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Water-use efficiency in cork oak (*Quercus suber*) is modified by the interaction of water and light availabilities

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Summary   We studied the interaction of light and water on water-use efficiency in cork oak (*Quercus suber* L.) seedlings. One-year-old cork oak seedlings were grown in pots in a factorial experiment with four light treatments (68, 50, 15 and 5% of full sunlight) and two irrigation regimes: well watered (WW) and moderate drought stress (WS). Leaf predawn water potential, which was measured at the end of each of two cycles, did not differ among the light treatments. Water-use efficiency, assessed by carbon isotope composition (δ^{13}C), tended to increase with increasing irradiance. The trend was similar in the WW and WS treatments, though with lower δ^{13}C in all light treatments in the WW irrigation regime. Specific leaf area increased with decreasing irradiance, and was inversely correlated with δ^{13}C. Thus, changes in δ^{13}C could be explained in part by light-induced modifications in leaf morphology. The relationship between stomatal conductance to water vapor and net photosynthesis on a leaf area basis confirmed that seedlings in higher irradiances maintained a higher rate of carbon uptake at a particular stomatal conductance, implying that shaded seedlings have a lower water-use efficiency that is unrelated to water availability.

Keywords: carbon isotope composition, uptake, drought, specific leaf area, photosynthesis, shade, stomatal conductance, water potential.

Introduction

Water-use efficiency (WUE) is positively related to water-stress tolerance (Ehleringer 1993). The increase in the ratio of carbon fixed to water consumed may be adaptive in drought-prone environments (Dawson and Ehleringer 1993, Donovan and Ehleringer 1994), although not always (Nguyen-Queyrens et al. 1998, Pita et al. 2001). Water-use efficiency has frequently been analyzed by studying the variability of a single environmental resource (Hanba et al. 1997, Le Roux et al. 1999, Le Roux et al. 2001), considering water stress as the main source of variation (Ehleringer 1993, Damesin et al. 1998, Ferrio et al. 2003). However, the availability of nutrients, light and CO_2 may also affect WUE (Jackson et al. 1993, Abrams and Mostoller 1995, Valladares et al. 2000, Niinemets and Valladares 2004).

Instant gas exchange measurements are not always representative of the WUE sustained by the plant throughout the growing season (Dawson et al. 2004) because they do not account for carbon losses associated with respiration or for the transient decoupling of net photosynthesis and stomatal conductance under non-steady state conditions of gas exchange. Isotopic discrimination of ^{13}C in leaf dry matter offers a more realistic and holistic integration of the long-term balance between carbon assimilation and water loss in plants. It is based on the relationship that links ^{13}C depletion along the CO_2 diffusion pathway, as well as the biochemical discrimination by Rubisco, to the long-term intercellular CO_2 concentration, C_i, which is inversely related to the WUE when photosynthesis is limited by stomatal conductance (Farquhar et al. 1989). However, several reports have provided evidence of variation in the relationship between WUE and the δ^{13}C signal (Donovan et al. 2000), particularly as a result of changes in the internal resistances of the leaf to CO_2 diffusion from intercellular spaces to carboxylation sites (Ethier and Livingston 2004, Warren and Adams 2006).

In Mediterranean areas, seedlings in the forest understory are subjected to scarcity of soil water and other resources, especially light, during the dry summer months (Aranda et al. 2002, Valladares and Pearcy 2002, Sack et al. 2003). The success of long-term advanced regeneration in the Mediterranean region therefore depends on the capacity of seedlings to survive prolonged drought and deep shade. To gain a deeper understanding of seedling responses to limited resources, we examined if limiting availabilities of light and water have differ-

ent effects on seedling WUE. We also determined what type of trade-off is established between drought tolerance and shade tolerance when WUE is considered a set point for the control of water consumption by the plant.

