

# Microclimatic conditions and plant morpho-physiological development within a tree shelter environment during establishment of *Quercus ilex* seedlings

Juan A. Oliet<sup>a,\*</sup>, Douglass F. Jacobs<sup>b</sup>

<sup>a</sup> E.T.S. Ingenieros Agrónomos y de Montes de la Universidad de Córdoba, Avda. Menéndez Pidal s/n, 14071 Córdoba, Spain

<sup>b</sup> Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA

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## Abstract

Tree shelters are used commonly under a wide range of agriculture and forestry applications, and the presence of the shelter is known to influence plant development through shelter–plant interactions that affect microclimatic conditions. However, effects of tree shelter microclimates on development of relatively slow-growing forest tree species characteristic of semi-arid Mediterranean climates have yet to be examined. Under a controlled environment using mesic and xeric watering regimes, we examined microclimatic conditions within light and dark tree shelters relative to non-sheltered conditions and corresponding influences on morpho-physiological development of holm oak (*Quercus ilex* L.) seedlings during initial establishment. Despite relatively low photosynthetic rates ( $<1 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $\text{CO}_2$  concentrations within shelters oscillated widely during the day, reflecting respiration from late afternoon through night and rapid assimilation during the first 2–3 h after sunrise that dropped sharply around noon. In dark shelters within the mesic watering regime, reduced vapor pressure deficit (VPD) was associated with high plant transpiration. Higher irradiance inside light shelters stimulated photosynthesis, although higher VPD under xeric conditions led to decreased mid-day xylem water potential within light shelters. Additionally, decreased light transmittance among shelter treatments (control > light > dark) did not affect plant N or starch concentrations nor total plant biomass, but resulted in increased height, as well as leaf area production and shoot:root ratio under mesic conditions, morphological adaptations likely to increase drought susceptibility under hot, dry conditions characteristic of late summer in this region. We conclude that microclimatic conditions within tree shelters and corresponding influences on plant morpho-physiological development are interrelated through complex phenomena dependent upon environmental conditions and phenotypic characteristics of a given plant species. Consequentially, shelter configurations must be adapted to match climatic conditions of the outplanting site with species plasticity; we recommend light shelters with high light transmittance for planting holm oak in Mediterranean areas.

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## 1. Introduction

Tree shelters were introduced in England in 1979 as a means to alleviate animal browsing pressures of planted forest tree seedlings (Potter, 1988). Unexpected benefits were realized when planted *Quercus* spp. exhibited improved growth in tree shelters (Tuley, 1983, 1985),

\* Corresponding author. Tel.: +34 957 218655; fax: +34 957 218563.

E-mail address: [joliet@uco.es](mailto:joliet@uco.es) (J.A. Oliet).

which was later attributed to the formation of a greenhouse-simulated microclimate within the shelter favorable to plant growth. Tree shelters have since been used extensively for forestry, horticultural, and agronomic applications.

Research on tree shelter microclimatic conditions and corresponding influences on plant morphology and physiology has been nearly exclusively confined to species of temperate regions (Potter, 1991; Kjelgren and Rupp, 1997; Kjelgren et al., 1997; Bergez and Dupraz, 1997; Swistock et al., 1999; Dupraz and Bergez, 1999; Bergez and Dupraz, 2000). Previous studies with temperate zone species have demonstrated the influences of variation in light transmittance on plant response via effects on light intensity, light quality (e.g., red:far red wavelength), or air temperatures (Potter, 1991; Kerr, 1996; Kjelgren et al., 1997; Jacobs and Steinbeck, 2001; Sharew and Hairston-Strang, 2005). Other studies have examined interrelationships between vapor pressure deficits and CO<sub>2</sub> levels associated with air circulation within the sheltered environment and corresponding influences on plant gas exchange and biomass allocation (Dupraz and Bergez, 1999; Bergez and Dupraz, 2000).

Forest tree species of semi-arid Mediterranean regions, characterized by generally lower transpiration rates resulting from reduced soil moisture availability and higher temperatures and evaporative demand, likely interact differently with shelter microclimatic conditions. Previous studies with Mediterranean species have reported higher temperatures and/or vapor pressure deficits in tree shelters compared to ambient levels (Bellot et al., 2002; Oliet et al., 2003). In these regions, plant response to tree shelters appears to be species-specific, with many species exhibiting improved survival and growth when protected by tree shelters (Marques et al., 2001; Oliet et al., 2003, 2005). However, little research has studied the effects of tree shelter microclimatic conditions and soil moisture on plant physiology and growth of Mediterranean species (Bellot et al., 2002; Navarro et al., 2005). We are aware of no published research that has examined the interrelationship between CO<sub>2</sub> concentration, soil moisture, and plant development within tree shelters under environmental conditions characteristic of Mediterranean areas. Thus, improved understanding of tree shelter microclimatic conditions and influences on plant physiology and growth during the seedling establishment phase is needed to help design tree shelters that optimize environmental conditions and thereby enhance field performance of planted Mediterranean species.

Holm oak (*Quercus ilex* L.) is a widespread sclerophyllous evergreen Mediterranean oak that occurs in mesic through xeric habitats (Archibold, 1995), and is an important species for the restoration of heavily deforested areas and abandoned croplands of the Mediterranean basin (Rodà et al., 1999; Rey Benayas and Camacho-Cruz, 2004). Holm oak is relatively difficult to establish in the field, however, with generally lower survival and growth rates compared to other species options for planting on these sites, such as *Pinus* spp. (Baeza et al., 1991; Vallejo and Alloza, 1999). Poor field performance is related to the vulnerability of this species to abiotic stress factors during early life stages, particularly during the first summer drought following planting (Villar-Salvador et al., 2004). Holm oak response to tree shelters appears to be dependent upon the specific climatic conditions of the planting site, with improved survival of sheltered trees only under harsh site conditions (Oliet et al., 2003).

Our objective was to critically evaluate microclimatic conditions within tree shelters varying in light transmittance versus external conditions and examine corresponding influences on morpho-physiological responses of holm oak seedlings during the early establishment period (i.e., before the onset of summer drought). Because moisture limitations often dictate the capacity of *Q. ilex* seedlings to successfully establish on Mediterranean planting sites, the experiment was conducted with varying soil moisture levels.

## 2. Materials and methods

### 2.1. Cultural conditions and experimental design

The experiment was conducted in a greenhouse at Purdue University in West Lafayette, IN, USA (40°25'N, 86°55' W). Acorns of *Q. ilex* (provenance Region Leonesa, Zone 1b Sayago-Salamanca, Jiménez-Sancho et al., 1996) were sown into trays with 220 ml Superblock<sup>®</sup> (Beaver Plastics Inc., Canada) 12 cm depth cells, at a 284 plants m<sup>-2</sup> density and grown for 5 months. Before planting, 20 plants were randomly sampled to assess baseline seedling attributes. Mean values (±S.E.) for primary traits were: 14.3 (0.9) cm height, 4.7 (0.2) mm root collar diameter, 4.1 (0.2) g plant dry weight, 1.1 (0.1) g g<sup>-1</sup> shoot to root ratio and 17.3 (0.2) mg g<sup>-1</sup> foliar N concentration.

Seedlings were planted in mid-October 2003 into 12 l plastic pots (23 cm width × 39 cm depth) filled with a 3:1 mixture of 50:25:15:10 (v/v/v/v) bark-coconut coir pith-peat moss-perlite (Metro-Mix<sup>®</sup> 560, Scotts Co., Inc., Marysville, OH, USA) and washed

river sand. After planting, all pots were saturated by fertigation with a nutrient solution that supplied 370 mg N per pot. To facilitate early seedling establishment, an additional 100 mg N fertilizer dose was applied 35 days after planting (DAP). A 15-5-15 CalMag Excel (Scotts Co., Inc.) fertilizer was used in both applications. Day (06:00–22:00 h solar time) and night temperature were set at 25 and 20 °C, respectively.

To characterize average radiation inside the greenhouse during the experiment, photosynthetic photon flux density (PPFD) outside the greenhouse was determined using a LI-190SB quantum sensor (Li-Cor, Inc., Lincoln, NE, USA) and transformed to the internal greenhouse PPFD mean values by fitting a regression model ( $R^2 = 0.97$ ,  $n = 17$ ) with data recorded manually (LI-250 quantum sensor, Li-Cor, Inc.) in 16 points inside the greenhouse evenly distributed at different hours of the day during 3 days. Additional light was applied during the entire experiment with 1000 W high pressure sodium lamps during the day (06:00–22:00 h solar time). Average radiation ( $\pm$ S.E.) at plant height was 15 (0.4) mol m<sup>-2</sup> d<sup>-1</sup>, with minimum and maximum values of 10 and 24 mol m<sup>-2</sup> d<sup>-1</sup>, respectively, during the study period.

