RESPONSES OF QUERCUS SUBER SEEDLINGS UNDER DIFFERENT LIGHT AND WATER TREATMENTS. GREENHOUSE AND FIELD EXPERIMENTS

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ABSTRACT

The objective of this study is to understand the response of *Quercus suber* seedlings to light and water, two limiting factors of their growth and survival in Mediterranean woody plants. Greenhouse and field experiments were conducted to contrast the results from both types of experiments.

At the greenhouse experiment, *Quercus suber* seedlings were grown at three treatments of light (3%, 27% and 100% of incident light) and daily irrigation. When plants had four months old, half of the individuals did not receive any water, so we had then two water treatments (water and drought) within each light treatment. After two months, different variables were measured.

At the field experiment, *Quercus suber* acorns were sown in three microhabitats with different light conditions (open, under tree and deep shade) in “Los Alcornocales” Natural Park (Cádiz). These microhabitats have similar light radiation than the treatments used at the greenhouse experiment.

The greenhouse experiment results showed that the acorn mass was important for determining the seedling mass on the first stages, and that importance increased with light limitation. Thus, a greater seedling biomass was strongly associated with a bigger acorn mass under deep shade, but with increasing light levels the association between seedling and acorn mass became lower and non-significant. *Quercus suber* seedlings responded to light restriction with a decrease in the relative growth rate (RGR) and then the seedlings had lower biomass. This decrease in RGR was mainly caused by a decrease of the net assimilation rate (NAR). However, specific leaf area (SLA) and stem mass ratio (SMR) increased under shade as a way to enhance light capture. The response to water limitation was a general decrease of RGR, but interestingly, the effect of drought was negligible under deep shade conditions.

The results of the field experiment were similar to those in the greenhouse experiment, which reinforce the conclusions of this study. Seedlings from open microhabitats (with more light) had a greater biomass than seedlings under tree or deep shade microhabitats. Also, the response to light limitation was an increase of specific leaf area (SLA), an increase in stem mass ratio and stem length. In contrast with the growth data, the survival rate of seedlings after the summer drought was greater under trees than in the open habitats.

Then, it seems to exist an uncoupling between growth and survival of *Quercus suber* seedlings under different conditions. In this way, the best microhabitat for the growth (open microhabitat) is not the best for seedling survival, while medium shade microhabitat seems to be the best suited for *Quercus suber* regeneration.

Keywords: relative growth rate, specific leaf area, growth, acorn, photosynthesis, stomatal conductance.
INTRODUCTION

Water is one of the most limiting resources for plants in Mediterranean habitats (Aschmann, 1973). Under forest canopies, light is also an important limiting factor. Both resources affect the growth and survivorship of seedlings and saplings (Lambers et al., 1998) and therefore influence on the regeneration of *Quercus suber* forests.

The effect of the interaction of water and light is not well understood and is under controversy. For example, the trade-off hypothesis predicts that deep shade will aggravate the stress imposed by drought, based on the proposed trade-off mechanism that shaded plants allocate more to shoot, and to leaf area, than to root, thereby diminishing the ability to capture water (Smith and Huston, 1989). Supporting this hypothesis, Aranda et al. (2005) found, in a controlled experiment, that *Quercus suber* seedlings grown in shade were less efficient in developing physiological mechanisms of water tolerance, as osmotic adjustment and effective control of water loss.

A contrary hypothesis predicts that under limiting light availability, the shortage of another resource such as water should have less impact on plant performance (Canham et al., 1996). In addition, shade by the tree canopy has indirect effects, such as reducing leaf and air temperatures, vapour pressure deficit, and oxidative stress, that would alleviate the drought impact on seedlings in the understorey (Holmgren, 2000). In fact, empirical evidence of facilitation effects of shrubs and trees on seedlings in the understorey in Mediterranean environments has been widely documented (e.g., Castro et al., 2002; Gómez-Aparicio et al., 2004).

A third group of hypotheses posits that the effects of shade and water-shortage are independent, that is, their impacts are orthogonal (Sack and Grubb, 2002; Sack, 2004). For example, combination of light and water in a study of 13 temperate woody species showed changes in functional morphology (specific leaf area, leaf area ratio, biomass allocation) without interaction between light and water treatments (Sack, 2004).

