunlight (Table 2). Oaks had lower LAR values than pines in 1% of full sunlight, which led to a negative relationship between survival in deep shade (1% of full sunlight) and LAR (Figure 2). Species ranking based on shoot/root ratio was *Q. pyrenaica < Q. robur < P. sylvestris < P. pinaster* for all treatments except for 20% of full sunlight, where the ratios for the two pines were undistinguishable (Table 2). Again, we found a negative relationship between shoot/root ratio and survival in deep shade (Figure 2). *Pinus pinaster* had the highest slenderness index for all treatments, with highest values in the intermediate irradiance treatments. Compared with allocation patterns in deep shade, oaks allocated carbon mainly to structural biomass and much less to leaves and fine root biomass, whereas pines allocated carbon mainly to leaves (Figure 3). Among oaks, *Q. pyrenaica* allocated more to structural biomass than *Q. robur*. Among pines, *P. sylvestris* allocated more to fine root biomass than *P. pinaster* (Figure 3).

### Physiological variables

Values of $F_v/F_m$ were lowest in deep shade (1% of full sunlight) in all species except *Q. robur*, which had the lowest $F_v/F_m$ values in both deep shade and 100% of full sunlight (Table 2). There were no differences in NPQ either among species or among irradiance treatments (Table 2). The carotenoids/chlorophylls ratio did not show a clear trend with decreasing irradiance except in *P. pinaster*, where values were higher in 100% of full sunlight than in 1% of full sunlight (Table 2). A similar pattern was observed for the chlorophyll a/b ratio: only *P. pinaster* exhibited lower chlorophyll a/b ratios in 1% of full sunlight than in 100% of full sunlight. We also found differences among species, with *P. sylvestris* exhibiting the highest ratios of chlorophyll a/b in 1% of full sunlight. Total chlorophyll concentration increased with decreasing irradiance in all species (Table 2). Among species, *P. pinaster*, the most shade-intolerant (based on survival data in deep shade), had the lowest total chlorophyll concentration. Only *P. sylvestris* increased its total carotenoid concentration in response to shade: the other species did not exhibit significant changes in this variable in response to irradiance (Table 2). *Pinus sylvestris* had a significantly higher total carotenoid concentration in deep shade (1% of full sunlight) than *Q. pyrenaica*.

### Phenotypic plasticity

Species differed significantly in their phenotypic plasticity. *Pinus pinaster* exhibited the highest phenotypic plasticity for all types of plasticity examined (i.e., morphological, physiological or overall), whereas *Q. pyrenaica* exhibited the lowest

Table 2. Mean values and standard errors (SE) of the morphological and physiological variables examined. First letter code indicates differences between light treatments and the second letter (between brackets) indicates differences between species (one-way ANOVA, Fisher-test, $P < 0.05$). The ANOVAs were performed with log-transformed variables when neccesary. A total of 30 seedlings per species and light treatment ($n = 30$) were measured for total biomass, height, collar diameter, slenderness index and shoot/root ratio; $n = 15$ for specific leaf area (SLA), leaf area ratio (LAR) and total leaf area; and $n = 5$ for all the physiological variables. Abbreviations: NPQ = non-photochemical quenching; and DM = dry mass.