The experimental design was a factorial with three levels of tree shelter protection: dark (D, brown color), light (L, white color) and no shelter (N); and two levels of water availability (mesic, m and xeric, x). The experiment was arranged as a randomized complete block design with four replications (blocks). Eight plants from the same tree shelter  $\times$  water regime combination (Nm, Nx, Dm, Dx, Lm, and Lx treatments) in a block were placed in a row. Tree shelters were standard unventilated, translucent, circular, single-walled polyethylene tubes 64 cm tall  $\times$  11 cm wide (TreePro Inc., West Lafayette, IN, USA). The tree shelters were sealed at the bottom with soil media. Light transmittance into the shelters (calculated as the average of several PPFD readings with a LI-250 quantum sensor) was 55 and 70% of full sunlight for the dark and light shelters, respectively.

The mesic water regime was adjusted by drying the pots to a pre-determined growing media volumetric water content (VWC) of 15–20%, which represents

typical soil water content in Mediterranean areas in spring and early summer (Martínez-Vilalta et al., 2003). Pre-dawn water potential of plants in the mesic regime reached mean values ( $\pm$ S.E.) of  $-1.01$  (0.05) MPa at day 48 and  $-0.80$  (0.07) MPa at day 99 (Table 1), suggesting that mild to moderate water stress was incurred (Pesoli et al., 2003; Villar-Salvador et al., 2004). Hand irrigation with 0.5–1 l water per plant was used to avoid nutrient leaching from the pots. After watering to field capacity at planting, plants in the mesic watering regime received 6.5 l in nine irrigations, while those in the xeric regime received only 0.5 l when fertigated 35 DAP. Growing media moisture availability during the experiment was assessed using time domain reflectometry (Hydrosense Water Content Measurement System, Campbell Scientific Inc., Logan, UT, USA). Three rooting depths were evaluated: 0–12 cm and 12–20 cm (calculated as  $2VWC_{0-20} - VWC_{0-12}$ ) with the rods inserted vertically in the pot, and at 30 cm by inserting the 12 cm length rod horizontally in two openings created on the sides of the pot at 30 cm depth. One pot from each treatment replication was sampled and measurements were always conducted on the same pot.

## 2.2. Evaluation of microclimatic conditions inside shelters

To assess the effect of shelter radiation transmittance combined with water regime on microclimate conditions inside the tree shelters, air temperature and relative humidity (RH) data logger sensors (Hobo H08-003-02 Onset Computer Co., Cape Cod, MA, USA), were installed in the shelters during three sampling periods across the duration of the experiment: 23–24, 65–66 and 115–116 DAP. One plant per treatment was randomly chosen within a randomly selected experimental block (four plants total) and placed together in a row in the greenhouse for several days per period. Hobo probes were installed at the upper one-third of the canopy height, adjacent to the seedling foliage, oriented to the north, and contact with the wall of the shelter was avoided by holding the probes in place with a steel rod. An additional probe was installed at the same height and

Table 1

Effects of tree shelters and water regime on predawn water potential (MPa) at days 48 (48 plants total) and 99 (24 plants total)

Number of days after planting	No shelter		Dark shelter		Light shelter		$P > F$	Mesic		Xeric		$P > F$
	Mean	S.E.	Mean	S.E.	Mean	S.E.		Mean	S.E.	Mean	S.E.	
48	-1.25	0.08	-1.07	0.06	-1.12	0.05	0.092	-1.01	0.05	-1.27	0.05	< 0.001
99	-1.14	0.14	-1.01	0.11	-1.12	0.16	0.468	-0.80	0.07	-1.38	0.07	< 0.001

shaded to record external air temperature and RH. These variables were scanned at 1 min intervals, and recorded as 8 min averages. Temperature and RH of the air were combined to calculate vapor pressure deficit (VPD). At each sampling period, values from two consecutive sunny days were averaged to calculate VPD, and hourly means calculated from 8 min values are presented (Fig. 2). In addition, mean VPD, RH and temperature data between 09:00 and 18:00 h (when the greenhouse receives direct sunlight), and mean VPD from 22:00 to 06:00 h of the two consecutive sunny days from each sampling period are presented in the text for discussion. Additionally, Hobo probes were installed in the same manner to characterize microclimatic conditions in the shelters at the time CO<sub>2</sub> measurements were conducted (see next section).

### 2.3. CO<sub>2</sub> concentration inside the shelters and photosynthesis measurements

Measurements of CO<sub>2</sub> concentration inside the shelters were conducted 34 and 104 DAP using a portable open differential system infrared gas analyzer (LCA-4, Analytical Development Co., Huddleston, UK). One plant per block from each treatment (excluding non-sheltered plants) was randomly chosen (i.e., 16 total plants). Plants were placed in a row on a bench and randomly distributed by blocks. Daily measurements were initiated at predawn and continued until approximately 1 h after sunset. Plants were measured repeatedly by introducing the air supply tube of the infrared gas analyzer into the shelter through a small hole (6 mm diameter) in the shelter wall at mid-height and placing the end of the tube at midcanopy. After extracting approximately 1 l of air at an air flow rate of 250 ml min<sup>-1</sup>, CO<sub>2</sub> concentration of the air was recorded. For comparison purposes, external air was analyzed by connecting the air supply tube to a 4 m height probe at the beginning, in the middle and at the end of measuring a set of 16 plants. This procedure required approximately 1.5 h; mean values per set are presented. Additionally, at each CO<sub>2</sub> measurement, external PFD was determined using the PFD probe placed on the leaf chamber of the gas analyzer. To characterize microclimatic conditions inside the shelters during CO<sub>2</sub> measurements, a Hobo probe was installed in one randomly chosen plant per treatment (four plants total) among the plants selected for CO<sub>2</sub> (see previous section for details). Hourly PFD mean and mean of the four temperature values during the diurnal course are presented for reference (Fig. 4).

Photosynthesis measurements were conducted during two sampling periods. The first period was 46–47 DAP and the second period occurred at 98, 106 and 111 DAP. Dates within a sampling period were staggered because only three plants per treatment could be measured in 1 day. Net photosynthetic rate on a leaf area basis ( $A_n$ ) was measured using the infrared gas analyzer LCA-4 equipped with a leaf chamber. The day before measurements, one plant per block and treatment was randomly chosen from three blocks (18 plants total), and a 14 cm long × 10 cm wide window was opened at mid-height of the plant by cutting the wall of the shelter (except the top side), and immediately sealing with tape. The measurements were conducted between 1030 and 1500 h solar time the following day under greenhouse conditions. Prior to measurement of a sheltered plant, the top of the shelter was blocked with a plastic sheet to avoid creating an upward draft effect; then, the tube for the air supply of the analyzer was inserted through a small hole (6 mm diameter) in the wall of the shelter at mid-height and the end placed near the interior top of the shelter (when a non-sheltered plant was measured, the external air was drawn from a height of 4 m). The window was then opened and the chamber (covered by the window) was introduced in the shelter. A fully expanded leaf from the upper half of the shoot was inserted into the chamber, while ensuring that the leaf maintained its orientation. Several minutes were required to allow the leaf to adjust to the chamber microclimate; the air flow rate through the leaf chamber was set at 250 ml min<sup>-1</sup>. After leaf photosynthetic rates stabilized (i.e., once the value for sub-stomatal cavity CO<sub>2</sub> concentration settled), measurements were recorded. This process required 5–10 min following chamber closure. Temperature inside the chamber was checked to avoid differences greater than 4 °C from external air temperature (Valladares et al., 2000). In cases where photosynthetic stability was not reached, the plant sample was excluded. Additionally, the unit recorded incident PFD on the leaf and chamber temperature, measured by sensors mounted on the leaf chamber, as well as the CO<sub>2</sub> concentration of the air coming from the air supply tube inserted in the shelter. Estimates of leaf  $A_n$  per plant were calculated as the mean of several stable values, along with the mean of leaf chamber temperature, PFD and CO<sub>2</sub> concentration. Measurements were conducted consecutively by experimental blocks and plants were randomly measured within a block. The same blocks were used in both sampling periods. After each measurement was completed, the leaf inside the chamber was excised and stored in aluminum foil at –18 °C until sampled to

determine leaf area (LI-3000 Portable Area Meter, Li-Cor, Inc., Lincoln, NE, USA).