The objectives of this work are to understand the responses of *Quercus suber* seedlings to different levels of light and water and the interactions between both resources. We measured photosynthetic rate, relative growth rate, final biomass and survivorship. In two other chapters of this book, we present the effects of light and water treatments on physiological and morphological leaf variables (Quero et al., 2008) and the effects of different microsites (differing in light availability) on five regeneration stages (Pérez-Ramos et al., 2008). In this study, two different approaches were followed: greenhouse and field experiments. Greenhouse experiments have the advantage of a good control of environmental variables but with a lack of realism, while field experiments are the opposite. There are not many studies comparing the results of greenhouse and field experiments, so our aim is to corroborate our results using
these two complementary approaches (see preliminary results for *Q. suber* and other species in Marañón et al. 2004, 2005).

**MATERIAL AND METHODS**

**Greenhouse experiment**

The experiment was carried out in a greenhouse of the University of Córdoba (Spain), with automatic irrigation system and regulation of air temperature. Single acorns were individually weighed, and then sown (on December 2002) in pots of 3.9 liters and 50 cm height (to avoid as much as possible the interference during root growth). Pots contained a mixed soil of 2/3 sand and 1/3 peat. Ten g of a slow-release fertiliser (Plantacote® Pluss NPK: 14-9-15) were added at the middle of the experiment. Other three species (*Q. ilex*, *Q. pyrenaica* and *Q. canariensis*) were studied in the same experiment, but we are presenting here only the results of *Quercus suber*.

*Quercus suber* seedlings were subjected to three light levels: 1) “Full-light” treatment (L), receiving the 100% of the available radiation inside the glasshouse, and equivalent to open field conditions; 2) “Medium shade” treatment (S), covered by a green screen, and receiving about 27% of full-light radiation; and 3) “Deep shade” treatment (DS), covered by a green cloth, and receiving only about 3% of full-light radiation. The mean ± s.d. of photosynthetic active radiation measured (with EMS7, canopy transmission meter, PP-system, UK) on May 28, 2003, in each light treatment were 760 ± 424 (Full light), 187 ± 76 (Medium shade) and 23 ± 5 (Deep shade) µmol m⁻² s⁻¹ respectively.

Four months after sowing (at the end of April 2003), half of the pots stopped receiving any watering (drought treatment) while the other half was kept continuously moist (water treatment). These treatments simulated therefore a typical Mediterranean situation of summer drought versus a summer with reduced or no drought. The mean ± S.E. values of soil moisture (in volumetric water content), measured at 20 cm depth with a TDR (mod 100, Spectrum Technologies, Inc.) in July 2003, were 13.20 ± 0.20% (for water treatment) and 2.96 ± 0.13% (for drought treatment). Replicates were randomly mixed within the greenhouse.

Seedlings were harvested on May and July (four and six months after sowing, respectively) to calculate the relative growth rate. Sixteen replicates per treatment were analysed. For more details of growth analysis methodology see Villar et al. (2004), Antúnez et al. (2001) and Ruiz-Robleto and Villar (2005). Physiological and structural leaf traits were measured at the end of July 2003 (two months after stopping irrigation), when seedlings were about six months old. In general, six plants per species and treatment combination were randomly chosen to measure photosynthetic activity in mature leaves, that were taken at plant mid-height. We used a gas-exchange portable analyser (Ciras-2, PP-System, UK), to measure photosynthetic rate and stomatal conductance. Quero et al. (2008) describe more exhaustively the methods of gas exchange and discuss more variables related to the physiological and morphological responses of leaves in different light and water treatments.

