

Growth, nutrient, water relations, and gas exchange in a holm oak plantation in response to irrigation and fertilization

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Received 18 September 2003; accepted in revised form 20 August 2004

Key words: Climatic conditions, Nutrient status, *Quercus ilex* L., Seedling growth, Seedling physiology, Water supply

Abstract. Eighty 6-years-old *Quercus ilex* L. subsp. *ballota* seedlings planted on a former agricultural land were subjected during two growing seasons to one of four treatments: fertilization and irrigation, irrigation, fertilization, and control. Seasonal and between-treatment variations on water relations, gas exchange parameters, growth and nutrient status were analyzed. Water potential (Ψ) was related to climatic conditions. Thus, the frequent rain storms during the summer allow seedlings to maintain relatively high Ψ values, joined to moderate photosynthetic activity. Differences on Ψ , photosynthesis and stomatal conductance due to irrigation were shown at the onset and end of growth, related with lower water availability. Fertilization had a greater effect on growth than irrigation. Both fertilization and irrigation positively affected the relative increment on leaf nutrient concentration at the end of the second year. The results indicate that water availability was not a limited factor, thus irrigation is not justified; while fertilization improve growth.

Introduction

Flanking measures were adopted in 1992 by the EU to accompany the CAP (Common Agricultural Policy) reform. As part of one of the main areas of reform, an aid to promote afforestation as an alternative use of agricultural land was included. Spain was highly benefited by this aid, with more than 200,000 ha of agricultural land afforested, which represented 47% of the total area afforested in the EU (Report to Parliament 1997). The afforestation scheme seemed attractive for farmers, as woodlands were established preferably on low profitable permanent grasslands or on unproductive arable lands, usually located in poor sites, with irregular and limited rainfall. Overall, 56% of afforested areas in Spain involved broadleaved species, mainly mediterranean oak species, including *Quercus ilex* L. (hereafter referred to as holm oak). Mycorrhized holm oak seedlings have been commonly used for afforestation purposes; thus, farmers improve their land income by the short-term truffle yield. Holm oak is the most widely distributed species in the Iberian Peninsula, and the dominant tree species of most mature communities of the western Mediterranean basin (Terradas and Savé 1992; Sala and Tenhunen 1994).

Seedlings planted on former agricultural land (i.e., open areas) are exposed to extreme environmental conditions. The most dramatic ones are alterations in heat exchange processes and soil water relations caused by greater fluctuation in temperature and soil moisture (Grossnickle 1993; Oliveira and Peñuelas 2000), but also nutrient deficiencies. Agricultural exploitations modify the natural fertility of the soil, thus affecting later production of the afforested land. Plant nutrient status may have a significant impact on drought tolerance (Kleiner et al. 1992). Plants growing under conditions of high nutrient availability can exhibit a greater ability to maintain growth, stomatal conductance and photosynthesis during drought in comparison with plants growing on infertile sites (Hechler et al. 1991), although plants adapted to low nutrient availability can maintain basic physiological processes under water stress.

Water is frequently limiting to plants because of weather conditions, low soil holding capacity and vegetation competition, creating a wide range of growth environments, even in small areas. Each species, and even the same species under different growing conditions, develop a particular behavior in response to water stress (Grossnickle 1993). Under natural conditions, holm oak avoids summer water deficits by stomatal closure (Romane and Terradas 1992), as occurred in most deep-rooted species (Hinckley et al. 1983). For this purpose, holm oak employs mechanisms to maintain positive turgor during periods of limited water availability and morphological adaptations (Terradas and Savé 1992). Differences in the ability to regulate stomatal conductance may determine individual species persistence on a changing environment (Abril and Hanano 1998).

Most of the studies on holm oak ecophysiology are conducted under natural conditions (Duhme and Hinckley 1992; Romane and Terradas 1992; Castell et al. 1994; Sala and Tenhunen 1994; Nardini et al. 2000; Oliveira and Peñuelas 2001), but little is known about the field performance of holm oak under the special conditions of afforested areas. Defining how holm oak seedlings respond to water and nutrient availability would improve our understanding of their ecological potential to grow under afforestation site conditions. This study investigated water relations, gas exchange parameters, nutrient content and morphological development during two growing seasons, of afforested holm oak seedlings submitted to irrigation and fertilization. The afforested land had been for more than 20 years used for low quality barley production. We tested the following hypothesis: (1) the microclimatic conditions in our site during the summer months (with surprising frequent rain and hail storms) can decrease the positive effect of irrigation, a common practice used in afforested lands with broadleaved species. If no major climatic constraints are identified (i.e., dry summer with no storms), holm oak seedlings will be able to maintain their photosynthetic capacity and to tolerate moderate water shortage; (2) holm oak seedling's growth and biomass are positively affected by fertilization and irrigation, mainly when seedlings are outplanted in old arable lands, with poor soils. Will the interaction of these two treatments have an additive and positive effect on seedling's growth? The potential of holm oak seedlings to utilize available nutrients in response to nutrient supply can be identified.

Materials and methods

Study site

The holm oak plantation was located at Teruel, Spain (40°21'N, 1°16'W, elevation 1100 m). The average slope was 2%, facing East–Southeast. Around the site, scattered trees of *Q. ilex*, *Q. faginea*, *Juniperus thuriphora*, *J. phoenicea* and *J. communis* were found. Soil is haplic cambisol. Annual mean temperature is 11.8 °C and annual mean rainfall is 375 mm. Mean temperature during the growing season (April–October) is 16.2 °C. Maximum and minimum absolute temperatures are 30.4 °C (July) and – 2.1°C (January). The period with frosts goes from December to February, but frost are probable between February and May and again between October and November. The dry season occurs from mid-June to mid-July (Figure 1).

Compared to the last 20-year mean precipitation during the growing season (April to October, 283 mm), precipitation during 1997 growing season was higher than the mean (313.8 mm), but lower in 1996 (207.1 mm). Annual precipitation was 359.8 mm in 1996 and 467.7 mm in 1997, thus precipitation during the growing season represented 58.6% in 1996 and 67.5% in 1997. Compared to mean air temperature, mean value during 1996 growing season was normal (16.0 °C), but was higher than the mean in 1997 (17.2 °C). Storm

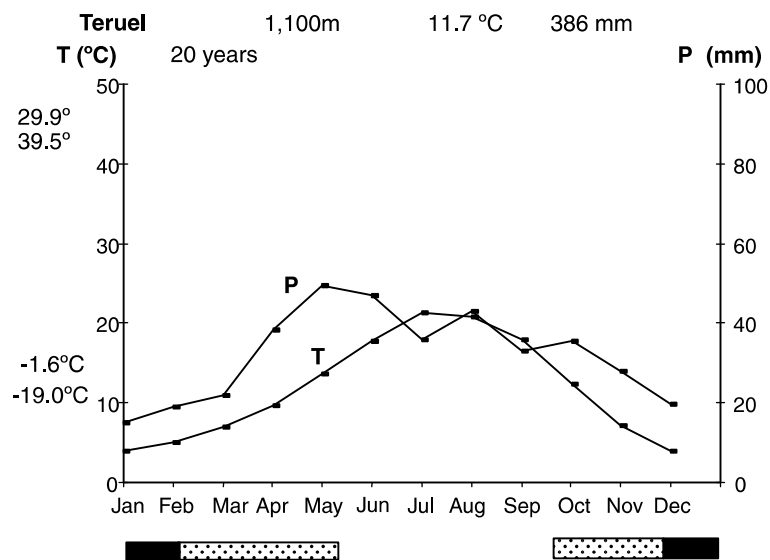


Figure 1. Climate diagram of the study site between 1977 and 1997. Redrawn from Walter et al. (1975). Mean temperature: 11.7 °C; mean annual rainfall: 386 mm; maximum absolute temperature: 39.5 °C; minimum absolute temperature: –19.5 °C; mean maximum in the hottest month: 29.9 °C; mean minimum in the coldest month: –1.6 °C. T: temperature, R: rainfall. ■: period with frost; ▨: period with possible frosts.

events occurred between June and September. They represented 46% in 1996 and 66% in 1997 of days with rainfall, which corresponded to 82% in 1996 and 94% in 1997 of total precipitation during this period.

