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Relating leaf photosynthetic rate to whole-plant growth: drought and shade effects on seedlings of four *Quercus* species

José L. Quero[A,B,E,F], *Rafael Villar*[B], *Teodoro Marañón*[C], *Regino Zamora*[A], *Dolores Vega*[B] *and Lawren Sack*[D]

[A]Grupo de Ecología Terrestre, Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain.
[B]Área de Ecología, Facultad de Ciencias, Universidad de Córdoba, 14071 Cordoba, Spain.
[C]Instituto de Recursos Naturales y Agrobiología, CSIC, PO Box 1052, 41080 Seville, Spain.
[D]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606, USA.
[E]Present address: Forest Ecology and Forest Management Group, Centre for Ecosystem Studies, Wageningen University, PO Box 47, 6700 AA Wageningen, The Netherlands.
[F]Corresponding author. Email: jose.queroperez@wur.nl

Abstract. Understanding the impacts of combined resource supplies on seedlings is critical to enable prediction of establishment growth, and forest dynamics. We investigated the effects of irradiance and water treatments on absolute growth, and relative growth rate (RGR) and its components, for seedlings of four *Quercus* species differing in leaf habit and with a wide variation in seed mass. Plants were grown for 6.5 months at three levels of irradiance (100, 27, and 3% daylight), and treated during the last 2.5 months with two watering treatments (frequent watering *v.* suspended watering). Both shade and drought reduced seedling growth rates, with a significant interaction: under full irradiance the drought treatment had a stronger impact on RGR and final biomass than under deep shade. For three species, seed mass was positively related to absolute growth, with stronger correlations at lower irradiance. The evergreen species grew faster than the deciduous species, though leaf habit accounted for a minor part of the interspecific variation in absolute growth. Seedling biomass was determined positively either by RGR or seed mass; RGR was positively linked with net assimilation rate (NAR) and leaf mass fraction (LMF), and seed mass was negatively linked with RGR and LMF, but positively linked with NAR. Seedling RGR was not correlated with light-saturated net photosynthetic rate, but was strongly correlated with the net carbon balance estimated, from photosynthetic light-response curves, considering daily variation in irradiance. These findings suggest an approach to applying short-term physiological measurements to predict the RGR and absolute growth rate of seedlings in a wide range of combinations of irradiance and water supplies.

Additional keywords: biomass allocation, carbon balance, growth analysis, leaf habit, Mediterranean oak, relative growth rate, seed mass, specific leaf area.

Introduction

Seedlings' rates of growth and survival are strongly determined by irradiance (e.g. Poorter 2001; Montgomery 2004; Sánchez-Gómez *et al*. 2006), and water availability (Grant *et al*. 2005; Matthes and Larson 2006; Engelbrecht *et al*. 2007). Recently, there has been strong interest in the combined effects of irradiance and water on plant performance across scales, from leaves (Aranda *et al*. 2005; Quero *et al*. 2006), to plants (Sack and Grubb 2002; Sack 2004; Sánchez-Gómez *et al*. 2006), to communities (Zavala and de la Parra 2005). This understanding is critical as droughts are occurring with increasing severity (Piñol *et al*. 1998; De Luís *et al*. 2001; Peñuelas *et al*. 2002); climate change scenarios predict a 20% decrease in rainfall in Mediterranean areas in the next century (IPCC 2007). Irradiance and water supplies may vary strongly even at the meter or centimetre scale (Maestre *et al*. 2003; Quero 2006), driving coexistence of different species (Montgomery and Chazdon 2002; Valladares 2003; Sack 2004). Indeed, in different natural systems, irradiance and water supplies may vary independently, or they may be correlated positively or negatively (e.g. Abrams and Mostoller 1995; Valladares and Pearcy 2002; Niinemets and Valladares 2006). Given this complexity, we need to understand how plant responses to drought can vary across irradiances. Some authors have hypothesised that the impact of drought should be stronger in deeper shade (Smith and Huston 1989; Aranda *et al*. 2005). However, studies have shown that for leaf-level physiology and for absolute plant growth and survival, the impact of drought is typically reduced in shade (e.g. Canham *et al*. 1996; Holmgren 2000; Sack and Grubb 2002; Sack 2004). In a previous

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paper, we analysed the responses in seedlings' leaf structure and physiology for four Mediterranean *Quercus* species under controlled conditions (Quero *et al*. 2006) and found that the impact of drought on photosynthetic rate was strongest in high irradiance. In this contribution, our main objective is to examine the responses at whole-plant level, after 6.5 months of seedling growth in a combination of irradiance and water supplies.

First, we aimed to ascertain the variation in absolute growth and in relative growth rate (RGR) across species, irradiances and water supplies. We also analysed the effects of irradiance and water on growth components, distinguishing morphological traits: i.e. specific leaf area (SLA, leaf area/mass) and leaf mass fraction (LMF, leaf/plant mass), which together drive leaf area ratio (LAR, leaf area/plant mass; $\mathrm{LAR} = \mathrm{SLA} \times \mathrm{LMF}$), and physiological traits, i.e. the net assimilation rate (NAR), which reflects the balance of photosynthetic rate against respiration and tissue loss rates. It has been generally found that under low irradiance NAR decreases (Poorter 1999, 2001), and increases in SLA and LAR may play a compensatory role, partly associated with reduction of the root mass fraction (RMF, root/plant mass; Walters and Reich 1999). We tested the degree of such compensation across irradiance × water supplies.

Second, we determined the degree to which the influence of seed mass on seedling growth and morphology persisted across irradiance and water supplies. Large-seeded species such as *Quercus* strongly depend on seed reserves for their initial development, especially in deeper shade [as shown previously by analysis of short-term (50 days) seedling growth (Quero *et al.* 2007)]. Here, we tested for a middle-term influence (6.5 months) on seedling growth, and its variation across irradiance and water supplies.

Third, we determined whether leaf habit had any effect on absolute growth rates or RGR and its components. As a general trend, deciduous species tend to grow faster than evergreen species because on average they have higher stomatal conductance, photosynthetic and respiration rates, leaf nitrogen concentration, and SLA (Reich *et al*. 1992; Villar *et al*. 1995; Cornelissen *et al*. 1996; Takashima *et al*. 2004). Here, we compared two evergreens and two deciduous *Quercus* species.

Fourth, we investigated the causes of differences in RGR and absolute growth rate using correlations and causal models. We analysed whether the differences in RGR arose from variation in morphological traits, such as SLA, LMF or LAR or from changes in physiological traits as NAR. Across species SLA can strongly drive interspecific variation in RGR (Poorter and Remkes 1990; Marañón and Grubb 1993; Antúnez *et al*. 2001; Villar *et al*. 2004; Ruiz-Robleto and Villar 2005). However, several studies have shown that variation in NAR may increase in importance during ontogeny and at higher irradiance (Sack and Grubb 2001; Shipley 2002; Montgomery and Chazdon 2002; Villar *et al*. 2005; Shipley 2006); thus, we tested the importance of NAR on RGR variation across irradiance and water supplies.