#### 2.4. Evaluation of water potential, morphological development, nitrogen, and starch

Shoot predawn water potential ( $\psi_P$ ) was determined 1 or 2 days following photosynthesis measurements in the same plants, although additional plants were randomly chosen and included in the sample: two plants per block and treatment (48 plants total) were evaluated at the first measurement (48 DAP) and half of them (24) at the second measurement (99 DAP). Midday shoot water potential ( $\psi_M$ ) was measured at the end of the experimental period (117 DAP) from one randomly selected plant per block and treatment (24 plants total). Shoots were separated from roots before sunrise and at 13:00 h solar time for  $\psi_P$  and  $\psi_M$  estimation, respectively, wrapped in aluminum foil and stored at 0 °C until measured for shoot xylem water potential using a pressure bomb (Model 600, PMS Instruments, Inc., Corvallis, OR, USA).

Harvesting of plants for evaluation of morphology, nutrients or starch reserves was performed 112 DAP. Three plants per block and treatment (72 plants total) were randomly selected, including all the plants used for gas exchange measurements during these days. Seedlings were excavated from pots, washed free of media and measured for stem height, root collar diameter, number of leaves and leaf area. Seedlings were then dipped in distilled water for 5 min, oven-dried at 60 °C for 72 h and weighed. To determine nutrients and nonstructural carbohydrates (starch and soluble carbohydrates) concentrations, leaves of seedlings sampled in a block per treatment were pooled (three plants per composite unit, 24 composite units total) and ground in a mill to pass through a 20 mesh screen. Leaf N analysis of seedlings prior to planting was sampled on 20 plants pooled into four composite units (five plants per unit). Plant N was determined according to Association of Official Analytical Chemist (AOAC) methods. Total N was determined by combustion (“Dumas”) procedure (AOAC 968.06) using a LECO nitrogen analyzer (LECO Corp., St. Joseph, MI, USA). Additionally, plant samples were digested in nitric + perchloric acids (AOAC 935.13), and P and K determined using inductively coupled argon plasma (ICAP) analysis (AOAC 985.01). Starch was hydrolyzed with perchloric acid, and soluble sugars were extracted with ethanol, water and chlorophorm. Glucose concentrations were determined by the anthrone method (Spiro, 1966).

#### 2.5. Statistical analysis

Analysis of variance (ANOVA) was conducted on all measured physiological and morphological variables using SPSS Statistical Package Version 11.00 (SPSS Inc., Chicago, IL, USA). A general complete block design was used, corresponding to the following linear model for the ANOVA (Little and Hills, 1990):

$$Y_{ijkl} = \mu + \tau_i + \omega_j + \tau\omega_{ij} + \beta_k + \varepsilon_{(ijk)l}$$

where  $Y_{ijkl}$  is seedling physiological or morphological attribute estimated from the  $l$ th replicate, from the  $k$ th block, the  $j$ th water regime level ( $j = 1, 2$ ) and the  $i$ th tree shelter level ( $i = 1, 2, 3$ ). The number of replicates within a block and treatment ( $l$ ) was: 1 (no replications) for  $\psi_P$  99 DAP and  $\psi_M$ , VWC of the pots, and nutrients and carbohydrate determinations; 2 for photosynthesis measured during first period and  $\psi_P$  48 DAP; and 3 for photosynthesis measured during second period and for growth and morphology traits. Day of measurement within a period was considered a replicate in analysis of photosynthesis because no interaction between date and main factors (tree shelter and water regime) was detected and the main effect of date within ANOVA was non-significant (data not shown). The number of blocks ( $k$ ) was four for all traits except for photosynthesis ( $k = 3$ ). After photosynthesis measurements, several values were rejected and the sample size was reduced from 36 to 30 in the first period and from 48 to 47 in the second period. From the linear model presented above,  $\mu$  = overall mean;  $\tau_i$  = fixed effect of the tree shelter;  $\omega_j$  = fixed effect of the water regime;  $(\tau\omega)_{ij}$  = tree shelter by water regime interaction effect;  $\beta_k$  = fixed effect of block; and  $\varepsilon_{(ijk)l}$  = experimental error. No interactions between block and main factors appeared (data not shown). Any significant tree shelter  $\times$  water regime interaction was noted in the text. For each analysis, when the ANOVA was significant, statistically significant differences between means were identified using Fisher’s protected least significant differences (LSD) test. Regression analysis was used to explore relationships among the variables. Effects were considered significant when  $P < 0.05$ .

### 3. Results

No trees emerged from the top of the shelter during the experiment. Therefore, physiology and growth results are specific to the shelter microclimate.

### 3.1. Water availability in the soil and microclimate in the shelters

Interactions between water regime and tree shelter on VWC of the pots were not significant (data not shown). Water content of the pots varied by water regime, and differences at the same depth increased with time, becoming significant by day 20 after planting (Fig. 1). Within both the mesic and xeric regimes, VWC (after drying from saturation) at 12–20 cm depth was always greatest, while VWC at 0–12 cm was least (Fig. 1). With regard to tree shelter treatments a significant effect on VWC appeared at several measurements, particularly during the first 49 days of the experiment (data not shown). When differences were significant, VWC was always higher in pots with plants protected by tree shelters. Significant effects appeared primarily at 0–12 cm depth. Excluding day 1 after planting, the most pronounced difference occurred on day 49 at 30 cm depth, when VWC of pots with dark tree shelters was 17.6 (2.2)%, while non-sheltered pots reached only 13 (1.5)% ( $P = 0.019$ ). On this day, VWC at 0–12 cm exhibited more significant differences ( $P < 0.001$ ), although the absolute value of the differences between pots with and without tree shelters was lower. Apparently, tree shelter color did not affect VWC of the pot substrate (data not presented).

Diurnal VPD on sunny days varied during the experiment by treatments. At the beginning (23–24 DAP, Fig. 2A), no differences between treatments were observed. In the middle of the experiment (65–66 DAP,

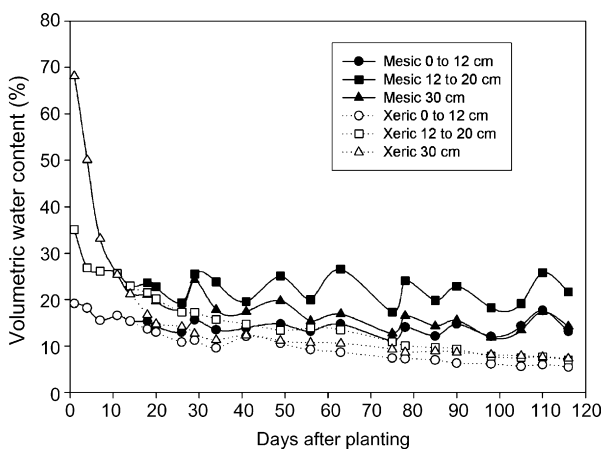


Fig. 1. Volumetric water content (VWC) during the study period at three rooting depths (0–12, 12–20 and 30 cm) as affected by water regime (mean of all tree shelter treatments,  $n = 12$ ). For each depth, VWC differences between the mesic and xeric regime were significant at  $P < 0.05$  from day 20 after planting, and at  $P < 0.001$  from day 29 to the end of the experiment.

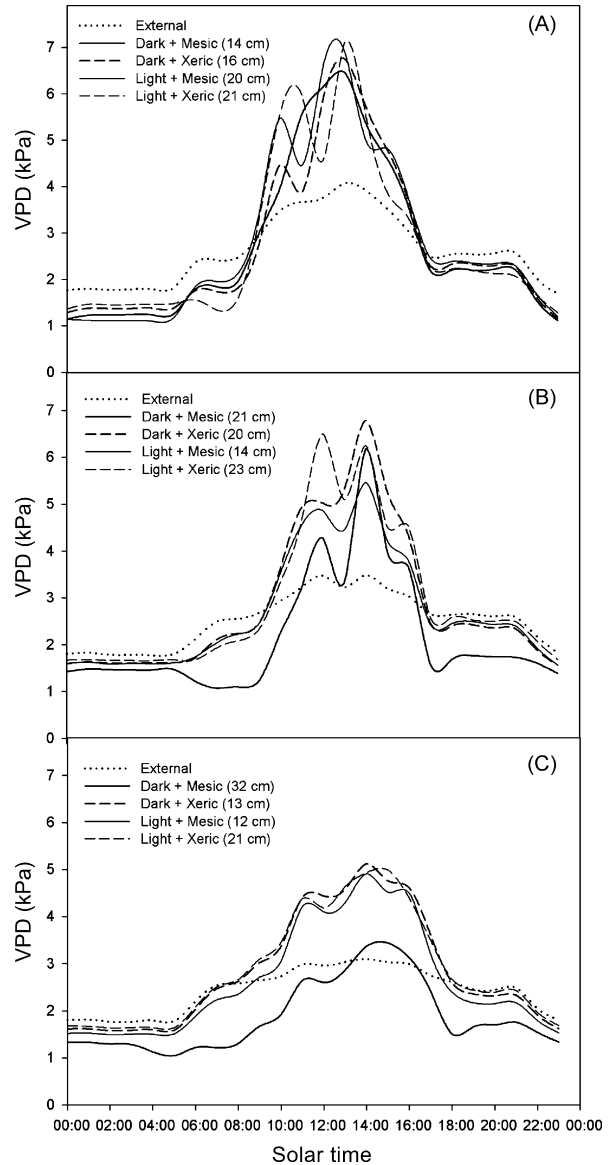


Fig. 2. Average daily time courses of vapor pressure deficit (VPD) during two consecutive sunny days according to shelter color (dark and light) and water regime (Mesic and Xeric) 23 and 24 (A), 65 and 66 (B), and 115 and 116 (C) days after planting. Parenthesis in legend indicates plant height.