Experimental design

Two-years-old holm oak (*Q. ilex* L. spp. *ballota*) seedlings were planted during March 1992 after site preparation, in a 7 m × 7 m spacing design, for a total of 20 ha, and irrigated immediately afterwards. Irrigation was repeated every summer until establishment of the experiment in 1996, but no fertilizer was added. Dead seedlings were replaced during the first 2 years. Four 250 m² measuring plots centered in the plantation were set up in January 1996 in already established seedlings, with a functioning root system. Eighty seedlings were arranged in a 2 × 2 factorial with treatments replicated in four blocks (measuring plots). Five seedlings per block were subjected to one of four treatments (i.e., 20 seedlings per treatment): unfertilized and non-irrigated (F− /W−); unfertilized and irrigated (F− /W+); fertilized and non-irrigated (F+ /W−); and fertilized and irrigated (F+ /W+). Seedlings in the W+ treatments received the equivalent to 40 l m^{−2} rainfall (10 l per seedling) on each irrigation date. Seedlings were irrigated monthly during May, June, July, Sept 1996, and April, June, July, Sept and Oct 1997, the day before physiological measurements. Water input in the W+ treatments (wat_{14d}) is considered to be the sum of irrigation and rainfall in the 2 weeks before the day of measurement. Water input in the W− treatments is just the rainfall in the 2 weeks before the day of measurement. A vertical-walled trench 0.5 m deep and approximately 1 m wide was dug around each seedling ensuring that sideways surface movement of water between irrigated and non-irrigated treatments could not occur. Each seedling in the F+ treatments received monthly 2.4 g of Peter's fertilizer 20:7:19 (0.1 g dm^{−3}) at the beginning of the growing season (May, June, July 1996 and April, June, July 1997) and 4:25:35, at the end (Sept 1996 and Sept, Oct 1997), just after irrigation. The fertilizer was applied in spade slits to avoid the risk of loss over the surface.

Resource measurements

Volumetric soil water content (SWC, %, v:v) and soil temperature (ST, °C) data were collected on the same days as measurements. Volumetric soil water content was measured at 15 cm depth with time domain reflectometry (TDR, Trase System I, Soil Moisture Equipment Corp., USA). Soil temperature was measured at 20 cm. Data of rainfall and temperature were gathered from a nearby meteorological station.

Soil pits were dug in representative sites on May 1996 to study the soil profile, and soil samples were collected for chemical and textural analyses. As

Table 1. Parameters obtained from the soil profile analysis

Horizon	Organic matter (%)	Soil moisture (%)	Active limestone (%)	N (%)	P (ppm)	K (ppm)	Texture classes
1	2.89	2.79	57.56	< 0.001	9.77	159.32	Loam
2	2.03	2.05	67.38				Loam
3	1.08	1.65	77.03				Silt loam
4	0.99	1.82	78.01				Silt loam

spatial variability was low, only two soil pits were considered. Each profile was divided in four horizons attending to the granulometric analysis. Parameters obtained on each horizon were: percentage of organic matter, percentages of sand, clay and silt, percentage of active limestone, and water content; and nutrient (N, P, and K) content. Texture was determined by sedimentation. CaCO_3 was determined by measuring the CO_2 developed after dissolution in HCl. Depth of the soil profile was between 2 and 2.5 m. Percentage of organic matter decreased with depth, while percentage of active limestone increased with depth (Table 1). Soil became more silty with depth. Soil was deficient in phosphorus and nitrogen. Potassium content was not so low. The land was poor in nutrients probably due to previous agricultural use.

Measurements of seedling physiological data

Leaf water potential (Ψ) and gas exchange parameters were measured in two different 2-years-old leaves per seedling, approximately the same size. Leaves were sampled from the middle canopy, exposed to full solar radiation. A pressure chamber (PMS 1000, Instrument Co., Corvallis, OR, USA) was used for leaf water potential measurements. Gas exchange parameters were measured with a portable IRGA (ADC-LCA4 Analytical Development Co. Ltd, Hoddeston, Herts., UK).

Predawn leaf water potential (Ψ_{pd} , MPa) and gas exchange parameters were measured every 40 days approximately from May to October 1996 and 1997. Daily minimum (midday) leaf water potential (Ψ_{min} , MPa) was measured in the same days as Ψ_{pd} , but not before July 1996. Seedlings darkened overnight with black plastic bags had Ψ_{pd} determined by 0600 h and Ψ_{min} and gas exchange were determined at 1300 h. Predawn leaf water potential was measured on 12 seedlings per treatment, and Ψ_{min} and gas exchange on 20 seedlings per treatment. Net photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and water vapor stomatal conductance (g_{ws} , $\text{mmol m}^{-2} \text{s}^{-1}$) were calculated. After each day of data collection, leaves measured for the gas exchange data were clipped and leaf area was determined with an image analyzer Delta-T, and gas exchange parameters were recalculated with actual leaf area (smaller than the standard 6.25 cm^2 area used by the IRGA software).

Measurements of seedling growth

All 80 seedlings were measured at d146 (25 May 1996), d412 (15 Feb 1997) and d642 (5 Oct 1997) for height and root collar diameter.

At the beginning and end of 1997 foliage density expressed as leaf area per crown volume was measured with a Li-Cor LAI-2000 plant canopy analyzer (Li-COR Inc., Nebraska). In one seedling of each treatment combination per plot, four LAI measurements on the north, west, south, and east were made below the first branch using a 180° view cap. Simultaneously, and for each seedling, above canopy light measurements were collected in the open field, where the light sensor had an unobstructed view of the sky.

Nutrient content in leaves

Nutrient concentration was determined in a sample of 15 current year, fully expanded leaves per seedling at the end of 1996 and 1997. Leaves were oven dried and ground to a fine powder (0.8 mm). Leaf tissue for each combination of treatment and block was pooled and then divided in three aliquots. Nitrogen was determined by acid digestion, distillation, and valuation by Kjeldahl method (Kjeldahl System 1026, Tecator). Phosphorus was determined by ammonium phosphomolibdate spectrophotometry (Shimadzu UV 1601), and potassium by flame photometry (Corning flame photometer 411). Leaves dry weight, nutrient concentration, and content were calculated. Total nutrient content was estimated as the product of concentration and dry weight.