Material and methods

Acorns were collected at the end of autumn in a natural population of *Quercus suber* L. at Valle del Tiétar in the Iberian Peninsula (5º6′ W, 39º58′ N) and stored at 4 ºC for six months. The seeds were germinated on a perlite seed-bed at 25 ºC. About 120 healthy germinated seeds with a mean radicle length of 2 cm were selected and placed in 3-l pots filled with a peat:sand mixture (3:1, v/v) containing 3 g l^{-1} of a slow-release fertilizer (N,P,K, 20,10,20 + micronutrients). The experiment was carried out in a greenhouse as a factorial design with four irradiances and two irrigation regimes. As epicotyls emerged, the seedlings were randomly assigned to one of four treatment groups and placed in 12 metal frames covered with neutral shade cloth. Photosynthetic photon flux (PPF) of full sunlight was measured on a sunny day with a quantum sensor (Li-190, Li-Cor, Lincoln, NE). In the light treatments, values of PPF relative to full sunlight and as a mean daily integral were 66-70% and 34.66 mol m^{-2} day^{-1} (L$_{68}$), 44–50% and 23.22 mol m^{-2}day^{-1} (L$_{50}$); 13.5–16% and 7.92 mol m^{-2} day^{-1}(L$_{15}$); and 5–6% and 2.59 mol m^{-2} day^{-1} (L$_5$). Plants were located in a randomized block design with three complete blocks per light treatment. Two irrigation regimes, well watered (WW) and moderate drought stress (WS), were established in each block.

One month after seeding, half of the seedlings (WS) were subjected to an initial cycle (51 days) of water stress until volumetric soil water content fell to 7–10%. After the target soil water content was reached in the first 15 days, plants were maintained between 7-10% until the end of the water stress cycle. Pots containing the remaining seedlings (WW) in all light treatments were maintained at a volumetric soil water content of about 25%. Soil water content was monitored twice weekly by time domain refractometry (TDR, Trase System I, Soil Moisture Equipment Corp., USA). To ensure that the water-stress treatment was applied approximately evenly across the light treatments, the pots containing the L$_{68}$WS, L$_{50}$WS and L$_{15}$WS seedlings were watered differentially twice a week, based on the soil water content of the pots in the L$_5$WS treatment. This protocol assured the maintenance of all seedlings within a similar range of water availabilities throughout the experiment, despite differences in the water consumption related to differences in the light environments (for more details, see Pardos et al. 2005).

After the first cycle of water stress (51 days), the WS seedlings were watered to the same volumetric soil water content (25%) as the WW seedlings, and then immediately subjected to a second water-stress cycle (38 days) until volumetric soil water content fell to 12%. At the end of each water stress cycle, and before re-watering, leaf predawn water potential (Ψ$_{pd}$) was measured with a pressure chamber (PMS 1000, Corvallis In-

struments).

Gas exchange

Gas exchange was measured at the end of each water stress cycle. Three to five seedlings per treatment were transferred to a controlled environment chamber the day before measurements were made. Leaves were enclosed in a broadleaf-type chamber for 15–20 min before measurements were made with a portable photosynthesis system (LCPro+, ADC BioScientific Ltd., Hoddesdon, U.K.) at 25 ºC ± 0.5, a vapor pressure deficit of 1.6 ± 0.1 kPa and a CO_2 concentration of 365 ppm. Seedlings from the the L$_{68}$ and L$_{50}$ treatments were exposed to a PPF of 1200 μmol m^{-2} s^{-1}, whereas seedlings in the L$_{15}$ and L$_5$ light treatments were exposed to an irradiance of 1000 μmol m^{-2} s^{-1}. Gas exchange was monitored until a constant net photosynthetic rate was reached. Photosynthetic rates were expressed on a leaf mass (A_m) and an area (A_a) basis.

Specific leaf area and N content

Two to three leaves per seedling, including the leaf used for gas exchange measurements, were sampled for nitrogen analysis and specific leaf area (SLA) determination. Leaf area was measured with an image analyzer (Delta-T). Leaves were then dried at 65 ºC for 72 h, finely ground with a mortar and analyzed for total nitrogen by the Kjeldahl method. Nitrogen concentration was expressed on a leaf mass (N_m) and an area (N_a) basis.