Finally, we aimed to determine the ability to predict from photosynthetic leaf responses the whole-seedling RGR. This is critical for a predictive ecophysiology, and has been previously demonstrated for predicting RGR across species (e.g. Kruger and Volin 2006). Our study provides a model relating leaf gas-exchange rates to whole-plant growth for woody seedlings in a range of irradiance and water supplies.

Materials and methods

Experimental design

Four oak species, two evergreens [*Quercus ilex* subsp. *ballota* (Desf.) Samp.and *Quercus suber* L.] and two deciduous (*Quercus canariensis* Willd. and *Quercus pyrenaica* Willd.), (nomenclature follows Amaral 1990) were used in this study. Acorns of each oak species were collected in forests of southern Spain (as described in Quero *et al*. 2006). Single acorns were weighed individually and sown on December 2002 in 3.9-L pots (one acorn per pot). We subsampled acorns of each species, separated these into seed and shell, and weighed seeds fresh and after drying at 70°C for at least 48 h. We estimated initial seed dry mass for each individual from the seed fresh mass, using species-specific regression equations (R^2 between 0.93 and 0.99; Quero *et al*. 2007).

The experiment was performed in a greenhouse at the University of Córdoba (Spain, 37°51′N, 4°48′W) with an automatic irrigation system and regulated air temperature. Seedlings were subjected to six combinations of irradiance and water treatments (three levels of irradiance × two levels of water). We used a split-plot design with three irradiance treatments: high-irradiance (HI) receiving full irradiance (no shade frame; 100% irradiance), medium-irradiance (MI; 27% irradiance) and low-irradiance (LI; 3% irradiance) imposed by using shade frames ($1.5 \times 1.2 \times 2$ m); the treatments were replicated four times, resulting in 12 shade-frame blocks in total. Plants of each of the four oak species to be subjected to each of the two levels of watering were randomly arranged within each shade-frame block. Water treatments were imposed in late April 2003: half the pots were not watered further (LW, low-water treatment) while the other half were kept continuously moist (HW, high-water treatment; Quero *et al*. 2006). The low-water treatment simulated a typical Mediterranean-climate seasonal drought, in which after rains stop the soil dries out to the levels achieved at the end of the experiment (Gómez-Aparicio *et al*. 2005). Irradiance levels simulated the range of typical values experienced in the forest understorey where oak seedlings grow (Marañón *et al*. 2004). Because the watering supplies were not applied differently across irradiances, we cannot exclude an interaction between water supply and irradiance (see Abrams *et al*. 1992; Sack 2004), especially at the beginning of the experiment, such that the soil dried faster in the high irradiance treatments, as found in some natural systems, e.g. early in the drought in gaps *v.* understorey in some forests (Ellsworth and Reich 1992). By the end of the experiment, the soil water content was similar across irradiances (Quero *et al*. 2006). This interaction does not affect the testing of hypotheses according to the objectives of our experiment (see Introduction).

We conducted an initial and final harvest of seedlings. The initial harvest was conducted in late April 2003 before the beginning of the drought treatment, by which time the seedlings had grown 4 months in the different irradiances; 15–17 seedlings per species and irradiance treatment were harvested. The second harvest was conducted in late July 2003, by which time the seedlings had grown ~6.5 months; 12–16 seedlings per species per irradiance and water treatment combination were harvested, evenly among the 12 shade-frame blocks. During each harvest, individual seedlings were carefully extracted from each pot, the soil was completely washed away from the roots, and leaves, stems and roots were separated. Leaves were scanned, and leaf area was measured using image analysis software (Image Pro-Plus v 4.5 Media Cybernetic Inc., Bethesda, MD, USA). Dry mass of the plant parts was determined after oven-drying at 70°C for at least 48 h. Specific leaf area (SLA) was calculated as leaf blade area/dry blade mass (Garnier *et al.* 2001), and root mass fraction (RMF), stem mass fraction (SMF), and leaf mass fraction (LMF) were calculated as the dry mass of root, stem and leaves, respectively, divided by seedling dry mass. Acorn remains were excluded from calculations.

Analysis of shade and drought effects at the end of the experiment

For each species, we tested the effects of shade and drought on absolute growth (final biomass) and biomass allocation at the end of experiment. We analysed the data with general linear models (GLM), considering the initial seed dry mass as a covariate, and considering irradiance and water treatments, and shade-frame block as categorical factors. As shade-frame block had no effect on any variable measured ($P > 0.05$ in all cases), we removed this factor and its interactions from the analyses (Zar 1984). We also analysed the data for the four species together testing for an effect of leaf habit (evergreen and deciduous), using GLM with species nested within leaf habit. Before the analysis, data were square-root-, arcsine-, or log-transformed to achieve normality and homoscedasticity (Zar 1984). Statistical analyses were performed with Statistica v 7.1 (Statsoft Inc., Tulsa, OK, USA). To control the inflation of type I error with repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was controlled at 5% using a standard step-up procedure (García 2004).

Calculations of growth components

Relative growth rate (RGR) during the growth period between harvests (~3 months), and its components (NAR, LAR, SLA, LMF, SMF and RMF) were calculated following the classical approach (Hunt *et al.* 2002; http://aob.oxfordjournals.org/cgi/content/full/90/4/485/DC1, accessed 31 March 2005).

Determinants of RGR and final biomass

To determine the causes of variation in RGR and final biomass across the four species grown in a wide variation of irradiance and water supplies we used two approaches. First, we applied Pearson correlation analyses to each pair of variables. However, these bivariate relationships cannot differentiate direct or indirect covariation between variables. Thus, we conducted a multivariate analysis, using Shipley's 'd-sep' method (Shipley 2000), to test different models to explain how differences in RGR and absolute growth may be caused by seed mass, biomass allocation and net assimilation rate.

Calculations of net carbon gain

Whole-plant level growth responses were calculated from leaf-level carbon gain following a similar approach to that by Holmgren (2000). Leaf-level net photosynthetic carbon assimilation was estimated using the model by Thornley (1976):

$$\mathrm{A(I)} = \frac{\Phi \mathrm{I} + A_{\max} - \sqrt{(\Phi \mathrm{I} + A_{\max})^2 - 4\theta \mathrm{I} A_{\max}}}{2\theta} - R_{\mathrm{d}}, \quad (1)$$