| Morphological and structural variables | Irradiance | Q. robur | | Q. pyrenaica | | P. sylvestris | | P. pinaster | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| SLA | 100 | 17.926 | 1.345 ab(a) | 10.226 | 0.143 a(b) | 5.708 | 0.034 a(c) | 4.781 | 0.032 a(d) |
| ($m^2\ kg^{-1}$) | 20 | 15.074 | 0.292 b(a) | 11.022 | 0.131 b(b) | 7.616 | 0.117 b(c) | 6.621 | 0.054 b(d) |
| | 6 | 17.906 | 0.322 a(a) | 11.846 | 0.098 c(b) | 9.341 | 0.250 c(c) | 8.804 | 0.020 c(c) |
| | 1 | 17.556 | 0.360 a(a) | 13.596 | 0.146 d(b) | 10.603 | 0.163 d(c) | 11.585 | 0.005 d(c) |
| LAR | 100 | 2.707 | 0.222 a(a) | 1.347 | 0.074 a(b) | 1.566 | 0.045 a(b) | 2.100 | 0.038 a(c) |
| ($m^2\ kg^{-1}$) | 20 | 2.530 | 0.156 a(a) | 1.632 | 0.085 ac(b) | 2.633 | 0.099 b(a) | 3.140 | 0.058 b(c) |
| | 6 | 4.084 | 0.239 b(a) | 2.476 | 0.139 b(b) | 4.217 | 0.162 c(a) | 2.482 | 0.391 c(b) |
| | 1 | 3.121 | 0.251 a(a) | 2.128 | 0.192 c(b) | 5.567 | 0.270 d(c) | 6.650 | 0.302 d(c) |
| Slenderness index | 100 | 54.899 | 2.780 a(a) | 45.153 | 2.750 a(b) | 38.359 | 2.068 a(b) | 66.887 | 2.233 a(c) |
| (cm mm$^{-1}$) | 20 | 51.320 | 2.013 a(a) | 40.205 | 2.176 a(b) | 49.576 | 2.589 b(a) | 119.137 | 4.327 b(c) |
| | 6 | 73.023 | 3.350 b(a) | 42.959 | 3.023 a(b) | 46.997 | 2.402 b(b) | 119.631 | 5.768 b(c) |
| | 1 | 58.025 | 2.534 a(a) | 48.947 | 4.775 a(b) | 51.667 | 2.181 b(ab) | 94.705 | 6.504 c(c) |
| Shoot/root ratio | 100 | 0.613 | 0.029 a(a) | 0.333 | 0.019 a(b) | 0.968 | 0.039 a(c) | 2.002 | 0.084 a(d) |
| | 20 | 0.688 | 0.035 a(a) | 0.357 | 0.018 a(b) | 1.386 | 0.091 b(c) | 3.585 | 0.159 b(c) |
| | 6 | 1.009 | 0.052 b(a) | 0.576 | 0.051 b(b) | 1.926 | 0.129 c(c) | 6.367 | 1.122 c(d) |
| | 1 | 0.865 | 0.045 c(a) | 0.485 | 0.039 b(b) | 2.367 | 0.237 c(c) | 5.892 | 0.836 c(d) |
| Total leaf area | 100 | 192.426 | 21.189 a(a) | 111.295 | 9.701 a(b) | 23.829 | 1.967 b(d) | 64.625 | 4.060 ab(c) |
| (cm$^2$) | 20 | 167.310 | 15.951 a(a) | 116.064 | 10.998 a(b) | 31.390 | 2.098 a(d) | 65.511 | 4.414 a(c) |