Fig. 2B), plants from the Dm treatment exhibited the lowest VPD values among protected seedlings during nearly the entire day, followed by the Lm treatment. During these two averaged days, maximum VPD differences (2.2 kPa) occurred at midday between the Dm plants and the Lx treatment (Fig. 2B). The mean VPD value of the former treatment from 09:00 to 18:00 h was 3.1 kPa, while mean values for the Lx and Dx treatments were 4.2 and 4.3 kPa, respectively. At the

end of the experiment (115–116 DAP, Fig. 2C), plants in the Dm treatment also experienced the lowest VPD values during the entire day. But in this period, greater sustained differences (compared to previous, Fig. 2B) between this treatment and the remainder of the protected treatments during daytime hours were observed: the mean VPD value of the air in the Dm treatment from 09:00 to 18:00 h was 2.6 kPa, while mean values for Lx and Dx treatments were 4.0 and 4.1 kPa, respectively. As in the previous period, plants in the Lm treatment also experienced lower values than those from the xeric water regime, but their VPD values were always higher than for plants in the Dm treatment (Fig. 2C). Mean temperatures from the 09:00 to 18:00 h interval within the shelters were very similar among treatments, with differences lower than 1.5 °C throughout the study (data not shown). Mean external temperature (09:00–18:00 h) at 23–24, 65–66 and 115–116 DAP (29.3, 28.0 and 27.3 °C, respectively) was always less than inside the tree shelters (34.6, 33.4 and 32.2 °C, respectively). Differences in RH among protected seedlings were much higher. After 23–24 DAP, mean RH (09:00–18:00 h) was greater inside Lx and Dx (34.7 and 34.3%, respectively) compared to Lm and Dm (30.7 and 28.6%, respectively). After 65–66 DAP, mean RH values for Dx and Lx were 28.1 and 28.5%, respectively, and values increased to 32.2 for Lm and as high as 52.0% for the Dm treatment. Values of RH 115–116 DAP were very similar: 25.3% (Dx), 25.4% (Lx), 28.6% (Lm) and 51.9% (Dm). Mean external RH values (23.1%, 23.2% and 24.7%), were lower than values of the air protected by shelters (see text above).

During the entire study, nighttime (22:00–06:00 h) VPD differences within shelters decreased to less than 0.6 kPa (Fig. 2). External air VPD consistently followed the same trend: from 18:00 to 09:00 h VPD was higher than inside the shelters, but at midday hours, VPD was markedly lower than inside the tree shelters, excepting the Dm treatment at 115–116 DAP, when VPD remained below external VPD values except from 14:00 to 16:00 h (Fig. 2C).

### 3.2. Water potential

Predawn xylem water potential was significantly affected by water regime 48 and 99 DAP (Table 1), although differences in  $\psi_P$  between the mesic and xeric regimes increased from 0.26 MPa at day 48 after planting to 0.58 MPa at day 99, in correspondence with fluctuations in VWC during the experiment (Fig. 1). Tree shelters did not significantly affect  $\psi_P$ ; however,

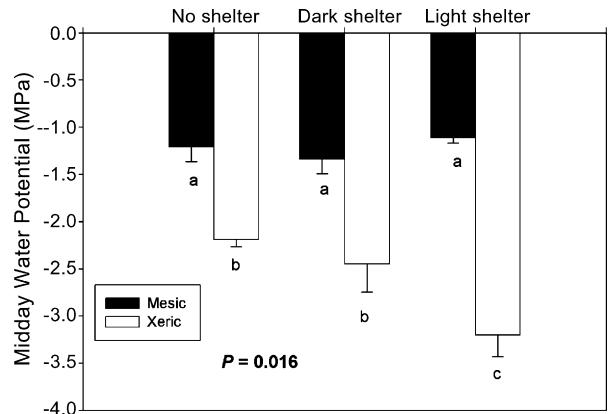


Fig. 3. Two-way interactive effect (mean and S.E.,  $n = 4$ ) of water regime and tree shelter on midday water potential of *Q. ilex* 117 days after planting. No significant differences in VWC of the growing media by tree shelter were found, according to time domain reflectometry measurements made on day 116 (data not shown). Means with different letters (a, b and c) indicate significant differences.

non-sheltered trees had lower  $\psi_P$  at day 48 and the effect was nearly significant ( $P = 0.092$ , Table 1).

Midday water potential was significantly affected by a tree shelter  $\times$  water regime interaction ( $P = 0.016$ , Fig. 3): while plants in the mesic regime maintained relatively stable  $\psi_M$  between  $-1.11$  and  $-1.34$  MPa, values in the xeric regime declined to lows of  $-2.19$ ,  $-2.45$  and  $-3.20$  MPa for Nx, Dx and Lx treatments, respectively (Fig. 3). Among these treatments significant differences appeared only between Lx and the others.

### 3.3. CO<sub>2</sub> concentration and photosynthesis

Diurnal patterns of CO<sub>2</sub> concentrations within the tree shelters differed from the external environment, with higher fluctuations inside at both 34 and 104 DAP (Fig. 4). Both sampling points exhibited the same pattern of CO<sub>2</sub> oscillation inside the tree shelters: a decrease at sunrise to a minimum before noon, followed by relatively constant CO<sub>2</sub> concentrations until 15:00 h solar time when concentrations increased to maximum values after sunset. The temperature inside the tree shelters responded to PFD fluctuations (Fig. 4), with maximum values reaching 35–40 °C and values over 30 °C being maintained during long periods of the day (i.e., 09:30–17:00 h solar time).

CO<sub>2</sub> values in light and dark shelter treatments were similar at 34 DAP, and sheltered treatments were greater than non-sheltered treatments. More pronounced differences were found between tree shelters in the mesic and xeric regimes 104 DAP, with minimum