where A is the photosynthetic assimilation rate; I, the photosynthetic active radiation (PAR); Φ, the apparent quantum yield; $A_{\max}$, the light-saturated photosynthetic assimilation rate; R_{d}, the leaf dark respiration rate; and θ, the 'bending degree' or curvature. We used the light curve parameters (Φ, $A_{\max}$, R_{d}, and θ) calculated for 3–6 leaves of seedlings of each species in each irradiance and water treatment, as reported by Quero *et al.* (2006). During the growth experiment, the photosynthetic photon-flux density (I) outside the greenhouse was measured each hour with a light sensor (Net Radiometer, Lambrecht, Germany). The shading obstruction (SO) of the greenhouse superstructure was calculated using hemispherical photography. Photographs were taken above seedlings using a horizontally-leveled digital camera (CoolPix 995 digital camera, Nikon, Tokyo, Japan) aimed at the zenith, using a fish-eye lens with 180° field of view (FCE8, Nikon). Hemispherical photographs were analysed with Hemiview canopy-analysis software v 2.1 (1999, Delta-T Devices, Cambridge, UK). Two of the irradiance treatments were applied using a green screen with different levels of light transmittance (LT) (27% of available radiation for MI and 3% for LI, quantified by a light PAR sensor EMS7, PP-systems, Hitchin, UK). The photosynthetic active radiation (PAR) received by each individual was calculated as $\mathrm{I} \times \mathrm{SO} \times \mathrm{LT}$ each hour from 22 April to 16 July. Leaf net carbon gain was modelled using the photosynthetic light curve (Eqn 1) and the PAR received each hour for each plant where photosynthesis rate was measured. Leaf R_{d} values were considered as constant and were applied to the carbon gain during the night; we did not consider root and stem respiration rates. Values of leaf net carbon gain for the whole growth period were calculated for given leaves and averaged for each species and treatment. These values were correlated with RGR for each species and treatment. Another approach was to estimate the leaf carbon gain per unit of plant mass. Therefore, the resulting leaf net carbon gain values of each leaf were multiplied by LAR values (of the final harvest) per individual seedling to scale from a leaf area basis to a whole-plant mass basis. This estimate involves some level of uncertainty, as it neglects differences among leaves on given plants in irradiance availability, in dark respiration, or in photosynthetic rate, and does not consider differences across treatments in root or stem respiration rates. This estimate of net carbon gain was averaged for each irradiance and water treatment and was tested for correlation with RGR in each treatment. Additionally, RGR values for each irradiance and water treatment were tested for correlation with $A_{\max}$ on an area and mass basis (taken from Quero *et al.* 2006).

Results

Effects of irradiance and water supplies on absolute growth, and on RGR and its components

Species differed in biomass at the end of the experiment, ranging from *Q. suber* (mean of 7.0 g) to *Q. canariensis* (mean of 2.0 g) (Fig. 1*A* and Table S1 in the accessory publication available from the online version of *Functional Plant Biology*). All species increased strongly in biomass with higher irradiance (explaining 64–86% of variance; Table 1; Fig. 1*A*). In contrast, the watering treatments had a relatively small effect on seedling biomass (explaining 3.4% of variance for *Q. pyrenaica* down to 0% for *Q. canariensis*).

Seedlings of *Q. suber* alone, and of all species together showed a significant irradiance × water interaction because the drought treatment had a stronger impact on both absolute growth and RGR under full irradiance (Fig. 1*A*), but a negligible impact under low irradiance (Fig. 1*A*; Table 1). However, the irradiance-water interaction explained only 0.6% of the variance in final biomass.

Just as for absolute growth, the low irradiance and water supply depressed seedling RGR (Fig. 1*B*). The RGR reduction was very strong from medium to low irradiance (RGR declined by 61% in *Q. canariensis* and up to 99% in *Q. pyrenaica*), and weaker from high to low water supply, averaged across irradiances (no decline for *Q. canariensis* and up to a decrease of 38% in the case of *Q. pyrenaica*). The impact of the drought treatment on RGR was strong under full irradiance conditions, but negligible under deep shade (Fig. 1*B*).

For all species the response of net assimilation rate (NAR) to irradiance paralleled that of RGR (Fig. 1*C*), declining strongly under low irradiance (by 92% on average). Averaged across irradiances, drought-treated plants showed an 18% lower NAR than well watered plants. As for RGR, the impact of the drought treatment on NAR was negligible under deep shade. By contrast, the leaf area ratio (LAR) increased at lower irradiance (Fig. 1*D*), and was in general unaffected by drought (Fig. 1*D*; Table 1). LAR is the product of the specific leaf area (SLA) and the leaf mass fraction (LMF). Oak seedlings responded to shade by increasing SLA (Fig. 1*E*; Table 1), while LMF remained relatively stable across treatments (Fig. 2*A*; Table 1). However, under deep shade, oak seedlings showed an increased SMF (Fig. 2*B*; Table 1), and a reduced RMF (Fig. 2*C*) relative to seedlings under full irradiance. The two evergreen species (*Q. suber* and *Q. ilex*) increased their RMF in the drought treatment (Fig. 2*C*; Table 1).

Effect of seed mass on seedling growth

Species varied in the importance of seed mass determining absolute growth after 6.5 months of growth (Table 1), ranging from a very strong dependence for *Q. canariensis*, the species with smallest seedlings, to almost complete independence for *Q. pyrenaica*, the species with largest seedlings. On average, seed mass contributed significantly to the differences among seedlings in absolute growth (Table 1; Fig. 3). There was a strong effect of irradiance on the importance of seed mass as a driver of absolute growth. The correlation coefficient of seed mass and final seedling biomass was higher at low irradiance (LI) than at higher irradiance (MI and HI) for all species but *Q. pyrenaica* (Fig. 3*D*). The proportion of variance of final seedling biomass explained by seed mass was as high as 80% under low irradiance but near zero under high irradiance. The relationship of seedling biomass and seed mass was consistent between the two watering treatments (Fig. 3).

Association of leaf habit with absolute growth and RGR

A significant association of leaf habitat with absolute growth across species and treatments was found, explaining 7% of variance. Leaf habit also had a significant effect on final biomass; evergreen species had a higher final biomass than deciduous species, but leaf habit explained only 1% of variance in final biomass (Table 1; Fig. 1*A*). Deciduous species had higher SLA but lower LMF than evergreens (Table 1; Figs 1*E*, 2*A*); as LAR is equal to SLA × LMF, no differences in LAR were found between both functional groups (Table 1; Fig. 1*D*).

Determinants of RGR and final biomass

Strong correlations were found across all species and treatments among final seedling biomass, RGR, growth components and seed mass (Table 2). Final biomass was positively correlated with seed mass, RGR and NAR, but negatively with SLA and LAR. Variation in RGR was explained mainly by changes in NAR, and not by modifications of LAR (Fig. 4*A*, *B*). The variation in LAR was explained both by SLA ($r = 0.64$, $P < 0.001$) and by LMF ($r = 0.48$, $P < 0.05$). Across all species and treatments, SLA was negatively correlated with NAR ($r = -0.68$, $P < 0.001$) and with RGR ($r = -0.64$, $P < 0.001$, Fig. 4*C*), mainly due to SLA increasing, and NAR and RGR both decreasing in the shade relative to the high light treatments. Seedling RGR was negatively correlated with SMF, but did not show any relation with LMF or RMF. The SMF and LMF were positively inter-correlated and both were negatively correlated with RMF (Fig. 4*D*). Seed mass was negatively correlated with LMF ($r = -0.77$, $P < 0.001$).

When the two water treatments were analysed separately, the results proved to be very similar (data not shown), with the exception that under the drought treatment, the negative relationships between RGR with SLA and SMF were non-significant.

We tested different causal models linking final seedling biomass, RGR, growth components and seed mass following the d-sep method of Shipley (Shipley 2000; see Fig. S1; Table S2). For brevity, here we show the only model that cannot be rejected at the 5% level of significance among all of those tested ($\chi^2 = 15.62$, 2 d.f., $P = 0.209$, Fig. 5; see Fig. S1; Table S2 for a complete list of alternative models). According to this model, the final seedling biomass is directly, positively determined by RGR and seed mass. In turn, a higher RGR is directly determined by high NAR and higher LMF. There was a direct negative influence of SLA on NAR, and a direct negative influence of SLA on final biomass, not mediated by NAR and RGR. In contrast, seed mass directly, negatively affected RGR and LMF, but had a positive effect on NAR.