Statistical analysis

Measures of nutrient content, gas exchange parameters, leaf water potential, and soil traits (moisture content and temperature) were examined within and between sampling dates using mixed models, with irrigation and fertilization as the fixed effects, and blocks as a random effect (PROC MIXED). As there was no block effect for any measured variable ($\text{Pr } z > 0.05$), only results for the fixed effects are presented. LS means were used to observe differences between measuring dates and among treatments within a date. Linear correlations between environmental variables and leaf water potential were calculated.

Shoot height and diameter data were log-transformed for normality. Initial height and initial diameter (at d146) were introduced in the model of shoot and diameter growth as covariates. Shoot height and diameter were analyzed by mixed procedures for repeated measurements (RM in PROC MIXED). The RM in PROC MIXED tested the effect of time, irrigation, and fertilization, as well as their interactions, over shoot height and diameter. In all analyses a critical value of $p < 0.050$ was used. All analyses were performed using SAS.

Nutrient data in 1997 was interpreted by the use of vector diagrams (Haase and Rose 1995). The most obvious use of vector diagrams is in examining plant responses to fertilizer regimes. Changes in nutrients and leaf biomass were displayed in a single graph on which nutrient content (x), nutrient concentration (y), and leaf dry weight (z) satisfied the function $(x) = f(y,z)$. Vector analysis allows the simultaneous comparison of plant growth, nutrient concentration, and nutrient content in an integrated graphic form. Values for the F- /W- treatment were used as reference points in the vector diagrams and assigned relative values of 100% in order to facilitate comparisons with a common base. Interpretation of the vector diagram is based on the magnitude and direction of each vector. The longest vector identifies the most responsive treatment of those analyzed. Shifts to the right or left of a diagonal represent increases or decreases, respectively, in unit weight. Shifts along the horizontal indicate a change in content and shifts along the vertical indicate a change in concentration.

Results

Soil traits (moisture content and temperature)

Average soil temperature at 20 cm varied seasonally in 1996 and 1997, it increased until September and decreased afterwards. Total increase was more pronounced during 1997 (2.4 °C in 1996 and 9.9 °C in 1997). The high soil temperature in September 1997 was related to high maximum air temperature (26.7 °C).

Volumetric soil water content during the growing period was higher in 1997 than in 1996 (Figure 2a). Depletion of soil water content during summer months was more acute in 1997. Irrigation significantly elevated soil water content in all measured months for both years (Figure 2a and b).

Response to environmental variables

Within a year of measurement, volumetric soil water content was positively correlated with water input in the 2 weeks before irrigation (wat_{14d}) (Table 2). When data were pooled for both years, water input explained 65% of the variation in volumetric soil water content (Table 2). ST was inversely correlated to water input during 1997. Soil temperature and volumetric soil water content were inversely correlated at the beginning (April) and end (September and October) of 1997 growing period. There was weak significant and negative relationship between midday water potential and predawn water potential with water input for the pooled data. This correlation was stronger during 1997, at

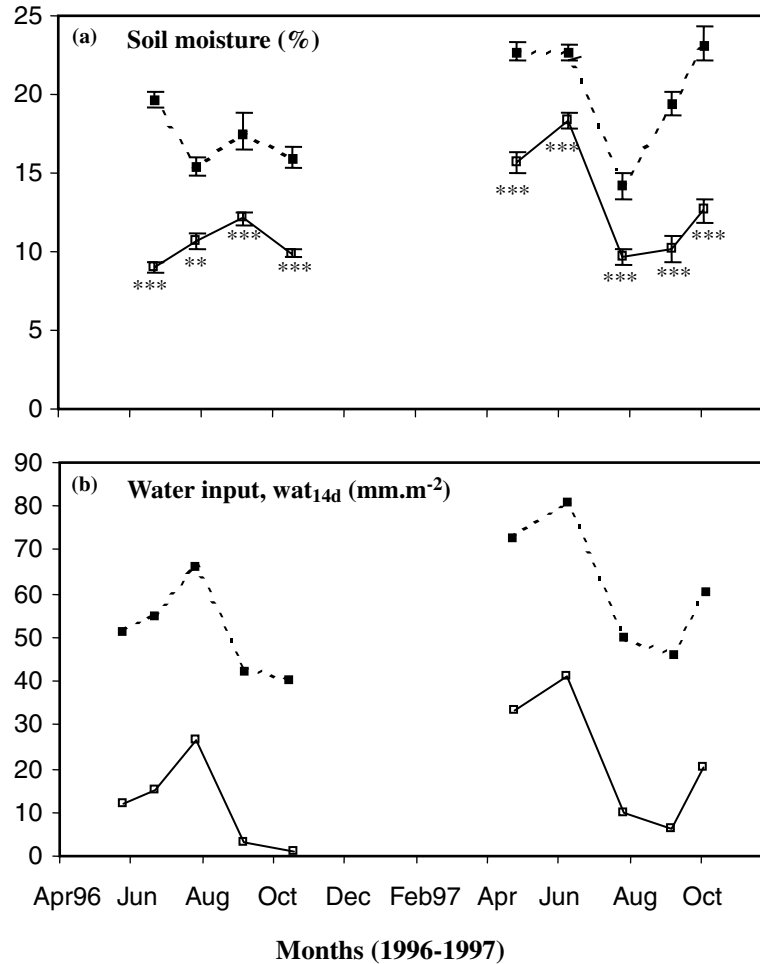


Figure 2. Volumetric soil water content (a) and water input in the two weeks previous to irrigation, wat_{14d} (b) from May to October 1996 and from April through October 1997, measured in (■) irrigated and (□) non-irrigated seedlings of a holm oak plantation. Symbols indicate the mean, I bars indicate the standard error. Significant differences between treatments within a date are shown by level of significance ($p < 0.001$ ***, $p < 0.01$ ** , $p < 0.05$ *, no asterisk: ns $p > 0.05$).

the beginning and end of the growing period. Predawn water potential was significantly related to midday water potential and inversely correlated to ST, for the pooled data. There was a linear relationship between midday water potential in 1997 and water input when irrigated and non-irrigated seedlings were separately analyzed (Figure 3). Both ST and water input (wat_{14d}) were also linearly related to midday water potential in the irrigated seedlings ($\Psi_{min} = -3.54 + 0.037wat_{14d} - 0.05 ST$, $r^2 = 0.50$, $p < 0.001$).

Table 2. Correlation values (Pearson's r) for leaf water potential (Ψ_{pd} and Ψ_{min}) and environmental measurements considering pooled data or separate years

	Ψ_{min}	ST	SWC	wat _{14d}
<i>Pooled data</i>				
Ψ_{pd}	0.46 (<0.001)	-0.41 (<0.001)	-0.16 (0.009)	-0.24 (<0.001)
Ψ_{min}		-0.38 (<0.001)	-0.48 (<0.001)	-0.47 (<0.001)
ST			-0.26 (<0.001)	ns
SWC				0.65 (<0.001)
<i>1996</i>				
Ψ_{pd}	ns	-0.31 (<0.001)	ns	-0.15 (0.023)
Ψ_{min}		ns	ns	ns
ST			-0.28 (<0.001)	0.26 (<0.001)
SWC				0.50 (<0.001)
<i>1997</i>				
Ψ_{pd}	0.16 (0.012)	-0.26 (0.024)	ns	-0.14 (0.035)
Ψ_{min}		0.53 (<0.001)	-0.45 (<0.001)	-0.59 (<0.001)
ST			-0.48 (<0.001)	-0.61 (<0.001)
SWC				0.83 (<0.001)

Probability values are shown in parenthesis ($p < 0.050$), ns non-significant.