| | 6 | 191.251 | 16.946 a(a) | 99.034 | 8.560 a(b) | 23.033 | 1.867 b(d) | 53.769 | 4.494 b(d) |
| | 1 | 101.043 | 10.009 b(a) | 67.172 | 8.350 b(b) | 18.910 | 1.527 b(c) | 36.064 | 3.104 ab(bc) |
| Total biomass | 100 | 6.706 | 0.396 a(b) | 7.912 | 0.413 a(a) | 1.513 | 0.112 a(d) | 3.106 | 0.186 a(c) |
| (g) | 20 | 6.504 | 0.485 a(a) | 6.920 | 0.456 a(a) | 1.219 | 0.074 b(c) | 2.129 | 0.149 b(b) |
| | 6 | 4.741 | 0.337 b(a) | 4.063 | 0.283 b(a) | 0.559 | 0.042 c(c) | 1.118 | 0.095 c(b) |
| | 1 | 3.195 | 0.217 c(a) | 3.129 | 0.209 c(a) | 0.366 | 0.036 d(b) | 0.546 | 0.053 c(b) |
| Height | 100 | 23.448 | 1.147 b(a) | 15.880 | 1.031 a(c) | 8.805 | 0.370 a(d) | 18.968 | 0.766 b(b) |
| (cm) | 20 | 23.724 | 1.292 b(b) | 14.540 | 1.037 ab(c) | 10.140 | 0.525 a(d) | 28.370 | 1.072 a(a) |
| | 6 | 29.476 | 1.437 a(a) | 12.693 | 0.900 b(b) | 7.348 | 0.414 b(c) | 26.652 | 1.465 a(a) |
| | 1 | 22.577 | 1.064 b(a) | 14.223 | 0.837 ab(b) | 5.853 | 0.273 c(c) | 14.750 | 1.395 b(b) |
| Collar diameter | 100 | 0.440 | 0.017 ab(a) | 0.351 | 0.013 bc(b) | 0.238 | 0.008 a(d) | 0.286 | 0.010 a(c) |
| | 20 | 0.460 | 0.018 a(a) | 0.362 | 0.015 c(b) | 0.210 | 0.007 b(d) | 0.247 | 0.010 b(c) |
| | 6 | 0.417 | 0.018 ab(a) | 0.305 | 0.012 a(b) | 0.161 | 0.007 c(d) | 0.223 | 0.008 b(c) |
| | 1 | 0.396 | 0.014 b(a) | 0.317 | 0.013 ab(b) | 0.114 | 0.003 d(d) | 0.157 | 0.012 c(c) |
| Physiological variables | 100 | 0.695 | 0.038 a(a) | 0.831 | 0.010 a(b) | 0.801 | 0.024 ab(b) | 0.755 | 0.019 a(ab) |
| | 20 | 0.804 | 0.014 b(a) | 0.793 | 0.018 ab(a) | 0.819 | 0.033 a(a) | 0.778 | 0.010 a(a) |
| | 6 | 0.804 | 0.014 b(a) | 0.829 | 0.014 a(a) | 0.740 | 0.011 b(b) | 0.727 | 0.027 a(b) |
| | 1 | 0.701 | 0.030 a(a) | 0.695 | 0.061 b(a) | 0.638 | 0.026 c(a) | 0.554 | 0.073 b(a) |
| NPQ | 100 | 1.353 | 0.166 a(a) | 1.685 | 0.083 a(a) | 1.493 | 0.346 a(a) | 1.026 | 0.253 a(a) |
| | 20 | 1.928 | 0.134 a(a) | 1.677 | 0.143 a(a) | 1.411 | 0.193 a(a) | 1.897 | 0.222 a(a) |
| | 6 | 1.831 | 0.241 a(a) | 1.701 | 0.042 a(a) | 1.845 | 0.439 a(a) | 1.684 | 0.230 a(a) |
