LEAF TRAITS AND PHOTOSYNTHETIC LIGHT RESPONSE OF QUERCUS SUBER L.
SEEDLINGS GROWN IN A COMBINATION OF LIGHT AND WATER REGIMES

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ABSTRACT

The aim of this study is to investigate the effects of two important factors: light and water availability on the leaf characteristics and the growth of *Quercus suber* plants. Seedlings of *Quercus suber* were grown under controlled conditions (greenhouse) with three light treatments (100, 27 and 3 % of incident light, open, shade and deep shade; respectively) and with daily irrigation. Since middle spring, one half of the seedlings were not watered, so we had two water treatments (water and no water) within each light treatment. We studied the photosynthetic response to light (light curves) using an IRGA (infra red gas analyser).

Leaves under deep shade showed lower values of light compensation point (LCP), light saturation point (LSP), photosynthetic and respiration rates per leaf area ($R_{area}$, $A_{area}$), N and C content per leaf area ($N_{area}$, $C_{area}$) than leaves under shade or open. However, leaves under deep shade showed a higher photosynthetic rate per unit dry mass ($A_{mass}$), specific leaf area (SLA), and N concentration than leaves in shade or full light treatments.

Water limitation induced a lower quantum yield ($\Phi$), LSP, photosynthetic and respiration rate (per unit area or dry mass), $g_{wmax}$, N concentration (per unit area or dry mass), photosynthetic nitrogen use efficiency (PNUE) and chlorophyll index. However, the leaves of plants under water limitation showed a higher water use efficiency (WUE). An interesting result was that plants under deep shade treatment did not show any differences between water and non-watered treatments in physiological traits, possibly indicating a beneficial effect of deep shade on drought resistance.

The simulation of carbon balance at leaf level was very well correlated with the relative growth rates (RGR) of seedlings, indicating that the leaf characteristics (variables obtained by the light curves and SLA) may be a good predictor of the relative growth rate under contrasting light and water conditions.

Keywords: cork oak, N concentration, WUE, PNUE, specific leaf area, shade, drought
INTRODUCTION

Traditionally, afforestation plans in the Iberian Peninsula have followed North-European criteria, removing plant cover to avoid competition from pre-establishment vegetation. Nevertheless, in Mediterranean areas where excess of light and/or lack of water can be stress factors, this type of forestry practices can cause frequent failures (Chaparro and Esteve 1996; Zamora et al. 2001). Moreover, some studies have demonstrated the beneficial effect of existing plant cover in seedling survivorship (Castro et al. 2002; Gómez-Aparicio et al. 2003).

There are few studies about the mechanisms of seedling responses to light and water factors, affecting leaf characteristics (physiological and structural) and growth parameters, to explain the observed facilitation and competition patterns. Some hypotheses predict that under limiting light availability, the shortage of another resource such as water should have less impact on plant performance (Canham et al. 1996). A contrary 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). A third group of hypotheses posits that the effects of shade and water-shortage are independent, that is, species showed changes in functional morphology without interaction between light and water treatments (Sack 2004).

We have designed an experiment with controlled conditions of light and water to explore the leaf responses of *Quercus suber* L. seedlings to six combinations of light (three levels) and water treatments (two levels). We measured physiological activities of the seedlings (e.g., photosynthetic and respiration rate, stomatal conductance, water-use efficiency, and photosynthetic nitrogen-use efficiency) and morphological and structural leaf traits (e.g., SLA and concentration of C and N).

Understanding these leaf-level responses would contribute to a better knowledge of the effects of these two important factors in plant performance and help to explain the regeneration processes in forest dynamics. In addition, it would help to establish specifics criteria of restoration in Mediterranean areas.

MATERIAL AND METHODS

Acorns of *Quercus suber* were collected in forests of Sierra del Aljibe (SW Spain) in Alcornocales Natural Park. This protected area contains the most extensive cork oak forest of Iberian Peninsula and is one of largest in the world. Single acorns were sown (in December 2002) in cylindrical pots (50 cm height, 10.5 cm diameter), thereby avoiding as much as possible 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. The experiment was carried out in a greenhouse of the University of Córdoba (Spain, 37º 51’ N, 4º 48’ W; at an altitude of 100 m. a. s. l.) with an automatic irrigation system and regulation of air temperature. Replicates were randomly set within the greenhouse.

Cork oak seedlings were subjected to three light levels: full light (L) 100% of the available radiation inside the greenhouse; shade (S) about 27% of the full light treatment; and deep-shade (DS) receiving only about 3% of the full light treatment. The experimental light treatments simulated the field conditions in the forest understorey, distinguishing three types of microhabitat: open (L), under single tree cover (S), and under dense forest (DS) (Marañón et al. 2004). Pots were watered weekly during the first stage of the experiment. Once the seedlings emerged, a drip-irrigation system was inserted in the pots. Four months after sowing, half of the pots stopped receiving any watering (NW, no-water treatment) while the other half was kept continuously moist (W, water treatment). 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. The dry treatment (NW) simulated a typical Mediterranean-climate situation of seasonal drought, compared with a continuously moist one (W) with reduced or no drought. For each water treatment, there were no differences in water content between the pots of different species or between the three different light treatments (data not shown).