Carbon isotope discrimination

A subsample of the ground tissue sample analyzed for leaf N was analyzed for δ^{13}C with an elemental analyzer (Carlo Erba 1108) coupled to a mass spectrometer IRMS (Isochrom, Micromass), at the SIDI of the Autonomous University of Madrid. The analytical method had a precision of ± 0.2 ‰.

Statistical analysis

Effects of sample date, light regime and irrigation treatment on gas exchange and δ^{13}C were evaluated by analysis of variance, using a completely randomized block design. The significance of differences among regressions between variables was tested by analysis of covariance (ANCOVA). If the interaction term was significant, differences among slopes were tested by the separate slope model. When the interaction term was not significant but the effect of light treatment was significant, the common slope model was used to test for differences in the intercepts of the regressions (Sokal and Rohlf 1995).

Results

The light treatments had no effect on seedling water status, assessed as leaf Ψ$_{pd}$, at the end of either water-stress cycle (Table 1). There were significant differences in leaf Ψ$_{pd}$ between watering regimes at the end of both water-stress cycles ($P < 0.05$ in both cases). Soil water content measurements during the experiment paralleled the Ψ$_{pd}$ results, indicating that Ψ$_{pd}$ was a reliable surrogate for soil water availability. Mean volu-

Table 1. Predawn water potential (Ψ_{pd}) assessed at the end of two waters stress cycles in cork oak seedlings growing in 68 (L_{68}), 50 (L_{50}), 15 (L_{15}) or 5% (L_5) of full sunlight. Values are means ± SE for $n = 3$–5. Letters indicate significant differences between treatments.

	L_{68}	L_{50}	L_{15}	L_5
Cycle 1				
Well watered (WW)	−0.49 ± 0.04 a	−0.47 ± 0.08 a	−0.53 ± 0.03 a	−0.54 ± 0.05 a
Drought (WS)	−0.79 ± 0.06 b	−0.93 ± 0.14 b	−0.83 ± 0.05 b	−0.85 ± 0.06 b
Cycle 2				
Well watered (WW)	−0.46 ± 0.01 a	−0.45 ± 0.02 a	−0.49 ± 0.01 a	−0.46 ± 0.04 a
Drought (WS)	−0.67 ± 0.04 b	−0.81 ± 0.05 b	−0.73 ± 0.11 b	−0.77 ± 0.05 b

metric soil water content of pots in the well-watered and moderately stressed treatments were 25–30 and 10–12%, respectively (data shown in Pardos et al. 2005).

Specific leaf area was highest in seedlings growing in the lowest irradiance (L_5) (Table 2). The irrigation regimes had no significant effect on SLA (Table 2). Although N_a changed with irradiance, N_m did not (Figure 1), indicating that the increase in N_a with irradiance was a consequence of the decline in SLA with increasing irradiance (Table 2).

Changes in A_a with changing irradiance in WW seedlings can be explained by the effect of light on SLA, because the light treatments had no effect on A_m (Table 3). Drought caused a decrease in A_a and A_m in seedlings in all light treatments, and the decrease was most marked during the first water-stress cycle. Stomatal conductance to water vapor (g_s) followed a similar pattern to A_a, reaching the highest and lowest values in seedlings in the L_{68} and L_5 irradiance treatments, respectively. The relationship between A_a and g_s was light dependent (ANCOVA, $P < 0.05$), with a lower slope for seedlings in the most heavily shaded treatments ($P < 0.05$). Thus, based on stomatal conductance to water vapor, carbon uptake was higher in seedlings in the L_{68} and L_{50} treatments than in the L_{15} and L_5 treatments (Figure 2).

Carbon isotope composition

Light and water availability had significant effects on $\delta^{13}C$ ($P < 0.05$, in both cases). The increase in $\delta^{13}C$ with increasing irradiance showed a hyperbolic trend in both water-stress cycles (Figure 3). Drought increased $\delta^{13}C$ in water-stressed seedlings, with a tendency to larger differences between well-watered and drought-stressed seedlings as light availability decreased, although the interaction between light and irrigation was not significant ($P > 0.05$). There was a negative relationship between $\delta^{13}C$ and SLA (Figure 4). Differences in $\delta^{13}C$ between irrigation regimes were explained by the different intercepts in the relationship between SLA and $\delta^{13}C$ (ANCOVA and common slope model).