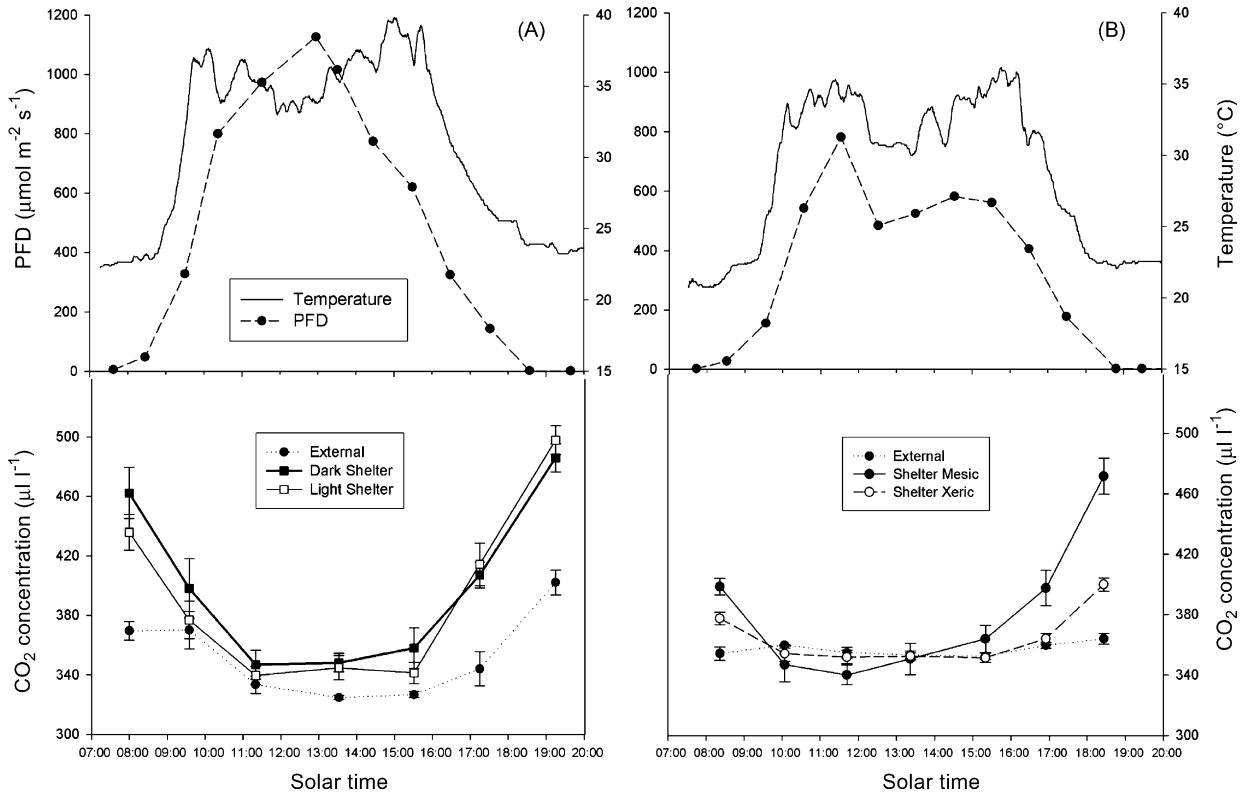


Fig. 4. Diurnal fluctuations in CO<sub>2</sub> concentration inside tree shelters (mean ± S.E., *n* = 8) in response to tree shelter color (34 days after planting, A) and water regime (104 days after planting, B). External air CO<sub>2</sub> concentration (mean ± S.E., *n* = 3), external hourly PFD radiation (calculated from hourly means during CO<sub>2</sub> measurements) and tree shelter temperature (mean of four probes placed inside tree shelters, see Section 2) are presented for reference.

values below external air concentration for shelters within the mesic regime (Fig. 4).

*A<sub>n</sub>* was significantly affected by tree shelter treatments 46–47 DAP, but not by water regime (Table 2). Net photosynthesis of plants inside light tree shelters was 259% and 412% higher than without shelters and in dark tree shelters, respectively. In contrast, 98–111 DAP, *A<sub>n</sub>* was nearly significantly affected by water regime (*P* = 0.091, Table 2), but not by tree shelter.

Among all environmental conditions evaluated during photosynthesis measurements, only mean PFD responded significantly to tree shelter treatments

(Table 3) during both periods. Mean CO<sub>2</sub> concentration of the air from the tree shelter during measurements was only affected 46–47 DAP, when the dark tree shelters had the highest CO<sub>2</sub> concentrations (Table 3). Differences between external and internal dark and light tree shelter CO<sub>2</sub> concentrations were 30.5 and 17.7 μl l<sup>-1</sup>, respectively. During the second period, although differences for this variable were not significantly affected by tree shelter treatments (*P* = 0.101, Table 3), a similar trend was found, with higher concentrations of CO<sub>2</sub> inside the tree shelters. Leaf chamber temperature when photosynthesis was measured was not affected by

Table 2

Effects of tree shelters and water regime on photosynthesis (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) at 46, 47 (first period, 30 plants total) and 98–111 (second period, 47 plants total) days after planting

Number of days after planting	No shelter		Dark shelter		Light shelter		<i>P</i> > <i>F</i>	Mesic		Xeric		<i>P</i> > <i>F</i>
	Mean	S.E.	Mean	S.E.	Mean	S.E.		Mean	S.E.	Mean	S.E.	
46, 47	0.51 b	0.26	0.32 b	0.14	1.32 a	0.37	0.028	0.55	0.25	0.83	0.22	0.242
98, 106, 111	0.20	0.12	0.47	0.16	0.48	0.20	0.375	0.54	0.17	0.23	0.07	0.091

Data from different days in each period were pooled. Within a row, means with different letters (a and b) indicate significant differences.

Table 3

Microclimatic conditions during measurements of photosynthesis conducted at the 46–47 and 98–111 day periods, as affected by tree shelter treatment

Number of days after planting	Variable (unit)	ANOVA significant ( $P > F$ )	Tree shelter treatment	$n$	Mean	S.E.	Minimum	Maximum
46, 47	PFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0.004	None	11	707 a	73	417	1199
			Light	9	643 a	82	260	963
			Dark	10	357 b	52	154	658
	Leaf chamber temperature ( $^{\circ}\text{C}$ )	0.549	None	11	35.0	0.6	32.9	38.5
			Light	9	34.3	0.9	28.6	36.5
			Dark	10	33.9	0.6	31.4	37.4
	[CO <sub>2</sub> ] air from tree shelter ( $\mu\text{l l}^{-1}$ )	0.001	None	11	338.4 b	4.0	328.3	368.7
			Light	9	356.1 a	6.1	338.5	393.1
			Dark	10	368.9 a	6.1	341.7	402.0
98, 106, 111	PFD ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0.001	None	16	853 a	83	378	1483
			Light	15	660 b	67	270	1049
			Dark	16	503 b	49	248	763
	Leaf chamber temperature ( $^{\circ}\text{C}$ )	0.637	None	16	34.4	1.0	27.8	40.0
			Light	15	33.6	1.0	28.4	39.2
			Dark	16	33.7	0.9	28.0	39.5
	[CO <sub>2</sub> ] air from tree shelter ( $\mu\text{l l}^{-1}$ )	0.101	None	16	371.3	4.9	345.1	401.5
			Light	15	379.7	7.2	328.9	454.8
			Dark	16	388.5	4.5	353.2	419.9

Data registered from LCA4 plant chamber sensors and analysis unit. Data from different days in each period were pooled. Within means column, values with different letters (a and b) denotes significant differences for the variable considered.

tree shelter at either sampling period (Table 3). Mean values were 34.4 and 33.9  $^{\circ}\text{C}$  for the first and second period, respectively.

#### 3.4. Tree growth, morphology and nutrient status

Plant height 112 DAP was significantly affected by both tree shelter ( $P = 0.048$ ) and water regime ( $P < 0.001$ ). Plants in dark tree shelters were 2.6 and

5.6 cm taller than those grown in light tree shelters and non-sheltered plants, respectively (Table 4). However, root collar diameter was not affected by tree shelters.

Plant biomass increased from 4.1 g prior to planting to a mean value of 6.2 g (Table 4) at the end of the experiment. By this time (112 DAP) total plant biomass was not affected by tree shelters. Plant dry mass in the mesic water regime was 14% greater than in the xeric regime, and differences were nearly significant

Table 4

Effects of tree shelters (T) and water regime (W) on height, root collar diameter, biomass by fractions and total, leaf N concentration and leaf starch concentration (mean and S.E.<sup>a</sup>) of *Q. ilex* 112 days after planting

	No shelter		Dark shelter		Light shelter		$P > F$	Mesic		Xeric		$P > F$	T $\times$ W $P > F$
	Mean	S.E.	Mean	S.E.	Mean	S.E.		Mean	S.E.	Mean	S.E.		
Height (cm)	18.9 b	0.9	24.5 a	2.4	21.9 ab	1.7	0.048	25.3	1.7	18.2	0.9	<0.001	0.128
Root collar diameter (mm)	5.2	0.3	4.9	0.2	5.5	0.2	0.265	5.2	0.2	5.2	0.2	0.810	0.876
Leaves dry weight (g)	1.8	0.1	2.2	0.2	2.1	0.2	0.175	2.3	0.2	1.7	0.1	0.010	0.148
Stem dry weight (g)	1.0	0.1	1.0	0.1	1.0	0.1	0.856	1.1	0.1	0.9	0.1	0.036	0.443
Shoot dry weight (g)	2.7	0.2	3.2	0.3	3.1	0.3	0.387	3.4	0.3	2.6	0.2	0.010	0.216
SLA ( $\text{cm}^2 \text{g}^{-1}$ )	59.8	1.4	64.7	1.9	61.2	1.3	0.053	64.3	1.3	59.5	1.2	0.005	0.048
Root dry weight (g)	3.5	0.2	2.9	0.2	3.2	0.2	0.110	3.2	0.2	3.2	0.2	0.883	0.173
Shoot:root ( $\text{g g}^{-1}$ )	0.9	0.1	1.1	0.1	1.0	0.1	0.120	1.1	0.1	0.8	0.0	0.005	0.095
Plant dry weight (g)	6.2	0.3	6.1	0.4	6.3	0.4	0.875	6.6	0.3	5.8	0.3	0.065	0.598
Leaf N ( $\text{mg g}^{-1}$ )	15.7	0.5	14.5	0.4	14.8	0.6	0.143	14.4	0.4	15.7	0.3	0.015	0.274
Leaf starch ( $\text{mg g}^{-1}$ )	33.2	1.5	33.0	1.8	32.1	1.2	0.801	35.5	0.9	30.0	1.0	0.001	0.531

<sup>a</sup> Sample size = 72 plants total, except for N and starch sample size, pooled in 24 composite units total (three plants per composite unit). Within a row, means with different letters (a and b) denotes significant differences.