Leaf carbon balance and seedling growth

We did not find any correlation between seedling RGR and maximum photosynthetic rate on an area basis or on a mass basis (Fig. 6*A*). However, RGR was positively related to average

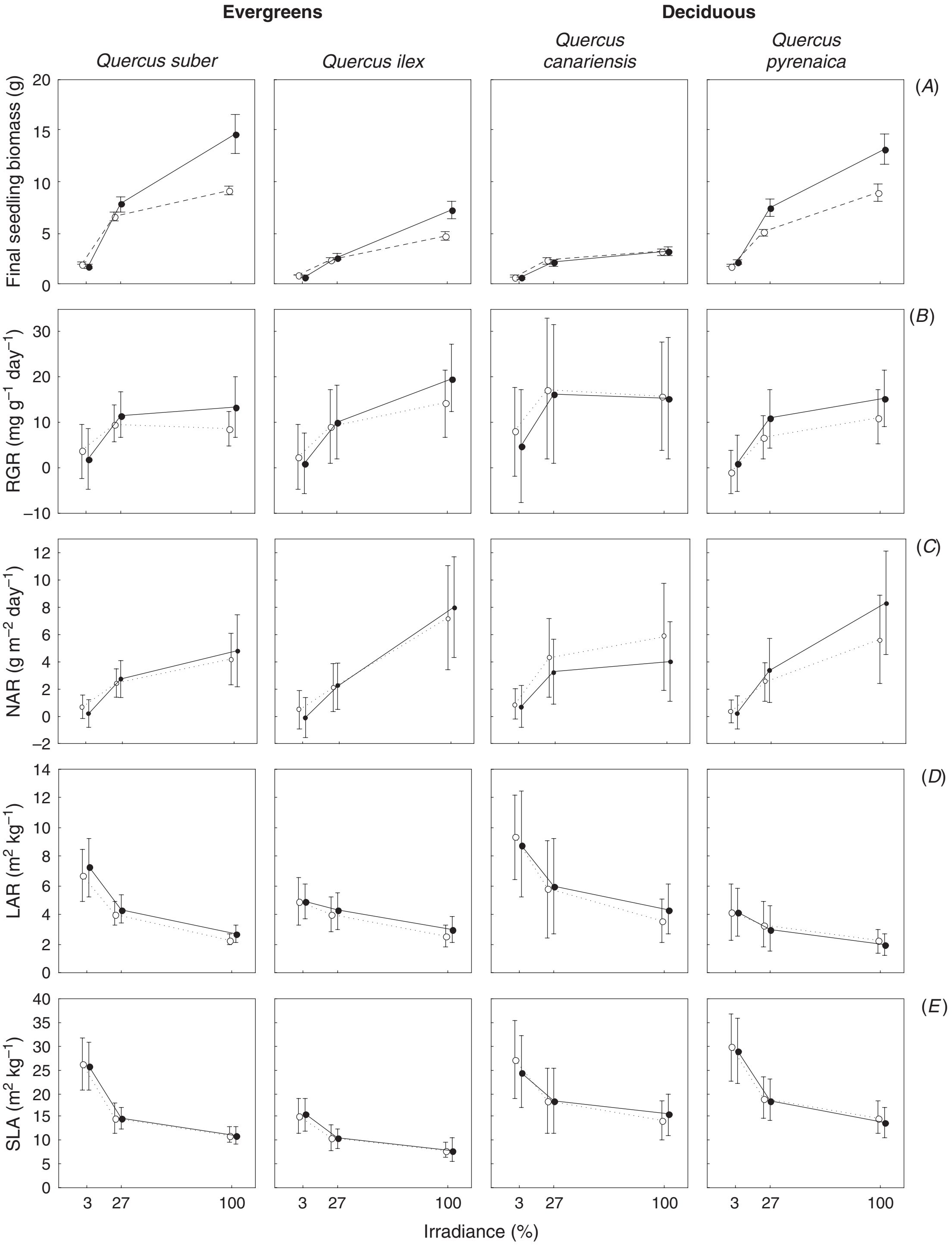

Fig. 1. Mean values and s.e. of (*A*) final seedling biomass, (*B*) RGR (relative growth rate), (*C*) NAR (net assimilation rate), (*D*) LAR (leaf area ratio) and (*E*) SLA (specific leaf area) at final harvest of after 6.5 months of growth under three irradiance levels: low (LI, 3%), moderate (MI, 27%) and full irradiance (HI, 100%), and two water treatments: continuously moist (closed symbol and solid line) or low water (open symbols and dashed line), for seedlings of four oak species.

net carbon assimilation on a leaf area basis ($R^2 = 0.60$; $P = 0.0003$) and even more strongly related to average net carbon assimilation on a plant mass basis ($R^2 = 0.66$; $P = 0.00003$; Fig. 6*B*).

Discussion

We found that multiple factors drive differences in absolute seedling growth and RGR in contrasting irradiance and water

Table 1. Factors explaining plant biomass and biomass allocation in four *Quercus* species

Results of 2-way (individual species) and 3-way (all species' data) ANCOVAs for final biomass, specific leaf area (SLA), leaf area ratio (LAR), leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF), for 6.5 month-old oak seedlings ($n = 12-16$), with factors species (S), irradiance (I) and water (W) treatments. Initial seed mass was used as covariate. For the analysis with all species, we considered species nested within leaf habit. The proportion of the explained variance (SS_x/SS_{total}) and the level of significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) for each factor and the interactions are indicated; those values not significant after controlling the false discovery rate are underlined. R^2 (×100) is the proportion of total variance accounted by the model