Discussion

Little information is available on the physiological mechanisms underlying the different responses of plants to the interactive effects of above- and belowground resources (Holmgren 2000, Brett and Knapp 2001, Prider and Facelli 2004, Aranda et al. 2005b). We found that shaded plants had a lower WUE when exposed to drought than plants in full sunlight. Water-use efficiency, assessed by carbon isotope composition, increased in response to drought. The WUE response to irradiance was similar to that previously reported (Schleser 1990, Berry et al. 1997, Hanba et al. 1997, Le Roux et al. 2001). The contrasting responses of WUE to drought and shade can be explained on the basis that the interaction between irradiance and

Table 2. Specific leaf area (SLA) and leaf nitrogen concentration (N_m, mass basis and N_a, area basis) of well-watered and drought-stressed cork oak seedlings grown in 68 (L_{68}), 50 (L_{50}), 15 (L_{15}) or 5% (L_5) of full sunlight. Values are means ± SE for $n = 3$–5. Within a column, different letters indicate statistically different values for light treatments on each water stress cycle at $P < 0.05$ based on a least significant difference test of treatment means.

Cycle	Treatment	SLA ($m^2\ kg^{-1}$)		N_m ($mg\ g^{-1}$)		N_a ($g\ m^{-2}$)	
		Well watered	Drought	Well watered	Drought	Well watered	Drought
1	L_{68}	7.16 ± 0.27 a	8.44 ± 0.8 a	18.4 ± 1.1 a	21.6 ± 1.1 a	2.61 ± 0.41 a	2.61 ± 0.36 a
	L_{50}	5.94 ± 0.76 a	8.13 ± 0.37 a	16.9 ± 1.1 a	18.6 ± 1.1 a	3.03 ± 0.32 a	2.41 ± 0.36 a
	L_{15}	10.05 ± 0.84 b	10.87 ± 0.52 b	16.2 ± 1.1 a	21.8 ± 1.1 a	1.65 ± 0.36 b	2.04 ± 0.36 b
	L_5	12.79 ± 1.07 c	14.05 ± 2.04 c	18.6 ± 1.1 a	20.0 ± 1.0 a	1.50 ± 0.32 b	1.54 ± 0.32 b
2	L_{68}	8.41 ± 1.34 a	9.06 ± 1.04 a	20.6 ± 1.0 a	20.0 ± 1.1 a	2.40 ± 0.32 a	2.30 ± 0.36 a
	L_{50}	9.12 ± 0.34 a	8.6 ± 2.19 a	20.1 ± 1.3 a	21.3 ± 1.3 a	2.63 ± 0.32 a	3.08 ± 0.41 a
	L_{15}	11.92 ± 0.25 b	13.17 ± 0.51 b	20.6 ± 1.0 a	20.7 ± 1.3 a	1.73 ± 0.41 b	1.60 ± 0.41 b
	L_5	19.38 ± 1.75 c	17.03 ± 1.87 c	19.6 ± 1.0 a	20.2 ± 2.2 a	1.06 ± 0.32 b	1.35 ± 0.72 b

Table 3. Photosynthetic parameters for well-watered and drought-stressed cork oak seedlings growing in 68 (L_{68}), 50 (L_{50}), 15 (L_{15}) or 5% (L_5) of full sunlight. Values are means (± SE for n = 3–5). Within columns, different letters indicate statistically different values for light treatments within a water stress cycle at $P < 0.05$ based on a least significant difference test of treatment means.