Ψ_{pd} : predawn leaf water potential, MPa; Ψ_{min} : midday leaf water potential, MPa; ST: soil temperature, °C; SWC: volumetric soil water content, %v/v; wat_{14d}: water availability in the soil in the 2 weeks previous to irrigation, mm m⁻².

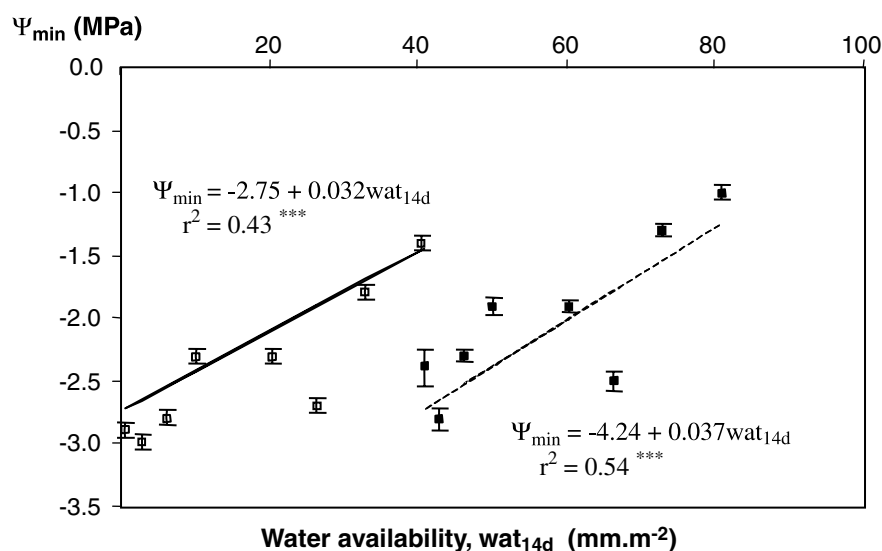


Figure 3. Relationships between midday water potential (Ψ_{min}) and water input in the 2 weeks previous to irrigation (wat_{14d}), from May to October 1996 and from April through October 1997 measured in (■) irrigated and (□) non-irrigated seedlings of a holm oak plantation. Each mean is comprised of the observations for a measuring date. Symbols indicate the mean, I bars indicate the standard error.

Leaf water potential

Patterns for Ψ_{pd} were similar for both years, although maximum values were higher in 1997 (Figure 4a). Mean Ψ_{pd} during the growing season of 1997 was between 32 and 70% of Ψ_{pd} during the growing season of 1996. Lower initial Ψ_{pd} values at the onset of growth in 1996 compared to 1997 were related to lower water available between October and April (1224 mm vs 2104 mm). At the end of the growing season of 1996 (Oct 1996, when the amount of water supplied to the soil was the lowest of all the growing period), a decrease on Ψ_{pd}

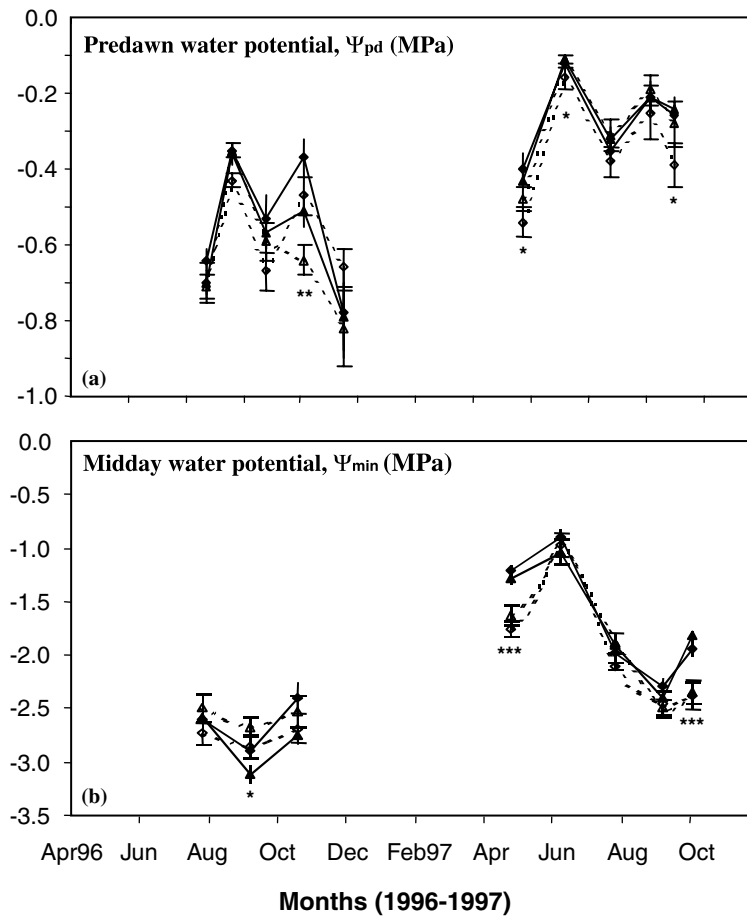


Figure 4. Predawn leaf water potential (a) and midday leaf water potential (b) from May to October 1996 and from April through October 1997, measured in (—▲—) fertilized + irrigated, (---△---) fertilized, (····◆····) irrigated and (—◇—) unfertilized and non-irrigated seedlings of a holm oak plantation. ($p < 0.001$ ***, $p < 0.01$ ***, $p < 0.05$ *, no asterisk: ns $p > 0.05$). Symbols indicate the mean, I bars indicate the standard error.

was observed for all seedlings, but mainly in non-irrigated seedlings ($p = 0.034$, Figure 4a). Significant lower values of Ψ_{pd} in the non-irrigated seedlings in comparison with irrigated seedlings were also apparent in Sept 1996 ($p = 0.010$), April 1997 ($p = 0.005$) and Oct 1997 ($p = 0.011$) (Figure 4a). The effect of fertilization on Ψ_{pd} was evident in Sept 1996 ($p = 0.001$) and June 1997 ($p = 0.034$) of the study. Fertilized and irrigated seedlings (F+/W+) showed the highest Ψ_{pd} values in Sep 1996, Apr 1997, June 1997 and Oct 1997 (Figure 4a).

Minimum (midday) leaf water potential (Ψ_{min}) during 1997 decreased from June to September, and recovered slightly at the end of the growing season (Figure 4b). The highest Ψ_{min} was shown in June 1997. Values in 1996 were lower than in 1997, related to lower water availability. The effect of irrigation on Ψ_{min} was evident at the end of growth in 1996 (Oct 1996, $p = 0.003$) and throughout all the 1997 measurements ($p < 0.001$). The sum effect of fertilization and irrigation on Ψ_{min} , with higher values, was shown in Sep 1996, Apr 1997, and Oct 1997. The highest daily amplitude on leaf water potential ($\Psi_{pd} - \Psi_{min}$) occurred in 1997 at the onset and end of growth ($p < 0.001$).