Species	Factor	Final Biomass	SLA	LAR	LMF	SMF	RMF
Quercus ilex subsp. *ballota*	Seed mass	8.1***	1.1	0.4	0.3	1.0	0.0
	Irradiance (I)	84.1***	77.2***	62.5***	7.2*	23.6***	4.9
	Water (W)	0.5*	0.1	3.0**	15.6***	2.3	12.3***
	I × W	0.4	0.1	1.6	8.0*	7.2*	18.0***
	R^2	93.1	78.5	67.5	31.0	34.1	35.2
Quercus suber	Seed mass	3.8***	0.6	1.8**	2.4	1.0	0.0
	Irradiance (I)	86.0***	87.0***	77.3***	15.2***	27.1***	18.8***
	Water (W)	1.2***	0.1	2.5**	14.9***	14.7***	21.1***
	I × W	1.3**	0.1	0.9	4.3	2.5	0.6
	R^2	92.3	87.7	82.5	36.9	45.3	40.5
Quercus canariensis	Seed mass	10.8***	1.8*	1.8*	0.7	0.2	0.1
	Irradiance (I)	63.7***	70.1***	59.8***	13.3**	21.5***	32.3***
	Water (W)	0.0	0.4	0.8	6.0*	0.0	2.9
	I × W	0.7	3.6**	3.9*	1.6	7.2*	3.0
	R^2	75.2	75.9	66.4	21.5	28.9	38.3
Quercus pyrenaica	Seed mass	0.6	0.4	1.8	1.5	0.0	0.1
	Irradiance (I)	77.9***	52.3***	47.0***	0.4	59.4***	43.0***
	Water (W)	3.4***	1.2	1.1	0.0	0.1	0.0
	I × W	0.4	0.3	1.3	3.3	0.1	1.4
	R^2	82.3	54.1	51.1	5.3	59.5	44.4
All species	Seed mass	3.6***	0.6*	1.1***	0.3	0.0	0.0
	Species (leaf habit) (S)	6.7***	10.9***	14.4***	25.7***	2.0**	15.2***
	Leaf habit (H)	1.0***	18.2***	0.3	21.1***	6.9***	15.1***
	Irradiance (I)	71.5***	48.7***	50.8***	1.9**	28.7***	13.4***
	Water (W)	0.5**	0.3*	0.5*	2.7***	1.2**	4.9***
	I × W	0.6**	0.1	0.6*	0.8	1.9**	1.9***
	H × I	0.3*	0.4*	0.9**	0.8	1.9**	1.1*
	H × W	0.0	0.1	0.7**	0.8*	1.5**	1.9***
	H × I × W	0.0	0.1	0.1	0.6	0.1	0.8*
	S × I	0.3	0.3	0.8	1.2	3.2***	1.0
	S × W	0.3*	0.0	0.4	0.4	0.7	0.4
	S × I × W	0.0	0.4	1.0*	0.9	1.0	2.1**
	R^2	85.1	80.0	71.5	57.2	49.1	57.9

supplies, with a special importance of seed mass and leaf habit. Additionally, we found that leaf-level carbon gain can predict RGR, across species and across contrasting light and water availabilities.

Effects of irradiance and water on absolute and relative growth

Growth and biomass allocation were affected by limiting irradiance and water, with irradiance having the greatest effect. Increasing irradiance had a non-linear effect on growth and biomass allocation. For example, we found a strong increase of RGR from 3% to 27% irradiance, but no further increase from 27% to 100% irradiance; this finding is similar to that shown by previous work in a range of systems (e.g. Poorter 1999; Holmgren 2000; Sánchez-Gómez *et al*. 2006). Although we found greater NAR at high irradiance, this increase was counteracted by the lower LAR and SLA. Similarly, under field conditions, seedling growth under moderate shade of pioneer shrubs is not reduced relative to open sites (Gómez-Aparicio *et al*. 2004, 2005).

Previous studies have reported a significant impact of drought on seedling growth across irradiance gradients, even in deep shade (Sack and Grubb 2002; Sack 2004; Valladares and Pearcy 2002). However, we found an impact of the drought treatment only in high irradiance and not in deep shade. This pattern may have arisen due to the drought being applied by suspending watering and thus the soil dried more rapidly under high irradiance, where the evaporative demand was higher, and the seedlings were larger (Abrams *et al*. 1992; Coomes and Grubb 2000; Sack and Grubb 2002). By the end of the drought period, the soil was similarly dry across irradiances, but the growth differences quantified over the whole experiment integrated the period in which the soil was moister in the shade treatments. This pattern of interaction of irradiance and water

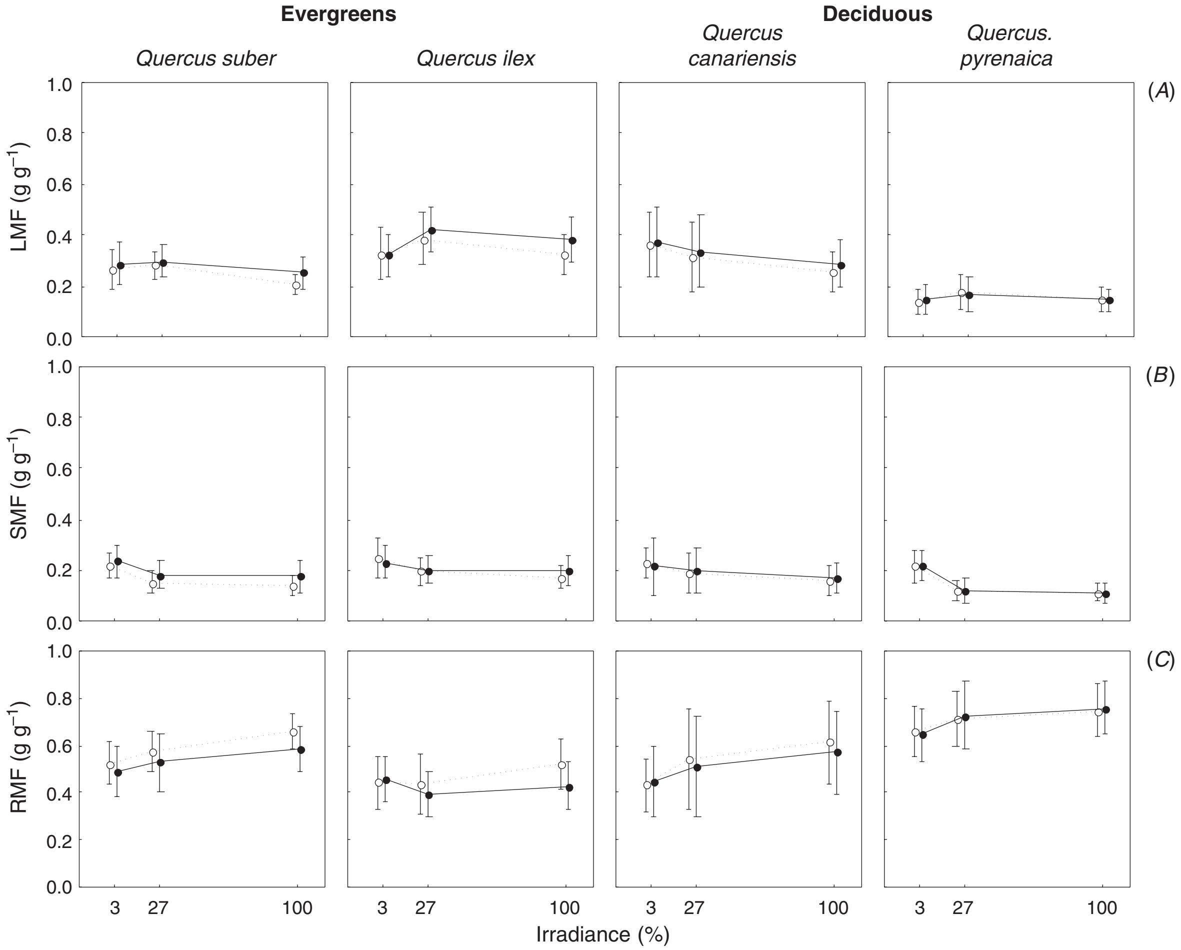

Fig. 2. Mean values and s.e. for biomass allocation to leaves (*A*, leaf mass fraction, LMF), stems (*B*, stem mass fraction, SMF), and roots (*C*, root mass fraction, RMF), under three irradiance levels: low (LI), moderate (MI) and full irradiance (HI), and two water treatments: high water (closed symbol) and low water (open symbols), for seedlings of four oak species.

treatments is similar to real-life scenarios in the field, in which soil dries between rainfall events and soil desiccates faster in clearings (e.g. Ashton 1992; Ellsworth and Reich 1992). We note that in several other field studies, the soil dries more rapidly in the understorey, due principally to greater root competition (Veenendaal *et al*. 1996; Valladares and Pearcy 2002). However, when the soil is moister in the shade, this represents a type of facilitation not typically described in the literature (cf. Holmgren 2000), and which would act simultaneously with other benefits of shade, including lower evaporative demand, and protection from the oxidative stresses associated with high irradiance (Demmig-Adams and Adams 1996; Tattini *et al*. 2005).