Six plants per 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, IRGA (Infrared gas analyser, Ciras-2, PP-System, UK). Photosynthetic rate was measured at ten light intensities (Fig. 1). Photosynthetic rate, transpiration rate, and intercellular CO₂ concentration were recorded three times, and the average value at each light intensity was calculated.

Figure 1. Photosynthetic light response and parameters obtained by the non-rectangular hyperbola model of Thornley (1976).
In the same leaves, a chlorophyll index was measured using a CCM-200 (Optic Science, USA). Then, leaves were collected and scanned, and the area was measured with an image analyser (Image Pro-Plus v 4.5 Media Cybernetic, Inc). They were oven-dried (at 80 °C for at least 48 hours) and weighed. The specific leaf area (SLA) was calculated as the ratio between the leaf area and dry mass. Leaves were ground with N liquid in an agate mortar, and analysed for N and C concentration using an elemental analyser (Eurovector EA 3000, EuroVector SpA., Italy).

For each leaf, the physiological parameters of the light curve were obtained by the non-rectangular hyperbola model of (Thornley 1976), adjusting the carbon assimilation data in response to light according to the following formula:

\[ A(I) = \frac{\Phi I + A_{\text{max}} - \sqrt{\left(\Phi I + A_{\text{max}}\right)^2 - 4 I A_{\text{max}}}}{2 I} - R_d \]

where \(A\) is the photosynthetic rate, \(I\) the photosynthetic photon flux density (PPFD), \(\Phi\) the apparent quantum yield, \(A_{\text{max}}\) (hereafter, \(A_{\text{area}}\)) the maximum light saturated assimilation rate, \(R_d\) the dark respiration rate and \(\theta\) the “bending degree” or curvature (Fig. 1). Parameters of the model were calculated by the non-linear estimation module (Statistica v 6.0). The variance explained by the non-linear model was very high (mean \(R^2 = 0.98 \pm 0.03\)). Using this formula, by definition, the maximum photosynthetic rate is obtained at the infinite light intensity, and then overestimated. Therefore, we recalculated \(A_{\text{area}}\) assuming a PAR of 2000 \(\mu\text{mol m}^{-2}\text{s}^{-1}\), the approximate maximum value for that season and latitude. The light saturation point (LSP) was calculated as the lowest value of PAR for which photosynthesis reached 90% of \(A_{\text{area}}\) (Fig. 1). Water-use efficiency (WUE) values were calculated as \(A_{\text{area}}/g_s\) ratio (Cavender-Bares and Bazzaz 2000) and photosynthetic nitrogen-use efficiency (PNUE) as \(A_{\text{mass}}/N\) concentration (Field and Mooney 1986).

Eighteen variables of plant performance were used in this study: 11 parameters of physiological activity and seven morphological and structural traits of the leaves (Table 1).

The differences among species and the effects of light and water treatments on each variable were analysed by two-way ANOVAs (light, and water as source factors) with Type III sums of squares. When the difference was significant, a multiple comparison of means test (post hoc Unequal N Tukey’s Honestly Significant Difference test) was carried out. Prior to ANOVA, data were square-root-, arcsine-, or log-transformed to satisfy the normality and homocedasticity assumptions (Zar 1984). The program Statistica v 6.0 was used for statistical analyses.

To scale up from leaf-level response to whole-plant level, a carbon balance was estimated using the light curve parameters, the SLA and the daily variation in photosynthetic photon flux density in each treatment. Calculations were done for each hour during April 22 to July 16 and the values were average. To validate this assessment, the carbon balance estimation was correlated with the relative growth rate (RGR) in each treatment, obtained from seedlings, which were harvested, oven-dried and weighted. RGR was calculated...

<table>
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<tr>
<th>Leaf Traits</th>
<th>Acronyms</th>
<th>Units</th>
<th>Light (L)</th>
<th>Water (W)</th>
<th>L x W</th>
<th>R²</th>
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<tr>
<td>Leaf area</td>
<td>Area</td>
<td>cm²</td>
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<td>7.3</td>
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<td>Specific leaf area</td>
<td>SLA</td>
<td>cm² g⁻¹</td>
<td>96.6</td>
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<td>N</td>
<td>mg g⁻¹</td>
<td>68.3</td>
<td>3.7</td>
<td>1.7</td>
<td>73.7</td>
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<tr>
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<td>C</td>
<td>mg g⁻¹</td>
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<td>26.0</td>
<td>9.3</td>
<td>35.5</td>
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<td>g N m⁻²</td>
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<td>0.0</td>
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<td>73.1</td>
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<td>Chl index</td>
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<td>Apparent quantum yield</td>
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<td>15.4</td>
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<td>18.8</td>
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<td>mmol H₂O m⁻² s⁻¹</td>
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<td>50.4</td>
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<tr>
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<td>9.4</td>
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<td>38.3</td>
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<td>2.4</td>
<td>45.5</td>
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<td>Ratio internal vs. external CO₂</td>
<td>Ca/Ca</td>
<td>no units</td>
<td>3.8</td>
<td>0.5</td>
<td>27.7</td>
<td>32.0</td>
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</table>

Table 1. Results of the two-way ANOVAs for structural and physiological leaf traits of Quercus suber seedlings, according to the factors light (L) and water (W) treatments. The proportion of the explained variance (SS/SS_total) and the level of significance (*: P<0.05; **: P<0.01; ***: P<0.001) for each factor and the interactions are indicated. R² is the proportion of total variance absorbed by the model.