Gas exchange

Water vapor stomatal conductance (g_{ws}), transpiration rate (E) and net photosynthetic rate (A) increased for both years with the onset of growth, peaked in the summer and decreased at the end of growth to values higher or similar to initial ones (Figure 5). The surprising high value of A in July 97 can only be explained through rainfall. Water availability in the 2 weeks previous to measurement was 10.1 mm, and 10 mm of them occurred 3 days before measurement (23 July). Both A and E responded to this rainfall, while g_{ws} remained in a similar range.

Differences in A between non-irrigated and irrigated seedlings were only significant in Sep 1997 ($p = 0.012$) (Figure 5a). Irrigation increased g_{ws} in April ($p = 0.024$), Sep ($p = 0.033$), and Oct 1997 ($p = 0.001$) (Figure 5b); in contrast, IWUE (intrinsic water use efficiency, $\mu\text{molCO}_2 \text{ mmolH}_2\text{O}^{-1}$) was reduced ($p = 0.002$). Fertilization reduced E in June 1997. Irrigation increased E in Oct 1996 (Figure 5c).

Seedling growth

The results of the repeated measures indicated that the F tests for the between-units effect Fertilization and for the interaction Irrigation \times Fertilization were significant, for both shoot height and diameter (Table 3). For within-units effects, the sphericity test rejected the null hypothesis of a Type H matrix ($p < 0.001$), thus, the multivariate analyses were used. The multivariate tests for the within-units effects time was significant for shoot height and diameter (Table 3).

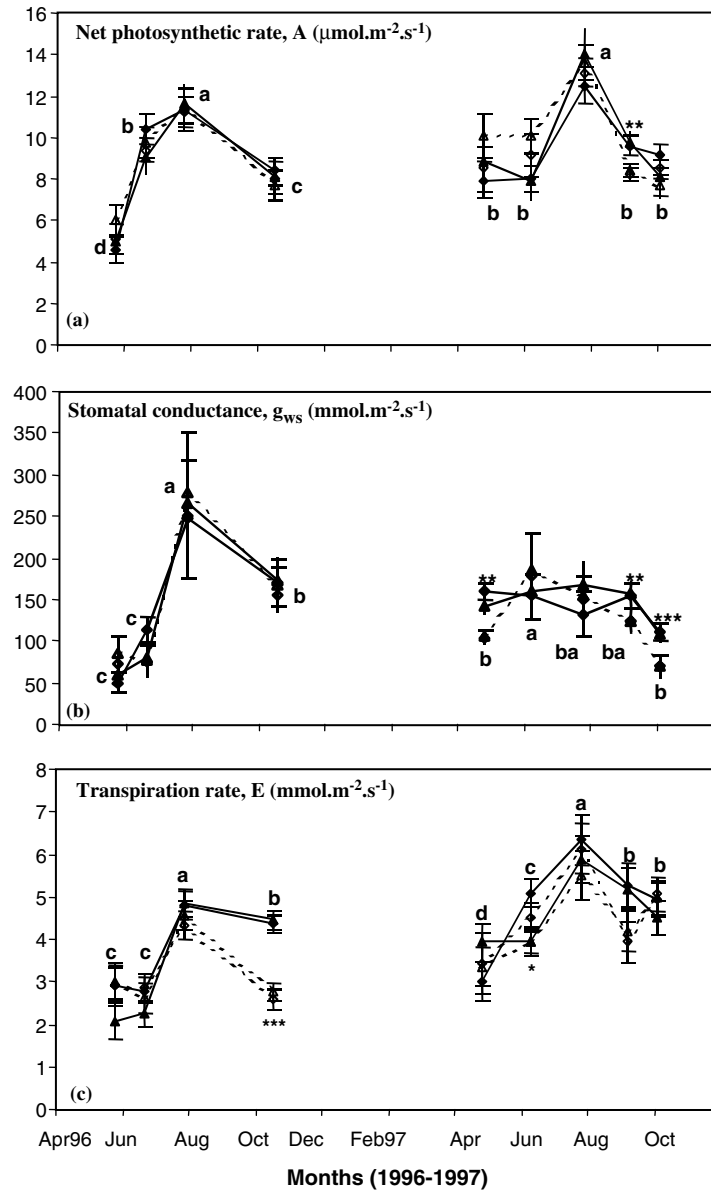


Figure 5. Net photosynthetic rate, A (a) and stomatal conductance to water vapor, g_{ws} (b) measured in leaves of (—▲—) fertilized + irrigated, (---△---) fertilized, (---◆---) irrigated and (---◇---) unfertilized and non-irrigated seedlings in a holm oak plantation, from May to October 1996 and from April through October 1997. Symbols indicate the mean, *I bars* indicate the standard error. Letters show differences in the mean between measuring dates, within a measuring season ($p < 0.05$, Tukey's Studentized range test). Significant differences between treatments within a date are shown by level of significance ($p < 0.001$ ***, $p < 0.01$ ** , $p < 0.05$ * , no asterisk: ns $p > 0.05$).

Table 3. Results of the repeated measures in PROC MIXED used for shoot height and diameter

Type III tests of fixed effects	Shoot height		Diameter	
	<i>F</i> value	Pr > <i>F</i>	<i>F</i> value	<i>p</i> > <i>F</i>
<i>Between subjects effects</i>				
Irrigation		ns		ns
Fertilization	17.93	< 0.001	26.55	< 0.001
Irrigation × Fertilization	6.32	0.012	4.15	0.043
<i>Within subjects effects</i>				
Time	29.49	< 0.001	34.66	< 0.001
Time × irrigation		ns		ns
Time × fertilization		ns		ns
Time × irrigation × fertilization		ns		ns

Only Tests of Fixed Effects are shown as the random effect (block) is not significant. Shoot height and diameter are log-transformed for normality.

Significant differences were observed in height and diameter for the fertilization treatment at the end of the second growing season (Oct 1997, d644). Diameter also varied significantly for the irrigation treatment in d644 (Figure 6). Fertilization had a significant and positive effect on shoot and diameter growth increment between Feb and Oct 1997 measurements, and in total increment (Figure 6). On average, fertilized seedlings showed nearly 79.4% increase in total shoot growth and 51% increase in total diameter growth in comparison with unfertilized seedlings.

There were no significant changes in relative foliage density increment between Feb and Oct 1997. Although the increment was positively affected by irrigation and fertilization, differences among seedlings were greater than differences between treatments due to a large within-treatment variation ($p > 0.050$). The increment in relative foliage density for the fertilized and irrigated seedlings ($F + /W +$, 156.72%) was twice that of irrigated ($F - /W +$, 72.33%) and fertilized ($F + /W -$, 71.02%) and 6-fold that of control ($F - /W -$, 24.47%).

Seedling nutrient status

Initial nitrogen, phosphorus, and potassium concentrations in leaves were normal for the species (N: 1.18%, P: 0.056%, K: 0.375%). Two years of fertilization had a significant and positive impact on nutrient concentration. At the end of 1997, relative increment on nutrient concentration was significantly influenced by fertilization and irrigation. Relative increment on P and K concentration were also affected by an irrigation × fertilization interaction ($p < 0.001$). These relative increments were greater for fertilized seedlings, but only when not irrigated (Table 4).