Integrating irradiance and water responses

The results allow us to propose a framework for understanding the impacts of natural combinations of irradiance and water on seedling RGR and absolute growth. First, when soil dries more slowly in shade, as in our study, and, potentially, facilitation or primary limitation effects also protect plants in shade, there would be a lower reduction of RGR and absolute growth due to dry soil relative to under high irradiance. This situation is most likely to occur if the drought is short, if the shade is very deep, or if the plants have inherent low growth rates (because small plants would deplete water less quickly, and would also be slower to manifest growth differences across irradiances). Alternatively, when soil dries equally across irradiances, the findings of previous studies suggest that RGR typically declines by the same proportion across irradiances, and notably, absolute growth declines much more strongly under high irradiance than in deep shade, because absolute growth is an exponential function of RGR (reviewed by Sack and Grubb 2002; Sack 2004). Finally, when soil dries more strongly in deep shade, due especially to stronger root competition (Veenendaal *et al*. 1996; Coomes and Grubb 2000; Valladares and Pearcy 2002), RGR and absolute growth would decline equally, or more strongly during the drought in deep shade, so depending on the degree to which the facilitation benefits provided by the shade can compensate for the stronger soil drought in the shade. This framework covers a wide range of shade and drought combinations, and predicts that plants are on the whole benefited during short-term drought by deep shade.

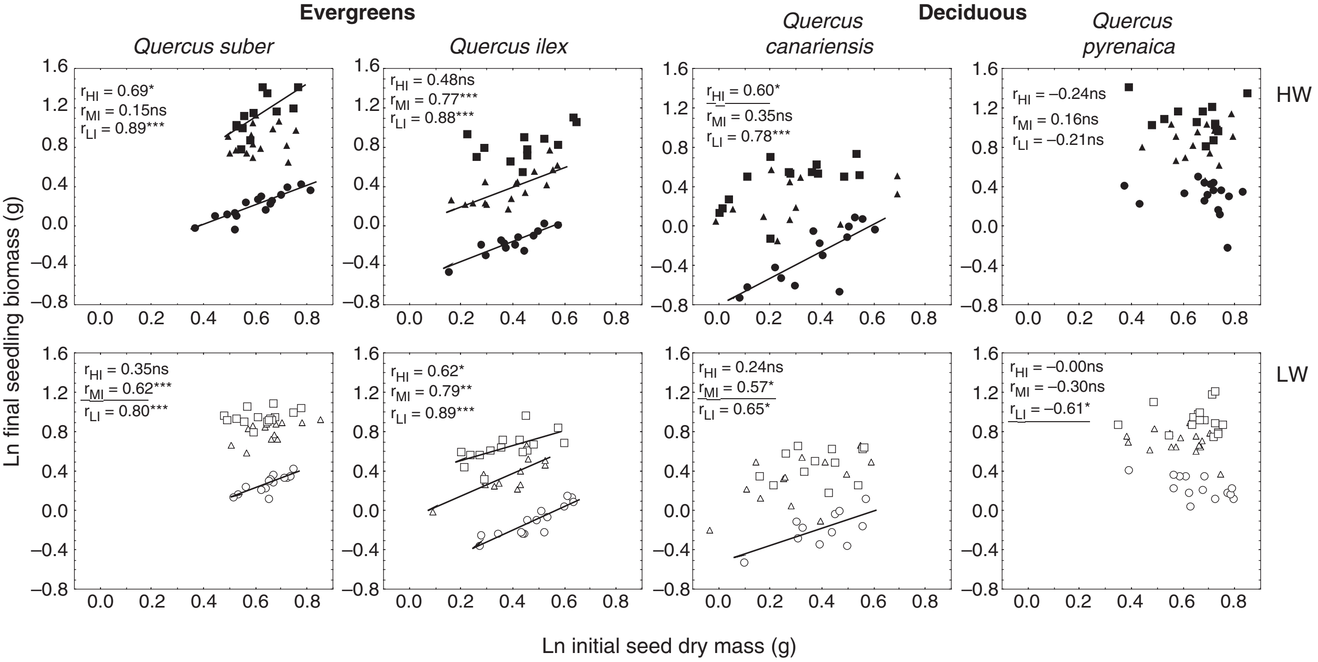

Fig. 3. Final seedling biomass after 6.5 months of growth in relation to initial seed mass for four *Quercus* species in irradiance × water treatments. Squares, high-irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings grown under high water (HW) conditions (closed symbols) are distinguished from those grown under low water (LW) conditions (open symbols). Levels of significance are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, and values not significant after controlling the false discovery rate (FDR) underlined.

Table 2. Correlations coefficients between plant variables in four *Quercus* species

Pearson correlation coefficients between seed mass, final seedling biomass, RGR and growth components for all species and irradiance and water treatments. The level of significance is indicated (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$); n.s., not significant ($P > 0.05$); underlining signifies a value not remaining significant after controlling the false discovery rate (FDR). RGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio; SLA, specific leaf area; LMF, leaf mass fraction; SMF, stem mass fraction; RMF, root mass fraction

	NAR	LAR	SLA	LMF	SMF	RMF	RGR	Seedling biomass
Seed mass	−0.07n.s.	−0.36n.s.	0.32n.s.	−0.81***	−0.38n.s.	0.74***	−0.36n.s.	0.49**
NAR	–	−0.64***	−0.68***	−0.11n.s.	−0.65***	0.32n.s.	0.85***	0.67***
LAR	–	–	0.64***	0.48*	0.71***	−0.62***	−0.35n.s.	−0.69***
SLA	–	–	–	−0.31n.s.	<u>0.42*</u>	0.07n.s.	−0.64***	−0.50*
LMF	–	–	–	–	0.56**	−0.95***	0.21n.s.	<u>−0.43*</u>
SMF	–	–	–	–	–	−0.79***	−0.48*	−0.72***
RMF	–	–	–	–	–	–	0.02	0.59**
RGR	–	–	–	–	–	–	–	0.46*

However, we note that even when the impact of drought is weaker in shade, the shade entails, in real terms, a reduction in carbon assimilation. During longer-term drought, the combined suppression of growth by drought and shade may filter out some species (Niinemets and Valladares 2004), though in many systems species can survive repeated occasional or seasonal strong drought in deep shade (Sack *et al.* 2003; Engelbrecht *et al.* 2007).