Cycle	Treatment	A_a (µmol m^{-2} s^{-1})		A_m (µmol kg^{-1} s^{-1})		g_s (mmol m^{-2} s^{-1})	
		Well watered	Drought	Well watered	Drought	Well watered	Drought
1	L_{68}	12.78 ± 1.32 a	5.60 ± 1.40 a	91 ± 11 a	55 ± 13 a	133 ± 11 a	58 ± 10 a
	L_{50}	9.66 ± 1.44 ab	5.44 ± 1.03 a	61 ± 17 a	43 ± 8 a	121 ± 21 a	56 ± 7 a
	L_{15}	7.01 ± 1.34 ab	4.36 ± 1.31 a	71 ± 18 a	45 ± 13 a	96 ± 26 a	58 ± 15 a
	L_5	6.73 ± 1.07 b	3.04 ± 0.45 a	85 ± 15 a	43 ± 8 a	87 ± 18 a	39 ± 5 a
2	L_{68}	12.07 ± 1.89 a	10.34 ± 1.28 a	100 ± 35 a	95 ± 19 a	161 ± 24 a	115 ± 18 a
	L_{50}	11.88 ± 1.64 a	6.59 ± 2.21 ab	96 ± 12 a	71 ± 37 a	142 ± 22 ab	74 ± 27 ab
	L_{15}	6.92 ± 1.79 b	3.83 ± 1.29 b	83 ± 27 a	54 ± 18 a	110 ± 32 ab	56 ± 23 ab
	L_5	5.94 ± 0.59 b	3.33 ± 1.20 b	115 ± 15 a	81 ± 18 a	85 ± 8 b	36 ± 14 b

drought on leaves differs depending on whether plants are grown in a high-light or a low-light environment (Niinemets et al. 2004). The increase in leaf $\delta^{13}C$ with irradiance indicates a decline in long-term integrated C_i, and therefore an increase in WUE (Israeli et al. 1996, Niinemets et al. 2004). This inference is reinforced by the slope of the A_a–g_s relationship at saturating irradiance, which was lower for seedlings grown in the most heavily shaded treatments (L_{15} and L_5) than in the sunlit treatments (L_{68} and L_{50}; Figure 2). This means that the

amount of carbon captured relative to the amount of water lost through transpiration was lower for leaves of seedlings in shade than in full sun.

The negative relationship between SLA and $\delta^{13}C$ demonstrates that WUE responds to variations in irradiance through modifications in leaf morphology (Vitousek et al. 1990, Sparks and Ehleringer 1997). The decrease in SLA with increasing irradiance is accompanied by a decrease in the leaf area:leaf volume ratio, thereby improving the potential for carbon uptake relative to transpirational water loss (Nobel 1991). Thus, changes in SLA resulting from modification of the leaf structure is the underlying mechanism facilitating acclimation
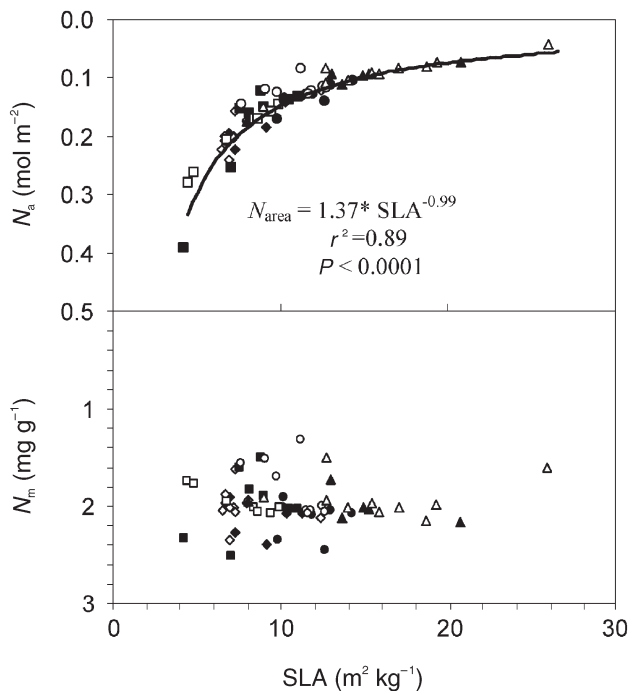