| | 1 | 1.530 | 0.252 a(a) | 1.658 | 0.268 a(a) | 1.100 | 0.204 a(a) | 0.889 | 0.493 a(a) |
| Carotenoids/ chlorophylls ratio | 100 | 0.563 | 0.095 a(a) | 0.658 | 0.060 ab(ab) | 0.871 | 0.037 a(bc) | 0.946 | 0.037 a(c) |
| | 20 | 0.802 | 0.095 a(a) | 0.729 | 0.037 b(a) | 0.802 | 0.194 a(a) | 0.712 | 0.034 b(a) |
| | 6 | 0.647 | 0.100 a(a) | 0.581 | 0.026 a(a) | 0.788 | 0.087 a(ab) | 0.905 | 0.079 a(b) |
| | 1 | 0.508 | 0.008 a(a) | 0.571 | 0.045 a(a) | 0.686 | 0.024 a(b) | 0.594 | 0.019 b(a) |
| Chlorophyll a/b ratio | 100 | 1.637 | 0.230 a(a) | 2.187 | 0.325 a(a) | 4.489 | 0.176 a(b) | 3.664 | 0.199 c(b) |
| | 20 | 4.100 | 0.902 a(a) | 3.274 | 0.313 b(a) | 3.154 | 0.488 a(a) | 3.165 | 0.272 bc(a) |
| | 6 | 4.757 | 1.854 a(a) | 2.487 | 0.223 ab(a) | 2.338 | 0.019 a(a) | 5.413 | 0.869 a(a) |
| | 1 | 2.033 | 0.057 a(a) | 2.099 | 0.327 a(a) | 2.968 | 0.219 a(b) | 2.218 | 0.122 b(a) |
| Total chlorophylls | 100 | 3.692 | 0.631 bc(a) | 2.704 | 0.179 a(a) | 1.314 | 0.046 a(b) | 0.878 | 0.056 a(b) |
| ($\mu mol\ g_{DM}^{-1}$) | 20 | 3.217 | 0.760 c(a) | 2.863 | 0.217 a(a) | 2.026 | 0.396 ab(ab) | 1.379 | 0.170 ab(b) |
| | 6 | 4.861 | 0.496 ab(a) | 4.239 | 0.340 b(a) | 2.322 | 0.167 b(b) | 1.848 | 0.184 bc(b) |
| | 1 | 7.114 | 0.261 a(a) | 3.734 | 0.230 b(b) | 3.353 | 0.113 c(b) | 2.164 | 0.331 c(c) |
| Total carotenoids | 100 | 3.932 | 0.531 a(a) | 4.133 | 0.466 a(a) | 2.557 | 0.330 a(b) | 3.093 | 0.294 a(ab) |
| ($\mu mol\ g_{DM}^{-1}$) | 20 | 3.299 | 0.550 a(a) | 4.092 | 0.366 a(a) | 2.980 | 0.730 a(a) | 4.737 | 0.875 a(a) |
| | 6 | 3.875 | 0.536 a(a) | 3.514 | 0.436 a(a) | 3.841 | 0.525 ab(a) | 3.844 | 0.880 a(a) |
| | 1 | 3.780 | 0.617 a(ab) | 3.251 | 0.515 a(a) | 4.918 | 0.452 b(b) | 4.088 | 0.511 a(ab) |
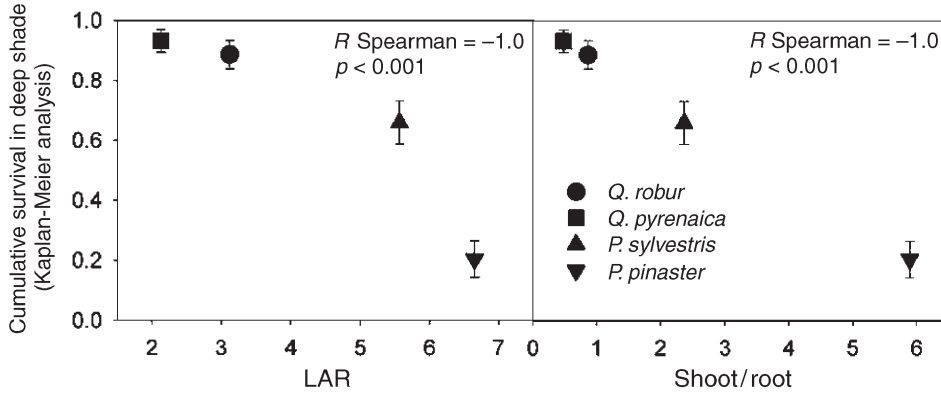
Figure 2. Spearman rank correlation between cumulative survival in deep shade, leaf area ratio (LAR) and shoot/root ratio in seedlings of the four species studied. Standard errors are shown as error bars.
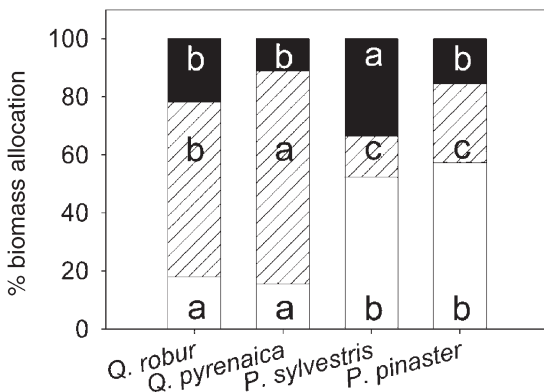