Seed mass and leaf habit as determinants of final seedling biomass

Seed mass is a crucial factor influencing seedling growth (Ke and Werger 1999; Poorter and Rose 2005). In general, for given species greater seed mass drives greater seedling size, and absolute growth, for months or several years (Poorter and Rose 2005). However, the initial strength of the effect may depend itself on the species' typical seed size; for example *Pinus sylvestris* (which has an average seed mass of 9 mg) showed no correlation of seed mass with seedling biomass after one growing season (Castro 1999). Our study shows that for *Quercus* species, seed size can impact on seedling biomass accumulation for 7 months or more.

We found that seedling biomass depended on seed mass more strongly under shaded conditions, for three of four species (Fig. 3). A similar effect was found in our previous study on 50 days growth (Quero *et al.* 2007), and for tropical woody plants

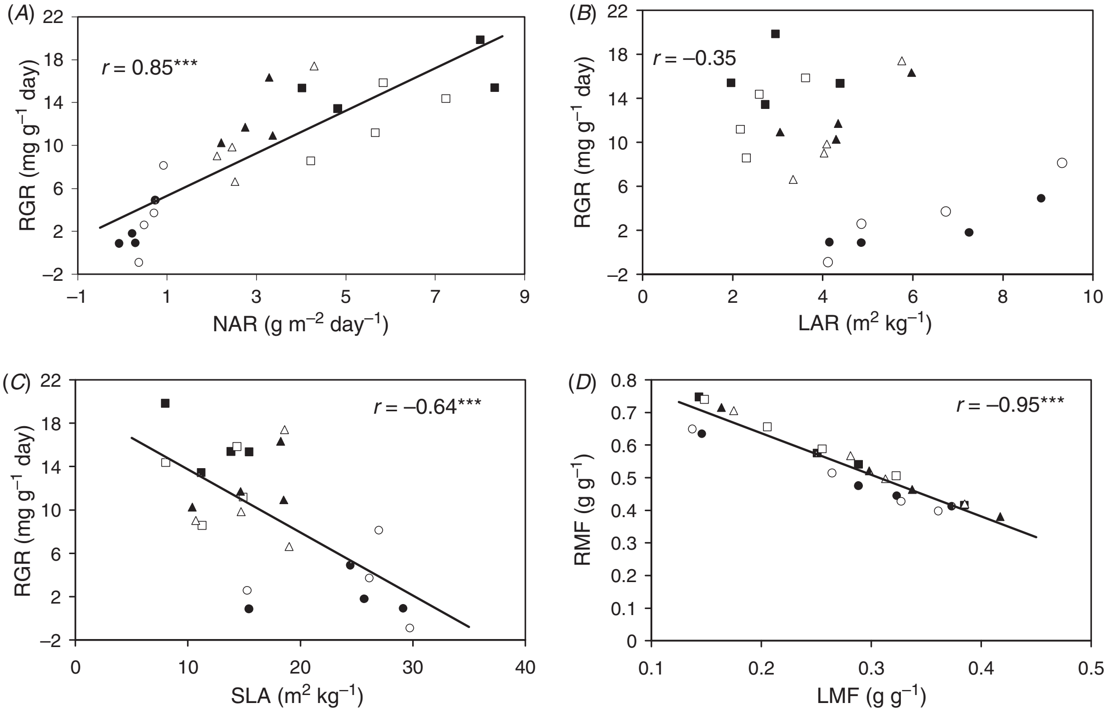

Fig. 4. Correlations between relative growth rate (RGR) and their components, (*A*) RGR-NAR, (*B*) RGR-LAR, (*C*) RGR-SLA and (*D*) correlation between LMF and RMF. Squares, high-irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings of four *Quercus* spp. grown under high water conditions (closed symbols) are distinguished from those grown under low water conditions (open symbols).

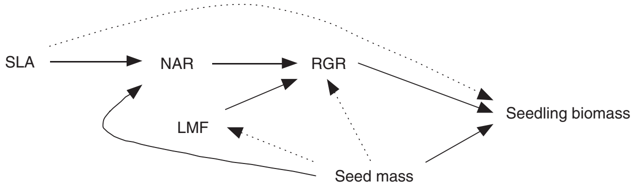

Fig. 5. Causal model linking different variables studied. For brevity, it only shows the model providing the best statistical fit to the dataset of *Quercus* seedling responses to water and irradiance treatments (see Fig. S1 and Table S2 for an extended list of models). Dashed and solid lines indicate negative and positive relations, respectively. SLA (specific leaf area), LMF (leaf mass fraction), NAR (net assimilation rate), RGR (relative growth rate).

(Paz and Martínez-Ramos 2003). Two factors may explain this effect. First, under deep shade where seedling growth is slower, initial effects of seed mass may persist longer. Second, under deep shade seedling growth becomes more dependent on cotyledon reserves. The persistence of an advantage for larger seeds would provide an ecological advantage in the understorey of Mediterranean forests and shrublands.

Leaf habit was also a significant driver of seedling growth, but explained only 1% of variation in growth. Evergreens had higher final biomass than deciduous species (Fig. 2*A*). As a general trend, deciduous species have higher SLA and RGR than evergreen species (Reich *et al.* 1992; Cornelissen *et al.* 1996). However, evergreen species can range widely in SLA and growth, and overlap with the range of deciduous species (Niinemets 2001; Wright *et al.* 2004). Our findings for four congeneric species is, thus, consistent with recent phylogenetically-controlled comparisons that showed no consistent, predictable differences in RGR between deciduous and evergreen species (Antúnez *et al.* 2001; Ruiz-Robleto and Villar 2005; Espelta *et al.* 2005).

Determinants of RGR and final biomass

We found complementary results for the determinants of RGR using the more intensive analysis of causal models with the d-sep method (Shipley 2000). Model G (Fig. 5) was the only non-rejected model. According to this model, seedling biomass across species and treatments was determined directly, positively by both RGR and seed mass. These results are consistent with previous studies showing the importance of seed mass (Ke and Werger 1999; Poorter and Rose 2005; Quero *et al.* 2007) and of RGR on final biomass (Van Andel and Biere 1989). Differences in RGR were caused by NAR and not by LAR. In previous studies, LAR has often been shown to be a strong determinant of interspecific differences in RGR (Poorter and Remkes 1990; Cornelissen *et al.* 1996), though the relative importance of LAR or NAR in determining RGR varies, depending on the irradiance (Shipley 2002; Kruger and Volin 2006), temperature (Loveys *et al.* 2002), nitrogen concentration (de Groot *et al.* 2002), and time scale (Sack and Grubb 2001; Villar *et al.* 2005). Shipley (2006) in a meta-analysis of growth including of 614 species found that in general, NAR was the best predictor of RGR, as found in this study (see also Antúnez *et al.* 2001; Ruiz-Robleto and Villar 2005). Notably, for the four