Figure 1. Relationship between specific leaf area (SLA; m^2 kg^{-1}, upper graph) and area-based leaf nitrogen concentration (N_a; mol m^{-2}) in leaves of well-watered (WW; open symbols) and water-stressed (WS; closed symbols) cork oak seedlings growing in 68 (L_{68}; □,■), 50 (L_{50}; ◇,◆), 15 (L_{15}; △,▲) or 5% (L_5; ○,●) of full sunlight. No relationship was observed for SLA and mass-based nitrogen concentration (N_m mg g^{-1}, lower graph).
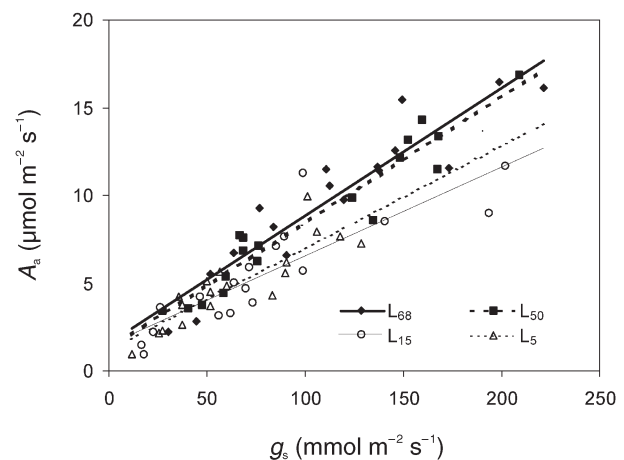

Figure 2. Relationship between net photosynthesis on a leaf area basis (A_a) and stomatal conductance to water vapor (g_s) in cork oak seedlings growing in 68 (L_{68}), 50 (L_{50}), 15 (L_{15}) or 5% (L_5) of full sunlight. The equations for the relationships are: 68%, A_a = 1.567 + 0.073 g_s, r^2 = 0.87 ($P < 0.001$); 50%, A_a = 1.249 + 0.072 g_s, r^2 = 0.93 ($P < 0.001$); 15 %, A_a = 1.502 + 0.050 g_s, r^2 = 0.74 ($P < 0.001$); 5%, A_a = 1.148 + 0.058 g_s, r^2 = 0.77 ($P < 0.001$). Based on comparisons of the slopes of the regressions, seedlings growing in the 68 or 50% light treatments differed significantly from seedlings growing in the 15 or 5% light treatments.
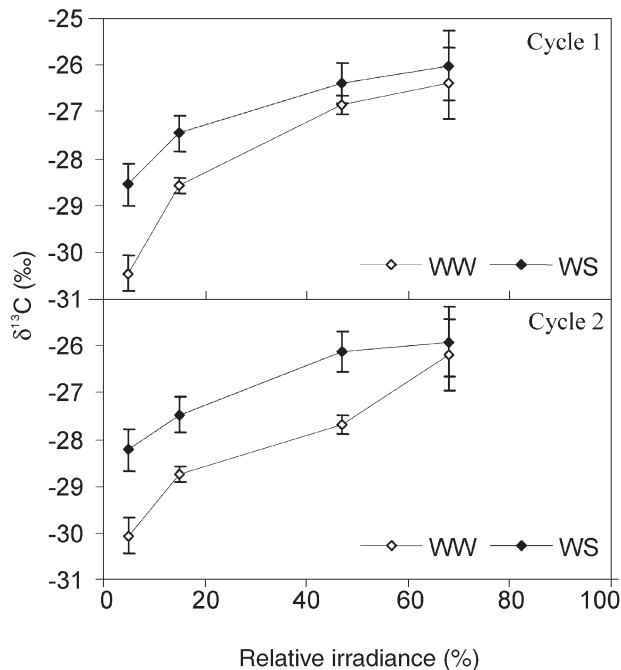
Figure 3. Leaf carbon isotope concentration (δ^{13}C) in leaves of well-watered (WW; $\diamond$) and water-stressed (WS; $\blacklozenge$) cork oak seedlings growing in 68, 50, 15 or 5% of full sunlight. Differences were highly significant for light environment and irrigation ($P < 0.0001$).