**RESULTS**

Most physiological variables showed strong interactions of light and water effects. That is, the effect of drought was dependent of the light treatments. For example, drought decreased A_max in full light and shade treatments but did not have any effect on plants under deep shade (Fig. 2).

However, structural variables changed mainly with light treatments, but less with water treatments, and consequently there are no interactions of these two factors (Table 1, Fig. 2).

As a whole, shade (light reduction) was a significant source of variation for structural leaf traits (five out of 7) and physiological traits (affected six out of 11) (Table 1). On the other hand, drought (shortage of water) significantly induced changes in two leaf structural traits and affected 9 physiological variables.
Figure 2. General variation in leaf traits (see meaning of abbreviation names and units in Table 1) of cork oak seedlings (means and 95 % confidence intervals bars for six replicates) in response to the six combinations of light and water treatments. Light levels are “Light” (L), “Shade” (S), and “Deep Shade” (DS), and water levels are “Water” (W, solid line) and “No Water” (NW, dashed line).
Leaves under deep shade showed lower light compensation point (LCP), light saturation point (LSP), photosynthetic and respiration rates per leaf area (R_area, A_area), N and C content per leaf area (N_area and C_area) than leaves under shade or full light treatments. However, leaves under deep shade showed a higher photosynthetic rate per unit dry mass (A_mass), stomatal conductance (gs_area), specific leaf area (SLA), and N concentration than leaves in shade or full light treatments.

On the other hand, droughted plants showed leaves with lower chlorophyll index, apparent quantum yield (Φ), photosynthetic and respiration rates per leaf mass and area (R_mass, A_mass R_area, A_area), stomatal conductance (gs_area) and photosynthetic nitrogen use efficiency (PNUE) than leaves under continuous irrigation (Fig. 2). In contrast, droughted leaves showed higher C content per mass (C_mass) and light compensation point (LCP) than irrigated leaves.

The prediction of carbon balance at leaf level was very well correlated with relative growth rates (RGR) of the seedlings (Fig. 3).

![Figure 3. Relative growth rate (RGR) vs. the carbon balance simulation (obtained by the light curve, SLA and the daily variation in photosynthetic photon flux density) in the different light and water treatments. DS, deep shade; S, shade; L, full light. W and NW, water and non-water treatment respectively. Error bars indicate standards errors of mean.](image)

**DISCUSSION**

Physiological variables under deep shade treatment did not show any differences between water and non-watered plants, however, slight differences on structural traits in water treatments were kept among light treatments. Drought therefore induced a relatively low response in structural leaf traits but a high one in physiological traits (Table 1, Fig. 2). Quero *et al.* (2006) has found similar result for other three Quercus species In this experiment, we
have simulated the Mediterranean-climate seasonal drought predictably occurring few months after seedling emergence. Thus, seedlings grown under varied irradiance conditions, and accordingly acclimated with different leaf structural traits, are all subjected to a water shortage. When drought stress becomes more severe, first-year seedlings have already finished their growth, and therefore have low ability to modify structural leaf traits, which usually have a large ontogenetic component. However, they show a high physiological responsiveness to optimise photosynthesis/transpiration ratios under drought conditions.

The ability to respond to light by modifying leaf structural traits may confer shade tolerance by increasing light-capture efficiency (Valladares et al. 2002a). At the same time, the relatively high responsiveness of leaf physiology may also indicate a tolerance to high irradiance (Valladares et al. 2002b). These leaf traits are associated to the plant's physiological response to the abundance of resources, and are related to the growth and survivorship of plants (Lambers and Poorter 1992; Wright et al. 2004). Concerning this, the importance to scale up from leaf level to whole plant level has been well documented in other works (see Valladares et al. 2005; Zavala and Bravo 2005). In our study, RGR of seedlings in the six combination of light and water, showed a good correlation with the carbon balance estimation, indicating that leaf characteristics may be a good predictor of the relative growth rate under contrasting light and water conditions.

In the closed forest understorey environment, deep shade, independently of water availability, can be a limiting factor in maintaining a positive carbon balance. On the other hand, extreme drought, at all levels of light, is a problem for the seedling in terms of avoiding water loss and maintaining carbon uptake, and therefore of biomass gain.

In conclusion, the results of this experiment showed that shade conditions seemed to ameliorate, or at least not aggravate, the drought impact on cork oak seedlings, and therefore, drought response on leaf and plant performance depends on the light environment.

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