**Field experiment**

The field study was carried out at Panera (36º 31’ N, 5º 34’ W), about 450 m altitude, in the Sierra del Aljibe (SW Spain). The average rainfall was 985 mm per year, and the mean tem-
perature was 17 °C; the mean of the minimum temperature in the coldest month was 3 °C and the mean of the maximum temperature in the hottest month was 34 °C (data from Pantano de los Hurones meteorological station). The main geologic substrate is sandstone, originating acidic and sandy soils. Cork oak (*Quercus suber*) and the deciduous oak *Q. canariensis* (in wetter and richer soils) are dominant trees. The three microhabitat types chosen in this forest were: 1) “Full light” (L), sites in open areas of bare ground or grassland; 2) “Medium shade” (S), sites under isolated trees without shrubs underneath; and 3) “Deep shade” (DS), sites inside dense forest patches with shrub understorey. At each microhabitat, we randomly assigned 20 sampling stations, in which we sowed seeds of the two most common tree species - *Quercus suber* and *Q. canariensis*, each in a frame of 20 x 30 cm. Five acorns were sown (on December 2002) per sampling station. Seeds were protected with a wire cage of 1.3 cm mesh size to exclude rodents. After sowing, sampling points were monitored periodically, noting emergence, survival, and cause of death. In this work we will present only the results of *Q. suber*.

**Data analysis**

In the greenhouse experiment, the effect of different light and water treatment was analysed by two ways ANOVA (with light and water as factors). In the field experiment, the effect of different microhabitats was analysed by one way ANOVA (with microhabitat as factor). Multiple comparison of means was analysed by post hoc Tukey test. Data were arcsine or log-transformed to satisfy the homocedasticity assumption (Zar, 1984).

Relative growth rate and biomass allocation was calculated with the spreadsheet in excel published in Hunt et al. (2002) (http://www.ex.ac.uk/∼rh203/growth_analysis.html).

Relationships between variables were analysed with Pearson correlations. The program STATISTICA v 6.0 was used for statistical analyses.

**RESULTS**

**Importance of seed mass on plant biomass**

In general, there was a positive correlation between seed mass and plant biomass for the first 50 days of growth (*r* = 0.60; *P* < 0.001) in greenhouse conditions. However, this positive correlation was weaker at 120 days of growth (*r* = 0.45; *P* < 0.05) or disappeared after 180 days of growth (*r* = 0.16; *P* = 0.39).

During the first 50 days of growth, the different light treatments affected the relationships between seed mass and plant biomass. Plants under deep-shade showed a stronger positive correlation between seed mass and plant biomass than in medium or full light treatments (Fig. 1). For medium shade and full light treatments the correlation between plant biomass and seed mass was not significant.

**Effects of shade and drought on physiological and morphological variables**

In the greenhouse experiment, there was a strong interaction of light and water treatments on maximum photosynthetic rate (*A*$_{max}$) (Fig. 2 A). Under deep-shade there was no effect of drought on *A*$_{max}$. However, under medium and full light conditions there was a strong decrease of *A*$_{max}$ with drought. Thus, these results do not support the hypothesis of trade-off between shade and drought. A similar result was found on stomatal conductance (Fig. 2 B).
Figure 1. Relationship between fresh plant biomass at 50 days of growth and the fresh mass of the acorn of Quercus suber in the greenhouse experiment at three different light treatments: Deep shade (3 % of full light), Medium shade (27 % of full light) and Light (100 % of full light).

Similarly, the relative growth rate showed a strong interaction of light and water treatments. That is, the effect of drought depends on the light treatments (Fig. 2 C). Under full light conditions there was a high decrease in RGR under drought, but under medium and deep-shade conditions the effect of drought was negligible. In general, there was a decrease in RGR with light limitation. The response of RGR to light and water treatments was mainly determined by the effect of these treatments on net assimilation rate (NAR) (Fig. 2 D).

Figure 2. (A) Maximum photosynthetic rate per unit area ($A_{\text{max}}$), (B) Stomatal conductance ($g_s$), (C) Relative growth rate (RGR) and (D) Net assimilation rate (NAR) in Q. suber seedlings in the greenhouse experiment with three different light treatments and two water treatments.
The biomass allocation was affected by the light treatments but not by the water treatments. For example, the proportion of biomass allocated to stems (the stem mass ratio) was higher under deep-shade conditions (Fig. 3 A) and also the stem length (Fig. 3 C). Specific leaf area (SLA) was also higher under light limitation and was not affected by water (see Quero et al., 2008).