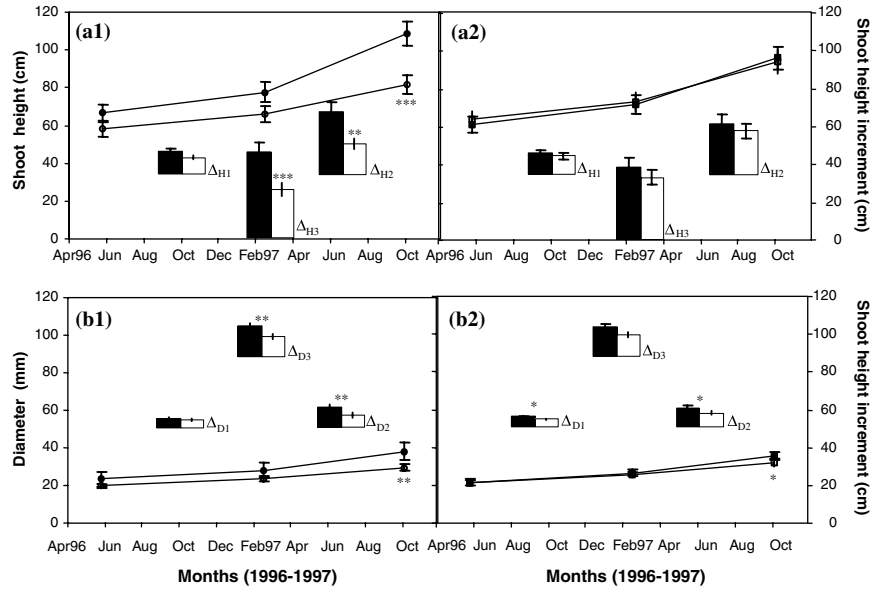


Figure 6. Shoot height (a) and diameter (b) in d146 (25 May 1996), d412 (15 Feb 1997) and d642 (5 Oct 1997), measured in (●) fertilized versus (○) non-fertilized seedlings (Figs. a1 and b1), and in (■) irrigated versus (□) non-irrigated seedlings (Fig. a2 and b2) of a holm oak plantation. Shoot height increment (Δ_{H_i} , $i = 1, 2, 3$) and diameter increment (Δ_{D_i} , $i = 1, 2, 3$) are also shown by vertical columns [data correspond to the right axes]. Δ_{H1} : Shoot height increment between d146 and d412; Δ_{H2} : shoot height increment between d412 and d642; Δ_{H3} : total shoot height increment; Δ_{D1} : diameter increment between d146 and d412; Δ_{D2} : diameter increment between d412 and d642; Δ_{D3} : total diameter increment. Each mean is comprised of the observations for a measuring date. I bars indicate the standard error. $p < 0.001^{***}$, $p < 0.01^{**}$, $p < 0.05^*$, no asterisk: ns $p > 0.05$.

Table 4. ANOVA results of the 2×2 factorial design with irrigation (W) and fertilization (F) as main effects treatments, presented as $P > F$ -values; and mean \pm SE of relative increment on nitrogen (ΔN , %), phosphorus (ΔP , %) and potassium (ΔK , %) concentration between 1996 and 1997, referred to concentration in 1996

	ΔN	ΔP	ΔK
Model	< 0.001	< 0.001	< 0.001
Irrigation (W)	< 0.001	< 0.001	< 0.001
Fertilization (F)	< 0.001	< 0.001	< 0.001
W×F	ns	< 0.001	0.005
F+/W+	31.3 \pm 1.5 b	15.9 \pm 3.3 b	23.3 \pm 0.2 a
F-/W+	38.6 \pm 4.7 b	19.0 \pm 4.6 b	16.8 \pm 1.1 b
F+/W-	77.1 \pm 0.9 a	74.3 \pm 0.1 a	23.9 \pm 0.1 a
F-/W-	25.8 \pm 5.3 b	33.6 \pm 0.7 b	19.2 \pm 0.7 b

($n = 8$ samples per treatment). Different letters show significant differences between treatments.

Vector shifts from final N, P, and K concentration, content and dry weight of leaves of F - /W - treatment to corresponding values at the other treatments are shown in Figure 7. Except for N, vectors showed that there was a

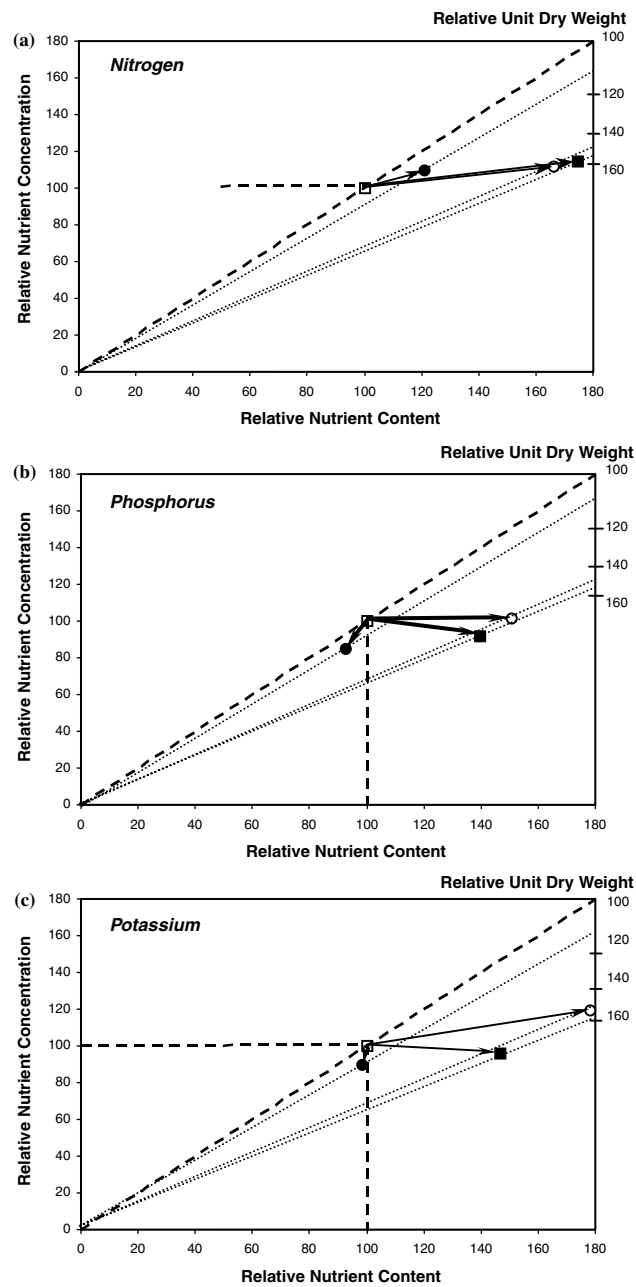


Figure 7. Vector shifts from final (a) nitrogen, (b) phosphorus, and (c) potassium concentration, content and dry weight of leaves at the F-/W- treatment to corresponding values at the other treatments. \square F-/W- ; \bullet F+/W+ ; \blacksquare F-/W+ ; \circ F+/W-.

downward shift in relative nutrient content and concentration for the F+ /W+ treatment. All treatments showed an upward shift in leaf dry weight relative to the F- /W- treatment. For P and K, the F+ /W- treatment showed an upward shift in relative nutrient content relative to the F- /W- treatment.