( $P = 0.065$ , Table 4). The biomass partitioning between shoot and root was strongly affected by water regime ( $P = 0.005$ ). Shoot to root ratio was 1.1 before planting, remained at 1.1 under the mesic regime and fell to 0.8 under xeric regime (Table 4).

A significant interaction between tree shelter and watering regime was detected for leaf area (Fig. 5A,  $P = 0.032$ ), specific leaf area (SLA, Table 4,  $P = 0.048$ ) and leaf area to root biomass ratio (Fig. 5B,  $P = 0.018$ ). Although no significant differences between Nx and Nm occurred, leaf area under Dm and Lm treatments was 83 and 52% higher than Dx and Lx, respectively, with differences being significant (Fig. 5A). In addition, while differences in leaf area between light and dark shelters in a particular water regime were non-significant, sheltered plants in the mesic regime developed more leaf area than sheltered plants in the xeric regime. The ratio of leaf area to root biomass and SLA showed the same trend (Fig. 5).

Foliar N and starch concentrations were not significantly affected by tree shelter treatments, although watering regime prompted different responses

for these traits (Table 4). Plants grown under the xeric regime contained 1.3 mg more N and 5.5 mg less starch per gram of dry leaf tissue than plants from the mesic regime. Soluble carbohydrates, P and K concentrations of leaves were unaffected by both tree shelter and water regime factors and interactions (data not shown).

## 4. Discussion

### 4.1. Air, soil and plant water status

Evaporative demand in the Dm treatment was lower compared to the other shelter treatments at 65–66 and 111–117 DAP. This trend became more evident with time, and VPD at 111–117 DAP in the Dm treatment was less than for ambient air for most of the day. However, VPD in treatments Lm, Dx and Lx was always greater than ambient conditions. This appears to be associated with plant transpiration rates: while VPD was reduced compared to external air in irrigated shelters or under high moisture availability (Kjelgren, 1994; Kjelgren and Rupp, 1997; Kjelgren et al., 1997), evaporative demand became higher in comparison to external air when no watering was applied (Kjelgren and Rupp, 1997); likewise, VPD was higher than external air in all experiments undertaken in Mediterranean conditions by Oliet et al. (2003). Dark shelters promote higher leaf surface area under mesic conditions (Fig. 5A). Additionally, leaves from the mesic regime must transpire at a higher rate than those from the xeric regime, according to Ohm's analogy law for plants (Cruziat et al., 2002). Therefore, transpiration rate at the plant level must be higher for the Dm treatment, reducing VPD around these plants. Thus, although the greenhouse effect within the tree shelter elevated temperature in a similar way in all the treatments (Section 3), the presence of a highly transpiring plant within the Dm treatment dramatically reduced VPD by increasing RH, with this effect becoming more evident as the plant increased in size (Fig. 2). A similar higher RH response was demonstrated by Oñoro et al. (2001), despite higher temperatures recorded inside tree shelters. These same authors also reported a significant positive relationship between shoot biomass and RH within the shelter, reflecting the transpiration effect cited by Potter (1991) at the whole plant level. Peterson et al. (1995) also registered higher midday RH values within tree shelters for two tree species, although tree shelter temperatures in this study were clearly affected by watering: temperatures 5 °C higher in non-irrigated shelters were found at midday compared to irrigated shelters. Costello et al. (1996) also reported higher

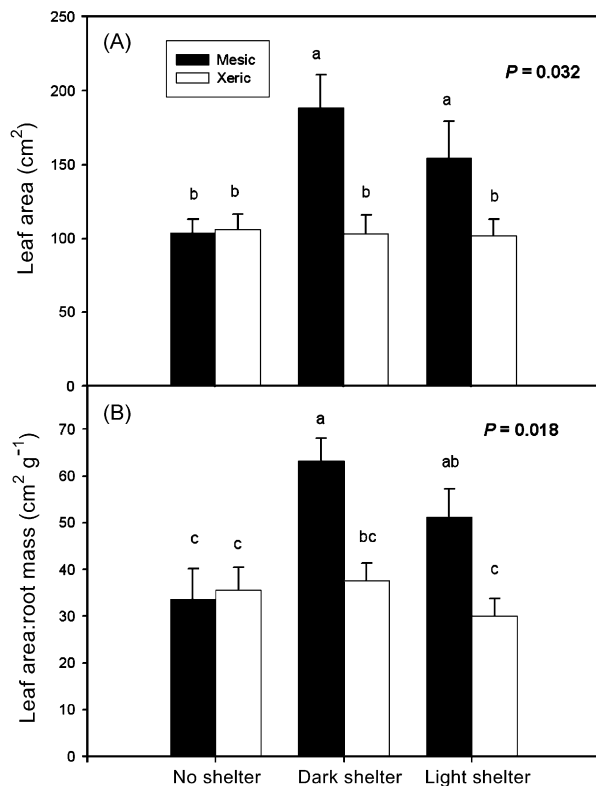


Fig. 5. Two-way interactive effect (mean and S.E.,  $n = 12$ ) of water regime and tree shelter on leaf area (A) and leaf area to root mass ratio (B) of *Q. ilex* 112 days after planting. Means with different letters (a, b and c) indicate significant differences.

maximum temperatures in non-irrigated trees compared to irrigated shelters in summer. Greenhouse conditions in our experiment (i.e., lower direct:diffuse irradiance ratio) could reduce the effect of incident radiation on temperature increases inside tree shelters. Thus, along with no significant response of temperature to watering regime, the effect of tree shelter color on temperature was also limited under the conditions of our experiment: no more than a 1 °C difference in mean temperature (09:00–18:00 h interval) was detected between dark and light tree shelters over the three sampling periods (data not shown). Conversely, under field conditions temperatures were 2–4 °C cooler in dark (brown) than in light (white) shelters (Kjelgren et al., 1997; Sharpe et al., 1999).

Tree shelter treatments affected VWC primarily during the first half of the experiment and this effect was most pronounced in the upper soil profile (0–12 cm). The shading of the pot resulting from the tree shelter could have reduced evaporation from the upper profile of the soil. However, this shade effect of tree shelters on VWC did not persist long-term as substrate from the upper 0 to 12 cm profile of the pot dried during the experiment (Fig. 1). This effect may be of less importance under field conditions because water diffuses across a wider soil volume, as shown by Bellot et al. (2002) under Mediterranean conditions.

Values of  $\psi_P$  recorded were consistent with VWC, as the former reflects soil water status (Cruziat et al., 2002). Predawn water potential of potted holm oak after 135 days in a shade and irrigation experiment was only affected by water treatment (Castro-Díez et al., 2006). However,  $\psi_M$  117 DAP was affected by the water regime  $\times$  tree shelter interaction (Fig. 3), reflecting a variable water status response of plants at midday that may integrate VWC of the soil, evaporative demand in the air and stomatal closure in response to light intensity (Acherar and Rambal, 1992; Sala, 1999). Despite low VWC of the soil under the xeric regime,  $\psi_P$  values (Table 1) were higher than critical values for stomatal closure of holm oak (Villar-Salvador et al., 2004). Under these circumstances, this species maintains high stomatal conductance for long periods in accordance with its drought-tolerance strategy (Savé et al., 1999), which results in low xylem water potential. This is particularly true for protected trees subjected to higher air evaporative demand (Fig. 2C). Among sheltered plants in the xeric regime, lower light levels within dark tree shelters could promote a decrease in stomatal conductance and transpiration rates, increasing  $\psi_M$  significantly with regard to plants inside light shelters (Fig. 3). Savé et al. (1999) and Sala (1999) also noted

stomatal closure of holm oak in response to light intensity, which decreased transpiration. Under the mesic regime, however, higher  $\psi_M$  values irrespective of shelter treatments indicates that water availability for roots compensates higher transpiration rates promoted by superior evaporative demand of the air. Thus, under medium or high water soil availability, sheltered holm oaks exhibit similar stress as non-sheltered seedlings, despite different evaporative demands.