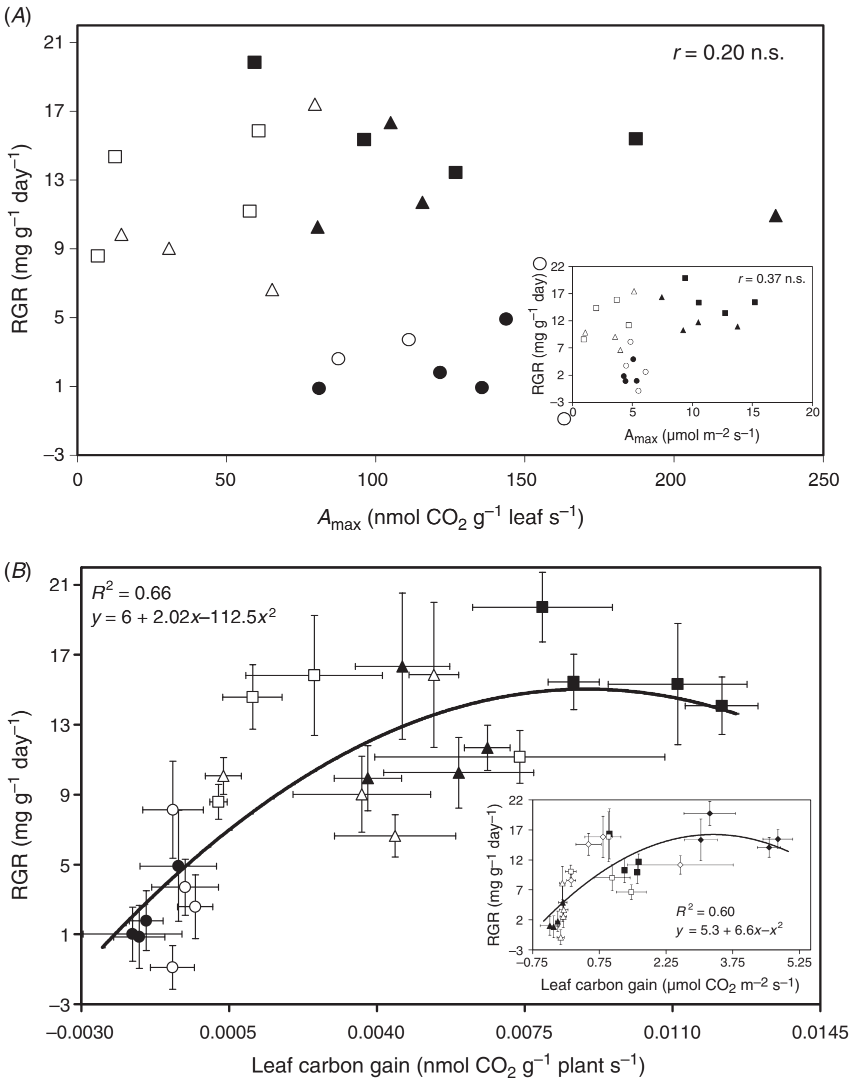

Fig. 6. (*A*) Correlations between relative growth rate (RGR) and maximum photosynthetic rate (A_{max} on a mass basis; data reported by Quero *et al.* 2006). Inset in the figure represents the RGR *v.* A_{max} on an area basis. (*B*) Relationships between seedling relative growth rate, for each oak species and irradiance and water treatments, with the average carbon gain at leaf level, calculated using the light curve parameters (reported by Quero *et al.* 2006), and the estimated total radiation reaching the leaf during the growth period studied. Inset in the figure represents the RGR *v.* carbon gain on an area basis. Mean and s.e. values are presented. Squares, high irradiance treatment (HI); triangles, medium irradiance treatment (MI); circles, low irradiance treatment (LI). Values for seedlings of four *Quercus* spp. grown under high water conditions (closed symbols) are distinguished from those grown under low water conditions (open symbols).

Quercus species, RGR was also driven positively by LMF; a similar pattern was found for large species sets tested (Cornelissen *et al.* 1996; Kruger and Volin 2006), but not all (Shipley 2006).

A direct negative influence of SLA on NAR was also found; most likely, this linkage arose because the shaded seedlings, with higher SLA, have the lowest photosynthetic rates (Niinemets *et al.* 2004; Quero *et al.* 2006). Shipley (2002) found also a trade-off between SLA and NAR as function of daily irradiance. In addition, the model showed a directly negative impact of SLA on final biomass; we cannot find a biological explanation for this relationship but it should be caused by indirect effect of light on both variables.

In contrast, seed mass was directly, negatively related to RGR and LMF, but was a positive driver of NAR. Seed mass was previously found to be a negative determinant of RGR across species (Marañón and Grubb 1993; Cornelissen *et al.* 1996) and on LMF, as seedlings from bigger seeds allocate more biomass to roots and less to leaves. The greater root allocation likely allows higher rates of water and nutrients assimilation, and higher NAR (Reich *et al.* 1998).

Leaf carbon balance predicting seedling growth

Our findings demonstrate that seedling RGR can be predicted from short-term measurements of leaf physiology, for closely related species across water and irradiance supplies. We found that RGR was strongly related to estimated leaf carbon assimilation even without accounting for differences across treatments in LAR. These findings were consistent with the finding that RGR across treatments was related to NAR but not to LAR, and signal an especially strong role for leaf-level physiology in defining performance across resource supply combinations (see also Quero *et al*. 2006).

Typically, the instantaneous maximum photosynthetic rate considered per leaf mass ($A_{\max}$) is assumed to be a strong determinant of species-differences in RGR (Givnish 1988; Poorter *et al*. 1990; Poorter 1999; Shipley 2002). However, we found no correlation between $A_{\max}$ and RGR across treatments (see also Montgomery 2004). Moreover, we found no correlation between RGR and other photosynthetic light parameters studied (Φ, R_d, and θ; $P > 0.2$; data not shown). We propose that a correlation between $A_{\max}$ and RGR would be strongest for plants of a range of species grown under high irradiance and water supply. However, across a gradient of resource supplies, the correlation would be weakened, due to interspecific variation in the plasticity of LAR and especially of physiology across resource supplies. Because resource supplies vary strongly in the field, a more broadly integrative estimate of leaf carbon gain, using photosynthetic light response curves as presented here, is needed to predict RGRs. Our model of leaf carbon balance assumed many simplifications since other components of plant respiration have not been considered. A more comprehensive model would include dynamics in stem and root respiration (Givnish 1988; Poorter *et al*. 1990; Kruger and Volin 2006). In addition, although this is not an analytical model but a correlative one based on relationships between variables, we note that the correlation of our carbon balance estimate with RGR was very high (even with NAR, $r = 0.66$, $P < 0.05$, data not shown), potentially suggesting either that the differences in carbon gain were more important than carbon loss in defining RGR, or that the rates of carbon loss across species and treatments were correlated with those of carbon gain (Walters and Reich 1999). Kruger and Volin (2006) found a strong interspecific correlation between estimated carbon gain with RGR and NAR; our results indicate that this approach can be extended for plants of different species across contrasting resource supply combinations. The ability to predict seedling growth responses from leaf-level gas-exchange measurements will have important application for predicting seedling performance across heterogeneous microsites. Our findings also indicate that extrapolations of plant performance from light-saturated net photosynthetic rates must be done with caution, as the whole range of environmental conditions need to be accounted for to accurately link leaf traits to whole-plant performance.

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