Figure 3. Stacked bars for percentage of biomass allocation to foliar biomass, structural biomass and fine root biomass in deep shade (1% of full sunlight). Letter codes indicate homogeneous groups (ANOVA, Fisher-test, $P < 0.05$) among species for each biomass compartment. Open areas represent % of biomass allocated to leaves, closed areas represent % of biomass allocated to fine roots and hatched areas represent % of biomass allocated to structure.

values for all types of plasticity considered (Table 3). Phenotypic plasticity to light was related to survival in 1% of full sunlight, but not in the other light treatments (data not shown). Global plasticity and morphological plasticity were significantly and inversely related to survival in deep shade ($R$ Spearman = –1, $P < 0.001$; Figure 4). Physiological plasticity was unrelated to survival in deep shade ($R$ Spearman = –0.8, $P = 0.333$), so the relationship between overall plasticity and survival in shade was due to the trend in morphological plasticity.

## Discussion

### Interspecific differences in ecophysiological traits and performance in low light

Interspecific differences in morphological and physiological traits along an experimental irradiance gradient were significant for the four species studied. Recent studies of seedlings have shown that shade tolerance is linked to conservative carbon allocation patterns that sacrifice growth potential in favor of persistence (Veneklaas and Poorter 1998, Walters and Reich 1999, Reich et al. 2003). That is, shade-tolerant species exhibit long-lived leaves of low SLA, low LAR, low shoot/root ratio and low respiration rates (Reich et al. 2003) but not necessarily traits associated with high carbon uptake efficiency.

In agreement with a conservative resource-use strategy, shade-tolerant oaks in our study had lower LAR, lower slenderness index and lower shoot/root ratio than shade-intolerant pines. In general, the ability to colonize low light environments is expected to be linked to reduced biomass turnover (Walters and Reich 1999) and high storage allocation (i.e., structural biomass: stems and thick roots). However, the mechanisms underlying this ability can vary among species depending on leaf habit. Low leaf allocation has been identified as a mechanism to minimize turnover in seasonally deciduous species (e.g., the oaks in this study), whereas evergreens achieve shade tolerance through long leaf life-spans (Walters and Reich 1999). Low leaf allocation and high storage allocation are traits linked to protection from aerial mechanical damage and persistence during periods of non-positive carbon uptake balance (Canham et al. 1999, Sack et al. 2003). We evaluated physiological performance in low light under con-

Table 3. Morphological, physiological and overall phenotypic plasticity to light of each species. Letter codes indicate homogeneous groups (ANOVA, Fisher-test $P < 0.05$). Overall plasticity was calculated as the average plasticity for all variables (i.e., morphological (only those standardized for size, e.g., slenderness, specific leaf area, leaf area ratio and shoot/root ratio) plus physiological variables).

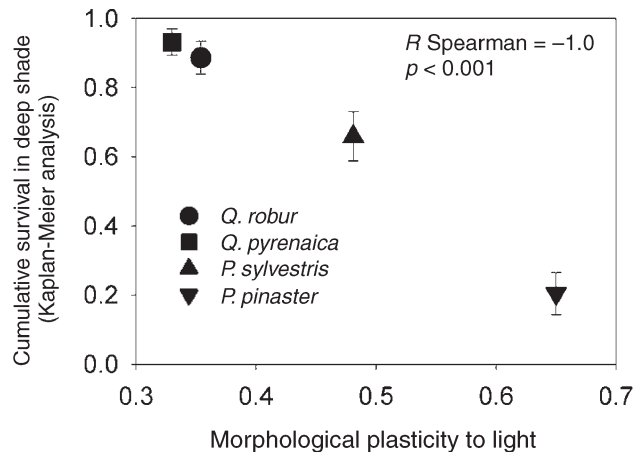|  | P. pinaster | P. sylvestris | Q. robur | Q. pyrenaica |
|---|---|---|---|---|
| Morphological plasticity to light | 0.651 a | 0.532 ab | 0.355 b | 0.327 b |
| Physiological plasticity to light | 0.531 a | 0.440 ab | 0.467 a | 0.225 b |
| Overall plasticity to light | 0.591 a | 0.486 ab | 0.411 bc | 0.276 c |

Figure 4. Spearman rank correlation between cumulative survival in deep shade and morphological plasticity to light in the seedlings of the four species studied. Standard errors are shown as error bars.