Discussion and conclusions

As expected, seasonal variation in rainfall promoted a significant variation in volumetric soil water content and, thus, in water input in the 2 weeks before measurement (wat_{14d}), to which it is positively correlated (Figures 2a, b). Minimum wat_{14d} occurred at the end of growth (Oct 1996, Sep 1997), when rainfall during the previous 2 weeks had been less than 6 mm and soil temperature was still relatively high.

Values of Ψ_{pd} never reached -0.9 MPa, even when seedlings were not irrigated. These values suggest a higher water availability in this plantation in comparison with values reported by Duhme and Hinckley (1992) for trees of this species during the summer (-1.2 to -1.8 MPa), indicating that our seedlings were not subjected to a severe water stress. Similarly, Nardini et al. (2000) found absence of dramatic water stress during summer on holm oak plants in Sicily related to milder weather conditions. Even under low water availability in the soil, the deep and extended root system of holm oak allows the species to contact with the moister deep soil layers, thus, not being so dependent on uncertain rains and surface water (Dickson and Tomlinson 1996). In addition, soil profile in the study site was adequate for the growth of this species, with a depth between 2 and 2.5 m, and a soil moisture in the last horizon of 2.38% (dry weight).

Differences in Ψ_{pd} due to irrigation were shown at the end of growth of 1996 and at the onset and end of growth of 1997, when soil water content was low, mainly at the end of growth. In the W- treatment, the decrease in soil water availability resulted in a small drop in Ψ_{pd} in these seedlings at the end of 1996 and 1997. Similar results were reported by Sala and Tenhunen (1994) for this species.

Although the effect of fertilization on Ψ_{pd} was low along the study, it influenced the response of plants to drought. Thus, the F+ treatment exhibited higher Ψ_{pd} than F- in response to drydown in the driest months. This trend is in agreement with the results by Kleiner et al. (1992) in fertilized vs non-fertilized chestnut oak.

Minimum water potential (Ψ_{min}) remained above -3.0 MPa under the driest conditions (end of growth in 1996 and 1997). It has been reported that even under extreme drought conditions ($\Psi_{min} < -3.0$ MPa) the deep roots of this species allow an adequate water supply (Castell et al. 1994). By stomatal regulation, the plant's water status is maintained within a security range of water potential in order to avoid xylem cavitation (Cochard et al. 1996); but if the threshold of -3.0 MPa is surpassed, cavitation may occur in holm oak

(Sala and Tenhunen 1994). Other results (Pigott and Pigott 1993) show that mature leaves of holm oak are not injured until $\Psi < -4.0$ to -4.5 MPa.

Minimum water potential was a good indicator of the water status of the plant, shown through the relationship between Ψ_{\min} and wat_{14d} . Differences in minimum leaf water potential between irrigated and non-irrigated seedlings during the end of growth revealed a quick response of seedlings to irrigation when the amount of water supplied to the soil was low. Similar response was obtained on Ψ_{\min} the day before and after the drought-breaking rain for red oak juvenile trees (Cavender-Bares and Bazzaz 2000).

The patterns of seasonal change for g_{ws} , E and A were similar for both years. Maximum values of A and g_{ws} during the growing period were close to the range reported in literature for this species under field conditions (Castell et al. 1994), but g_{ws} is higher to that reported after a drought period by Acherar et al. (1991). The species was able to maintain in July 1996 relatively high water vapor stomatal conductance (262 to 151 $\text{mmol m}^{-2} \text{s}^{-1}$) with quite low Ψ_{\min} (-2.3 to -2.5 MPa), which then recovered during the night (Ψ_{pd} between -0.55 and -0.63 MPa). These g_{ws} values in July allowed photosynthesis to continue at a high rate; thus, the resulting photosynthate would be available for continued water uptake by the growing root system. This is a common strategy for most evergreen oaks, which are able to maintain photosynthesis activity with relatively low water potentials (Abrams 1988; Acherar et al. 1991; Acherar and Rambal 1992).

By keeping stomata open during drought periods, plants avoid the carbohydrate starvation induced by extended closing (Hinckley et al. 1983). As water deficits were not so dramatic in our study, the reductions in gas exchange parameters in late summer were not as large as those usually reported for this species (86% reduction on g_{ws} (Acherar and Rambal 1992); 173% reduction on g_{ws} (Sala and Tenhunen 1994)). Normally, with the end of drying conditions, mediterranean species respond rapidly to soil drying and reduce leaf conductance. Seasonal variations in leaf gas exchange parameters suggested that both g_{ws} and A are sensitive to water availability in the soil, as shown by Mielke et al. (2000) in eucalypt. Since Ψ_{pd} represents an integrated response of a plant to soil moisture, a species' gas exchange response to changes in soil moisture is reflected by their response to Ψ_{pd} (Reich and Hinckley 1989).

The hypothesis that fertilization can increase drought tolerance of plants by ameliorating reductions in basic physiological processes (Abrams 1988) is only supported in June 1997, when transpiration rate (E) is reduced in F+ treatment in comparison with the F- treatment. The effect of irrigation on A and g_{ws} was shown at the beginning and end of the growing season, related to lower Ψ_{pd} in the F- treatment. The rapid recovery of g_{ws} after irrigation has been observed in other mediterranean species, and might indicate a robust, dehydration-tolerant photosynthetic apparatus (Lebourgeois et al. 1998).

The effect of fertilization on the increments of shoot growth and diameter were more pronounced than the effect of irrigation. It is widely known that nitrogen fertilization is a major determinant of seedling diameter. Similar

results were reported by Kleiner et al. (1992) in red oak and chestnut oak submitted to fertilization and irrigation. Although moisture stress have been found to negatively affect growth of mediterranean oaks (Pardos et al. 2001), leaf water potential in our study demonstrated that seedlings on the W-treatments were only submitted to a moderate water stress, thus seedling shoot growth was not much affected. On the contrary, diameter growth was reduced by 34% in the W- treatments, compared with the irrigated seedlings; thus, even a small decrease in water potential can induce a significant decrease in diameter growth. The fact that shoot elongation is less responsive to limiting water availability than diameter is very common (Kozłowski and Pallardy 1997) as elongation occurs at the beginning of the growing season, while diameter growth prolongues during the whole season.

Plant relative foliage density was increased, although not significantly, by the W+ treatment and the F+ treatment. It seems that species under water-limiting conditions (W-) produced leaves with smaller area (Abrams 1994). The increase in relative foliage density with increasing nitrogen availability (F+) indicated that consumption of nitrogen stimulated rapid growth on the F+ treatment, thereby resulting in less light capture. Similarly, Albaugh et al. (1998) found that fertilization increased leaf area, although the native level of nutrient availability can strongly limit leaf area production.