![Graphs showing biomass allocation and plant variables under different conditions.](image)

**Figure 3.** Comparison of different plant variables between the greenhouse and the field conditions. Graphs on the left are from the greenhouse experiment and those on the right are from the field experiment. (A and B) Stem mass ratio (proportion of biomass allocated to stems), (C and D) Stem length and (E and F) Plant biomass. Note the different scales for each graph.
The plant biomass after 6 months of growth was affected by the limitation of light and water (Fig. 3 E). Interestingly, the effect of water depended on the light treatment (i.e. under shade there is no significant effect of drought).

**Comparison between greenhouse and field experiments**

*Q. suber* seedlings grown in the field responded similarly as in the greenhouse experiment; the different microsites affected the stem biomass allocation (Fig 3 B) and the stem length (Fig. 3 D), being both higher under deep-shade microsites than in open microsites.

Considering the biomass of the seedlings in the field experiment, the trends were also similar to those found in the greenhouse experiment, although the plants were much smaller (between 5 to 20 times). Seedlings in deep-shade microsites were smaller than those in the open microsites (Fig. 3 F).

However, the survivorship rate after the summer drought in the field experiment was higher in the deep-shade microsites (Fig. 4 A). Thus, a negative correlation was found between survivorship rate and plant growth (i.e. the better microsites for growth were not the better for survival) (Fig. 4 B). Medium shade microsites appear as the best suited for *Q. suber* regeneration.

**DISCUSSION**

Seed mass has been widely recognised as being positively correlated with seedling biomass. However, most of the results have been obtained comparing different species. Paz and Martínez-Ramos (2003) found that positive correlation between seed and seedling mass for some species of tropical forest, but not for others. In this study, we have found that the importance of seed mass on seedling mass depends on the light conditions. When the light conditions are very limiting, the seed reserves are mobilised in a higher amount. Quero *et al.* (2008) also found similar results for other three Quercus species. Paz and Martínez-Ramos (2003) also found more positive correlations between seed and seedling masses under shade conditions than in open habitats. For afforestation practices it is commonly assumed that it is better to collect bigger seeds in order to have more vigorous seedlings; although this can
be true for the early stages, in favourable conditions the Q. suber seedlings quickly become independent from the seed reserve.

The effects of light and water on Quercus suber seedlings showed a strong interaction. That is, the effect of drought depends on the light treatment. The results are very sound as different variables showed the same interaction (e.g., photosynthesis rate, stomatal conductance, relative growth rate and plant biomass). Plants under deep-shade did not show any decrease with drought in these variables. In contrast, plants under medium or full light conditions showed a high decrease with drought in A\textsubscript{net}, gs, RGR or plant biomass.

What are the causes of the different responses of seedlings to drought in different light treatments? Under deep shade the plants under drought can maintain their stomata open, which determine that they can acquire carbon at the same rate that with water in the soil. Holmgren (2000) found similar results. However, Sack (2004) did not find any interaction between light and water treatments on relative growth rate. Contrary to our results, Aranda et al. (2005) found that Quercus suber seedlings under deep shade had lower tolerance to drought.

The severe limitation of light decreases growth and affect biomass allocation. This is a general result found in literature (Sack, 2004). Plants under light limitation respond increasing the stem biomass allocation and the specific leaf area to acquire more light. The results of both greenhouse and field experiments are very similar and strengthen the conclusions on the effect of light on growth and biomass allocation.

Interestingly, our results support the idea that in Mediterranean habitats a partial shade may be beneficial for plant performance, as it increases the survival (see examples in Zamora et al. 2001; Castro et al., 2002; and Gómez-Aparicio et al., 2004).

**Conclusions**

The results of greenhouse and field experiments were very similar, which strengthen the conclusions of our study. We have found that seed mass is important for seedling biomass under light limitation, but not in full light conditions. The severe limitation of light decreases growth and affect biomass allocation (increasing stem allocation and specific leaf area). Interestingly, the effect of drought depends on the light levels. Medium and deep shade plants do not suffer much impact of drought, thus they have higher survival probability. Growth and survivorship are two uncoupled processes in Mediterranean habitats. Medium shade appears the best suited microsite type for Quercus suber regeneration.

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