trolled conditions which partially limits the extrapolation of the results to shade tolerance under real field conditions. Nevertheless, the observed interspecific differences in performance in low light, evaluated in terms of survival, agreed with forestry knowledge on shade tolerance and with field observations in this region (Ruiz de la Torre 2001). The trends we found for SLA did not support the conservative resource-use hypothesis that predicts that shade-tolerant species have thick, long-life, low-nitrogen content leaves with low SLA (Veneklaas and Poorter 1998, Walters and Reich 1999, Reich et al. 2003). Contrary to this prediction, shade-tolerant oaks in our study exhibited higher SLA than shade-intolerant pines. A xeromorphic structure with a semi-cylindrical shape and a thick cuticle largely explains the lower SLA of pine needles compared with oak leaves (Fahn 1990, Reich et al. 1992). Also the high chlorophyll a/b ratio in shade-intolerant species is associated with the specific structural features of xeromorphic pine needles (Hansen et al. 2002). Variation in SLA along the irradiance gradient was moderate for *Q. robur*, contrasting with other studies (Niinemets 1997, Valladares et al. 2002*b*) showing high variation in this variable. Such discrepancies could be explained by different local adaptations among the populations studied and also by differences in plant age (i.e., seedlings in our study, saplings or adults in the cited studies). The first explanation suggests that caution must be taken when generalizing results to different populations of these species. The second explanation raises an important topic because not only do allocation patterns of species change with ontogeny but also the way plants respond to light during the early stages of development depends on the rate of ontogenetic change (Niinemets 2006). Moreover, correlations and constraints identified at early stages may weaken during later stages of growth when the linkage with seed size dissipates, leading to species crossovers in relative growth rates (Sack and Grubb 2001, Kitajima and Bolker 2003, Sack and Grubb 2003, Baraloto et al. 2005). Studies with seedlings have obvious limitations. Although germination and seedling establishment are

major demographic bottlenecks in Mediterranean ecosystems, further studies on saplings, juveniles and adults are required to gain a better understanding of the mechanisms underlying interspecific differences in performance along environmental gradients.

### Phenotypic plasticity and performance in low light

Shade-tolerant oaks in our study exhibited relatively low phenotypic plasticity regardless of the type of variable considered (e.g., physiological or morphological), indicating that plasticity was similar at the different organization levels considered (Robinson and Rorison 1988, Balaguer et al. 2001). Plasticity in shade-intolerant pines, particularly elongation in low light, is part of an avoidance mechanism rather than a persistence mechanism, and includes costs (sensu DeWitt et al. 1998) that prevent these species from surviving in low light. Low plasticity has been associated with a conservative resource-use strategy in Mediterranean oaks (Valladares et al. 2002*a*), and was found to be part of the shade-tolerance syndrome in tropical shrubs (Valladares et al. 2000*b*). Our findings agree with the proposal of Henry and Aarssen (1997) that phenotypic plasticity is adaptive only in successionally intermediate species but not in late successional species that cope well with deep shade. Our data also support the idea that reduced plasticity in response to light is part of a general suite of traits linked to a conservative use of resources and a high tolerance to low light stress (Grime and Mackey 2002).

Although plasticity of true shade-tolerant species (as opposed to plants that can be occasionally found in the shade) is lower than that of plants encountered in full sunlight (Valladares et al. 2000*a*, Grime and Mackey 2002, Valladares et al. 2005), there is a lack of consensus regarding plasticity of the different types of variable. Physiological plasticity (i.e., plasticity in traits related with gas exchange, photochemical efficiency and water relations) has been linked to an enhanced capacity to colonize gaps and grow in high irradiances, whereas morphological plasticity has been linked to an enhanced capacity to survive and grow in the understory (Valladares et al. 2002*b*, Niinemets and Valladares 2004). However, we have found a negative relationship between morphological plasticity and survival in deep shade, which agrees with the general expectation of reduced plasticity in shade-tolerant species but not with the prediction of enhanced morphological plasticity in understory plants. This discrepancy cannot be resolved by considering the particular morphological variable or variables studied in each case. In our study, plasticity either in those variables involved in shade avoidance (e.g., elongation) or in those variables enhancing light capture (e.g., SLA and LAR) was negatively related to enhanced performance in low light. More studies are needed to confirm the trend of reduced general plasticity in shade-tolerant plants across contrasting phylogenetic groups.

Finally, discrepancies among suites of traits in relation to a given functional strategy indicate that interpretation of adaptive traits along a unique evolutionary axis must take into account adaptations in response to multiple selective forces as well as phylogenetic differences among species. Despite the

limitations of our study—the inclusion of only four species and the confounding effects of shade tolerance and phylogeny—the results reveal the importance of ecophysiological variables as determinants of interspecific differences in seedlings survival in limiting light and provide relevant information for four important tree species that dominate the overstory along the cool temperate-Mediterranean forest transition zone in this region.

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