#### 4.2. $CO_2$ concentration and photosynthesis

The daily pattern of  $CO_2$  concentration inside tree shelters reflects the fluctuations of assimilation rate during the day (Bergez and Dupraz, 2000). A decrease in  $CO_2$  concentration appeared at sunrise (Fig. 4), indicating a positive net photosynthetic rate under very low radiation levels, which was reduced further inside the tree shelters. This trend shifted before midday (from 10:00 to 11:30 h solar time) when  $CO_2$  levels within the tree shelters reached a minimum that was maintained at a near constant level (indicating almost null assimilation rates) until increasing in the afternoon. A similar trend was found in other studies (Peterson et al., 1995; Dupraz and Bergez, 1999; Bergez and Dupraz, 2000), with the latter two studies reporting a greater reduction in  $CO_2$  concentration during sunrise and lower minimum values at midday inside tree shelters. These values were below  $CO_2$  compensation points for the species employed in these studies, *Prunus avium* L., which negatively affected assimilation rates. In our study, however, minimum midday  $CO_2$  concentrations were close to external values, in accordance with low  $A_n$  rates, and therefore  $CO_2$  concentrations are not likely to serve as primary limitation to assimilation. Dupraz and Bergez (1999) and Bergez and Dupraz (2000) concluded that without soil water limitations, assimilation inside shelters is chiefly limited by  $CO_2$  rather than PFD; however, when high temperatures occur, photosynthesis is impaired, and  $CO_2$  concentration inside tree shelters would therefore not likely fall below external levels. In our study, temperatures over 30 and 35 °C were reached soon after sunrise (Fig. 4). Because optimal temperature for assimilation processes of holm oak peaks below 25 °C (Gratani et al., 1998; Mediavilla et al., 2002), positive assimilation rates are restricted to early morning hours, when PFD values were low. Thus, maximum assimilation rates in sheltered plants occurred during only a short period of the day.

This may explain the relatively low  $A_n$  values measured, which are in agreement with low biomass

growth (2.1 g) during the course of the experiment. In spite of this, significantly higher photosynthetic rates inside light tree shelters 46–47 DAP were associated with the combined effect of higher light transmittance through the shelters along with less water stress incurred by sheltered plants during this period. A significant ( $P = 0.002$ ) linear regression model fitted for photosynthesis on PFD (Fig. 6) supports this contention. The lower  $\text{CO}_2$  concentration values measured in light tree shelters (especially during the first hours of the day, Fig. 4A) reinforces the hypothesis of a higher assimilation rate of plants in those shelters 46–47 DAP. During the 98–111 DAP sampling, neither  $\psi_P$  (measured on day 99) nor VWC (measured 98, 105 and 110 DAP) were affected by tree shelter, and no significant relationship between PFD and  $A_n$  was found.  $A_n$  was primarily affected by water regime during this time. Variation in  $\text{CO}_2$  cycle during this period, reaching minimum values at around midday (Fig. 4B), suggests higher  $A_n$  of protected plants in the mesic regime. Higher  $\psi_M$  values of plants from the mesic regime during this period (Fig. 3) also helps explain higher  $A_n$ . When excluding non-sheltered plants, the photosynthetic rate of those from the mesic water regime was increased to a mean of  $0.71 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , and differences with plants from the xeric regime were nearly significant ( $P = 0.051$ , data not shown). A rapid increase of  $\text{CO}_2$  concentration inside tree shelters for plants under the mesic regime, detected after 15:00 h solar time during the period (Fig. 3B), implicates increased respiration for these plants, which is consistent with higher  $A_n$  values of plants in the mesic regime measured before 15:00 h solar time (Chiariello et al., 1996).

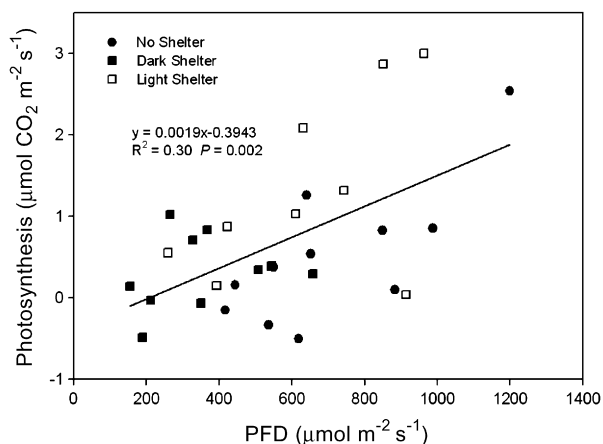


Fig. 6. Relationship between photosynthetic flux density (PFD) and net photosynthesis of *Q. ilex* seedlings 46 and 47 days after planting ( $n = 30$ ).

Mean  $\text{CO}_2$  concentration of air from the tree shelter during photosynthesis measurements (Table 3) is consistent with that shown in the diurnal pattern of  $\text{CO}_2$  concentration for both measurement periods (Fig. 4). Thus, current  $\text{CO}_2$  concentration of air passing through the leaf chamber as photosynthesis is measured is similar to that inside a tree shelter when no window is opened. Hence, our study demonstrates that measurements of current assimilation rate in shelters with an open photosynthesis system can be done without affecting  $\text{CO}_2$  content of sheltered air. Although daytime temperatures in shelters were higher compared to external air (Section 3), mean air temperature inside the leaf chamber during measurements in the shelters equalled external temperature (Table 3). This was probably due to higher incident radiation on the leaf in the chamber when not shaded by the shelter wall, as well as a decrease in temperature when a window in the shelter was opened. This effect is a source of experimental error that must be considered when gas exchange measurements are recorded in shelters.

#### 4.3. Tree growth, morphology and nutrient status

The increase in seedling height within a short-term duration (i.e., several months) inside tree shelters compared to those without shelters is consistent with many reports (Hammat, 1998; Sharpe et al., 1999; Bergez and Dupraz, 2000; Jacobs and Steinbeck, 2001; Bellot et al., 2002; Oliet et al., 2005). This response of protected plants is associated with reduction in light availability within the tubes, as plants had not yet reached the top of the shelters. The absence of height differences among shelter colors is in accordance with previous studies, where different height responses of various species were found only with much wider shelter light transmission gradients (Potter, 1991; Jacobs and Steinbeck, 2001; Oliet et al., 2003; Sharew and Hairston-Strang, 2005). The absence of differences in total biomass between protected and non-sheltered plants is in agreement with similar  $A_n$  values measured at the end of the experiment. Total biomass response seems to be primarily affected by watering regime, as also occurred with  $A_n$ . Holm oak seedling biomass is only reduced in response to light under much lower levels (Retana et al., 1999; Gómez-Aparicio et al., 2006). Other plant traits, however, like total leaf area, SLA and biomass allocation between shoot and root (particularly when the shoot variable represents leaf area) appeared to be affected by a tree shelter  $\times$  water regime interaction. This is supported by another study that showed holm oak had higher phenotypic plasticity

for these traits compared to other species of Mediterranean ecosystems (Valladares et al., 2005). In particular, the trend for decreasing response of SLA to radiation (Table 4,  $P = 0.053$ ) reflects the plasticity of leaves to optimize capture of light, photosynthetic capacity and leaf cooling under varying PFD (Castro-Díez et al., 2006). In the latter study, however, neither holm oak SLA nor shoot to root ratio varied significantly with watering regime after 8 months in pots (Castro-Díez et al., 2006); thus, our study illustrates that the effect of imposed drought on certain traits can vary according to drought severity. Leaf area to root mass ratio was higher under the mesic regime only for protected seedlings (Fig. 5B). Significantly higher leaf area of holm oaks in tree shelters (particularly in dark tree shelters) in the mesic regime (Fig. 5A) indicates that, under these water availability conditions, assimilates of shoots from protected plants are mainly mobilized to produce photosynthetic surface because no differences in stem dry weight by treatments were detected (Table 4). A similar trend was found for other Mediterranean species such as *Q. faginea* and *Q. coccifera* inside tree shelters (Oñoro et al., 2001; Bellot et al., 2002, respectively), as well as for temperate zone species (Dupraz, 1997). Such results are relevant only before the trees emerged from the shelter, which in Mediterranean conditions may occur after the second year after planting in 60 cm tall shelters (Oliet et al., 2003).

Higher leaf area to root biomass ratios of protected plants under the mesic regime reflects reduced capacity to tolerate drought, given that higher transpiration losses are not likely to be compensated for by root uptake; thus, plants grown in tree shelters under non-moisture limiting conditions during spring could incur more stress during subsequent summer drought periods. Well watered and moderately shaded (50% of external PFD) holm oaks exhibited greatest leaf area in July and subsequently had to reduce leaf area more than seedlings grown under full sun and low watering treatments to adjust shoot to root ratio to late-summer conditions (Castro-Díez et al., 2006). In our study, light tree shelters resulted in a more balanced leaf area to root biomass ratio compared to dark tree shelters.