Figure 4. Relationship between specific leaf area (SLA; $m^2\ kg^{-1}$) and leaf carbon isotope composition (δ^{13}C) in leaves of well-watered (WW; open symbols) and water-stressed (WS; closed symbols) cork oak seedlings growing in 68 (L_{68}; $\square$,$\blacksquare$), 50 (L_{50}; $\diamond$,$\blacklozenge$), 15 (L_{15}; $\triangle$,$\blacktriangle$) or 5% (L_5; $\circ$,$\bullet$) of full sunlight. The regression models for the well-watered (discontinuous line and open symbols) and water-stressed seedlings (continuous line and closed symbols) are shown. Intercepts were significantly different at $P < 0.001$.

to light (Le Roux et al. 1999, Le Roux et al. 2001, Robakowski et al. 2003, Aranda et al. 2005b). We predict that a similar mechanism operates in shade-acclimated plants whereby the increase in SLA in response to shade tends to maximize carbon gain. This prediction is supported by the finding that an increase in SLA in response to increasing shade is accompanied by an increase in leaf area ratio, which is related to the optimization of light capture in low-light environments (Poorter and Nagel 2000, Sack and Grubb 2001).

The decrease in SLA that occurs during photoacclimation results from increases in the thickness and density of the leaf blade (Aranda et al. 2005a), and a more developed palisade parenchyma (Ashton and Berlyn 1994, Niinemets 1999, Aranda et al. 2004), leading to an increase in mesophyll cell surface area, which is positively correlated with internal conductance to CO_2 (g_i) (Evans et al. 1994, Syvertsen et al. 1995, Hanba et al. 1999, Terashima et al. 2001) and, in turn, with photosynthetic capacity (Hanba et al. 2002, Piel et al. 2002). Based on our results and the cited studies, we speculate that mesophyll conductance (g_i) has a similar effect on WUE as it has on the photosynthetic capacity of well-watered plants. Furthermore, several papers have reported that the relationship between WUE and carbon isotope composition can be modified by g_i (Ethier and Livingston 2004, Warren and Adams 2006). It is unclear, however, if water stress modifies g_i, and to our knowledge the response of g_i to the interaction of light environment and water stress has never been analyzed.

The effect of shade on SLA of *Q. suber*—SLA was almost twice as large in 5% of full sunlight as in 68% of full sunlight
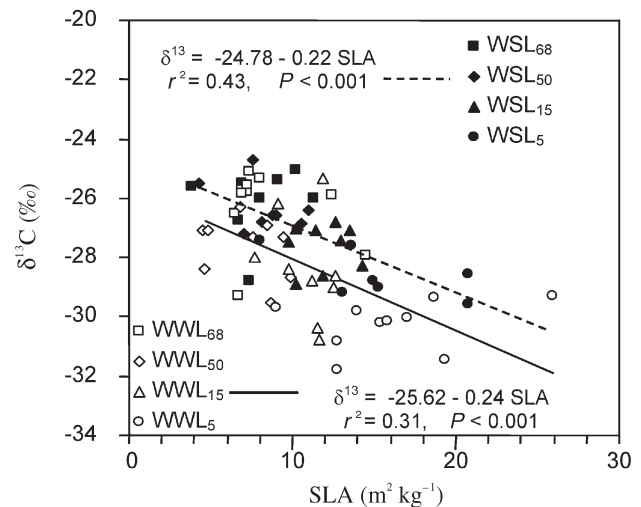
(Table 2)—is similar to that reported for other Mediterranean oaks such as *Q. ilex* and *Q. coccifera* (Valladares et al. 2000). The greatest morphological change in SLA occurred in response to irradiances of 15–50% of full sunlight. Most of the physiological parameters studied also showed a maximum change between 15 and 50% full sunlight, suggesting that, in cork oak, the potential for acclimation to a natural resource is non-linear, but is characterized by a threshold for adjustment that is specific for each resource and physiological or anatomical trait.

In summary, shade lowered WUE, inferred from δ^{13}C (Israeli et al. 1996, Hanba et al. 1997), even when plants were water stressed. This finding corroborates previous results showing that shade reduces drought tolerance through mechanisms such as osmotic adjustment (Augé et al. 1990, Aranda et al. 2001, Aranda et al. 2005b, Pardos et al. 2005).

Acknowledgments

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