Nutrient concentration increased over time in all treatments. The response of different plants to nutrient levels is related to their adaptation to soil conditions. Thus, those plants adapted to more fertile conditions show the greatest growth response to elevated nutrient rates (Robinson et al. 2002). The increment in N concentration was found the lowest in the F- /W+ treatment and the highest in the F+ /W- treatment. Numerous studies have demonstrated a decrease in nutrient concentration with an increase in the frequency of irrigation.

The vector diagrams showed that the P and K shifts tended to respond to the F+ /W- treatment. In contrast, vector shifts for P and K levels and leaf biomass were negative for the F+ /W+ treatment. It is clear that the F- /W+ treatment caused a shift in N concentration, content, and leaf weight, while the F+ /W+ treatment increased only slightly N concentration and content.

The values of the physiological parameters, which resulted in a suitable growth, show the good adaptation of *Q. ilex* to the ecological conditions found in the studied afforested site, formerly employed as low productivity agricultural land. Although such conditions are, in general, representative of the sites typically used for afforestation purposes (low productivity lands, with irregular and limited rainfalls), the frequent storms during summer are a typical characteristic of this particular site . These storms, which represent between 40 and 50% of precipitation during the growing season, allow seedlings to maintain relatively high Ψ_{pd} values and a sufficient daily amplitude on leaf water potential ($\Psi_{pd} - \Psi_{min}$) for seedling's survival and moderate growth. Water supplied by irrigation (between 13 and 19% of rainfall during the growing

period) was enough to provoke an immediate response of Ψ to it only when soil water content was low. Thus, seedling's growth response to irrigation was scarce. Fertilization had a more relevant effect on growth due to low fertility of the soil.

Acknowledgments

This study was partially supported by funds from the Diputación Provincial de Teruel. We express our gratitude to Antonio Alcalá for providing the study site and the irrigation system. We thank Rafael Calama for statistical advice and Catherine Collet for revision of the manuscript.

References

- Abrams M.D. 1988. Comparative water relations of three successional hardwood species in central Wisconsin. *Tree Physiol.* 4: 263–273.
- Abrams M.D. 1994. Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. *Tree Physiol.* 14: 833–842.
- Abril M. and Hanano R. 1998. Ecophysiological responses of three evergreen woody Mediterranean species to water stress. *Acta Oecologica* 19(4): 377–387.
- Acherar M., Rambal S. and Lepart J. 1991. Évolution du potentiel hydrique foliaire et de la conductance stomatique de quatre chênes méditerranéens lors d'une période de dessèchement. *Ann. Sci. For.* 48: 561–573.
- Acherar M. and Rambal S. 1992. Comparative water relations of four Mediterranean oak species. *Vegetatio* 99–100: 177–184.
- Albaugh T.J., Allen H.L., Dougherty P.M., Kress L.W. and King J.S. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient additions. *For. Sci.* 44(2): 317–328.
- Castell C., Terradas J. and Tenhunen J.D. 1994. Water relations, gas exchange, and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L.. *Oecologia* 98: 201–211.
- Cavender-Bares J. and Bazzaz F.A. 2000. Changes in drought response strategies with ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia* 124: 8–18.
- Cochard H., Bréda N. and Granier A. 1996. Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought: evidence for stomatal control of embolism? *Ann. Sci. For.* 53: 197–206.
- Dickson R.E. and Tomlinson P.T. 1996. Oak growth, development and carbon metabolism in response to water stress. *Ann. Sci. For.* 53: 181–196.
- Duhme F. and Hinckley T.M. 1992. Daily and seasonal variation in water relations of macchia shrubs and trees in France (Montpellier) and Turkey. *Vegetatio* 99–100: 185–198.
- Grossnickle S.C. 1993. Shoot water relations and gas exchange of western hemlock and western red cedar seedlings during establishment on a reforestation site. *Trees* 7: 148–155.
- Haase D. and Rose R. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *For. Sci.* 41: 54–66.
- Hechler W.D., Jeffrey O.D. and DeLucia E.H. 1991. Stomatal conductance of seedlings of three oak species subjected to nitrogen fertilization and drought treatments. In: McCormick L.H. and Gottschalk K.W. (eds), Proc. 8th Central Hardwood Forest Conference. USDA For. Serv. Gen. Tech. Rep. NE-148.188–193

- Hinckley T.M., Duhme F., Hinckley A.R. and Richter H. 1983. Drought relations of shrub species: assessment of the mechanism of drought resistance. *Oecologia* 59: 344–350.
- Kleiner K.W., Abrams M.D. and Schultz J.C. 1992. The impact of water and nutrient deficiencies on the growth, gas exchange and water relations of red oak and chestnut oak. *Tree Physiol.* 11: 271–278.
- Kozłowski T.T. and Pallardy S.G. 1997. *Physiology of woody plants*, 2nd edn. Academic Press, 441 pp.
- Lebourgeois F., Lévy G., Aussenac G., Clerc B. and Willm F. 1998. Influence of soil drying on leaf water potential, photosynthesis, stomatal conductance and growth in two black pine varieties. *Ann. Sci. For.* 55: 287–299.
- Mielke M.S., Oliva M.A., de Barros N.F., Penchel R.M., Martinez C.A., da Fonseca S. and de Almeida A.C. 2000. Leaf gas exchange in a clonal eucalypt plantation as related to soil moisture, leaf water potential and microclimate variables. *Trees* 14: 263–270.
- Nardini A., Salleo S., LoGullo M.A. and Pit F. 2000. Different responses to drought and freeze stress of *Quercus ilex* L. growing along a latitudinal gradient. *Plant Ecol.* 148: 139–147.
- Oliveira G. and Peñuelas J. 2000. Comparative photochemical and photomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) mediterranean woody species. *Acta Oecologica* 21(2): 97–107.
- Pardos M., Montero G. and Pardos J.A. 2001. Growth dynamics of cork oak under different water regimes. *Silva Lusitana* 9(1): 1–12.
- Pigott C.D. and Pigott S. 1993. Water as a determinant of the distribution of trees at the boundary of the Mediterranean zone. *J. Ecol.* 81: 557–566.
- Reich P.B. and Hinckley T.M. 1989. Influence of predawn water potential and soil-to-leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. *Funct. Ecol.* 3: 719–726.
- Report to Parliament and the Council on the application of Regulation (EEC) No. 2080/92 instituting a Community aid scheme for forestry measures in agriculture. 1997.
- Robinson D.E., Wagner R.G. and Swanton C.J. 2002. Effects of nitrogen on the growth of jack pine competing with Canada blue-joint grass and large-leaved aster. *Forest Ecol. Manage.* 160: 233–242.
- Romane F. and Terradas J. 1992. *Quercus ilex* L. ecosystems: function, dynamics and management, *Advances in vegetation science* 13. Kluwer Acad Publ., Dordrecht, Boston, London.
- Sala A. and Tenhunen J.D. 1994. Site-specific water relations and stomatal response of *Quercus ilex* in a Mediterranean watershed. *Tree Physiol.* 14: 601–617.
- Terradas J. and Savé R. 1992. The influence of summer and winter stress and water relationships on the distribution of *Quercus ilex* L. *Vegetatio* 99–100: 137–145.
- Walter H., Harnickell E. and Mueller-Dombois D. 1975. *Climate-diagrams maps of the individual continents and the ecological climatic regions of the earth*. Springer-Verlag, Berlin 36p. with maps.