Lack of differences in starch leaf concentration,  $A_n$  and total biomass associated with tree shelter application suggests that tree shelters do not affect C partitioning between reserve and structural carbohydrates. The tendency for lower foliar N concentrations of plants protected by dark tree shelters, however, is in correspondence with shoot to root ratio results: plants with lower values for this attribute have higher N uptake

in relation to shoot biomass, as further exhibited by differences in N concentration according to watering regime.

## 5. Conclusions

The performance of holm oak within tree shelters results from a wide variety of environmental factors, many of which are affected by the presence of the plant itself. A slight effect of tree shelter on soil water content was detected, although it is not possible to confirm whether this effect will be maintained under field conditions. In addition, we observed that, as the plant grows within shelters without water limitations (mesic regime), VPD of the air in the shelters decreases (particularly in dark shelters) due to higher transpiration rates at the whole plant level. Thus, protected plants in dark shelters could obtain benefits from both lower evaporative demand of the air, causing higher  $\psi_M$  under a xeric regime, as well as from low irradiance levels that promote height growth. Other traits, however, were negatively affected in dark shelters, such as photosynthetic rate. In addition, a notable trend was found along the gradient from no shelter to light shelter to dark shelter in regard to morphological balance under the mesic regime: as plants received less irradiance, they developed greater leaf area, as well as increased shoot to root ratio. This is a consequence of high phenotypic plasticity of this species, with large shifts at the plant level in response to relatively low changes in light conditions.

Tree shelter management must be adaptable and match environmental conditions of the planting locale to species plasticity. In this respect, our results suggest that holm oaks, particularly in dark shelters, may develop a less balanced shoot:root morphology during wet springs that could jeopardize late summer survival. This same consideration also applies to use of any other shading mechanism during field establishment for this species. Consequentially, light shelters with high transmittance are recommended for planting holm oak in Mediterranean areas characterized by relatively wet springs followed by hot, dry summers. The effect of higher transmittance of light on VPD and, hence, water status of the plant during that season, deserves more attention particularly in regard to the trade-off between higher evaporative demand and the acquisition of a more balanced shoot:root phenotype. Additionally, potential discrepancies in some environmental variables within a greenhouse versus field environment (e.g., differential action of advection on VPD and ventilation rates, lower direct:diffuse irradiance) necessitate further

examination of these responses specific to field conditions.

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## References

- Acherar, M., Rambal, S., 1992. Comparative water relations of four Mediterranean oak species. *Vegetation* 99–100, 177–184.
- Archibold, O.W., 1995. *Ecology of World Vegetation*. Chapman and Hall, London, 528 pp.
- Baeza, M.J., Pastor, A., Matín, J., Ibáñez, M., 1991. Mortalidad postimplantación en repoblaciones de *Pinus halepensis*, *Quercus ilex*, *Ceratonia siliqua* y *Tetraclinis articulata* en la provincia de Alicante. *Studia Oecol.* VIII, 139–146.
- Bellot, J., Ortiz De Urbina, J.M., Bonet, A., Sánchez, J.R., 2002. The effects of tree shelters on the growth of *Quercus coccifera* L. seedlings in a semiarid environment. *Forestry* 75 (1), 89–106.
- Bergez, J.E., Dupraz, Z.C., 1997. Transpiration rate of *Prunus avium* L. seedlings inside an unventilated tree shelter. *For. Ecol. Manage.* 97, 255–264.
- Bergez, J.E., Dupraz, Z.C., 2000. Effect of ventilation on growth of *Prunus avium* seedlings grown in tree shelters. *Agric. For. Meteorol.* 104, 199–214.
- Castro-Díez, P., Navarro, J., Pintado, A., Sancho, L.G., Maestro, M., 2006. Interactive effects of shade and irrigation on the performance of seedlings of three Mediterranean *Quercus* species. *Tree Physiol.* 26, 389–400.
- Chiariello, N.R., Mooney, H.A., Williams, K., 1996. Growth, carbon allocation and cost of plant tissues. In: Pearcy, R.W., Ehleringer, J.R., Mooney, H.A., Rundel, P.W. (Eds.), *Plant Physiological Ecology: Field Methods and Instrumentation*. Chapman and Hall, London, 457 pp.
- Costello, L.R., Peters, A., Giusti, G., 1996. An evaluation of tree shelter effects on plant survival and growth in a Mediterranean climate. *J. Arboric.* 22 (1), 1–9.
- Cruziat, P., Cochard, H., Ameglio, T., 2002. Hydraulic architecture of trees: main concepts and results. *Ann. For. Sci.* 59, 723–752.
- Dupraz, C., 1997. Abris-serres: ce quien pensant les arbres. *Revue forestière. française.* 49 (5), 417–432.
- Dupraz, C., Bergez, J.E., 1999. Carbon dioxide limitation of the photosynthesis of *Prunus avium* L. seedlings inside an unventilated tree shelter. *For. Ecol. Manage.* 119, 89–97.
- Gómez-Aparicio, L., Valladares, F., Zamora, R., 2006. Differential light responses of Mediterranean tree saplings: linking ecophysiology with regeneration niche in four co-occurring species. *Tree Physiol.* 26, 947–958.
- Gratani, L., Pesoli, P., Crescente, M.F., 1998. Relationship between photosynthetic activity and chlorophyll content in an isolated *Quercus ilex* L. tree during the year. *Photosynthetica* 35 (3), 445–451.
- Hammatt, N., 1998. Influence of tree shelters, irrigation and branch pruning on early field performance of micropropagated wild cherry cv. F12/1. *New For.* 15, 261–269.
- Jacobs, D.F., Steinbeck, K., 2001. Tree shelters improve the survival and growth of planted Engelman spruce seedlings in southwestern Colorado. *Western J. Appl. For.* 16 (3), 114–120.
- Jiménez-Sancho, M.P., Díaz-Fernández, P.M., Iglesias, S., De Tuero Y Reina, M., Gil, L., 1996. Las regiones de procedencia de *Quercus ilex* L. en España. ICONA, Madrid, 93 pp.
- Kerr, G., 1996. The history, development and use of tree shelters in Britain. In: Brissette, J.C. (Ed.), *Proceedings of the Tree Shelter Conference*. USDA Forest Service. NE Forest Experiment Station. GTR-NE-221. pp. 1–4.
- Kjelgren, R., 1994. Growth and water relations of Kentucky coffee tree in protective shelters during establishment. *HortScience* 29 (7), 777–780.
- Kjelgren, R., Rupp, L.A., 1997. Establishment in tree shelters I: shelters reduce growth, water use, and hardiness, but not drought avoidance. *Hortscience* 32 (7), 1281–1283.
- Kjelgren, R., Montague, D.T., Rupp, L.A., 1997. Establishment in tree shelters. II. Effect of shelter color on gas exchange and hardiness. *Hortscience* 32 (7), 1284–1287.
- Little, T.M., Hills, F.J., 1990. *Métodos estadísticos para la investigación en la agricultura*. Trillas, 2ª ed., 1ª reimp México, 270 pp.
- Marques, P.M., Ferreria, L., Correia, O., Martins-Louçao, M.A., 2001. Tree shelters influence growth and survival of carob (*Ceratonia siliqua* L.) and cork oak (*Quercus suber* L.) plants on degraded Mediterranean sites. In: Villacampa, Y., Brebbia, C.A., Usó, J.L. (Eds.), *Ecosystems and Sustainable Development III*. Wit Press, Southampton, Boston, pp. 635–644.
- Martínez-Vilalta, J., Mangirón, M., Ogaya, R., Saurer, M., Serrano, L., Peñuelas, J., Piñol, J., 2003. Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. *Tree Physiol.* 23, 747–758.
- Mediavilla, S., Santiago, H., Escudero, A., 2002. Stomatal and mesophyll limitations to photosynthesis in one evergreen and one deciduous Mediterranean oak species. *Photosynthetica* 40 (4), 553–559.
- Navarro, R.M., Fragero, B., Ceaceros, C., Del Campo, A., 2005. Establecimiento de *Quercus ilex* L. subsp. *ballota* [Desf.] Samp. utilizando tubos invernadero ventilados. In: *Actas IV Congreso Forestal Español*. Mesa 2. CD Proceedings of the Congress.
- Oliet, J.A., Navarro, R.M., Contreras, O., 2003. Evaluación de la aplicación de mejoradores y tubos en repoblaciones forestales. *Consejería de Medio Ambiente de la Junta de Andalucía*, 234 pp.
- Oliet, J.A., Planelles, R., Artero, F., Jacobs, D.F., 2005. Nursery fertilization and tree shelters affect long-term field response of *Acacia salicina* Lindl. planted in Mediterranean semiarid conditions. *For. Ecol. Manage.* 215, 339–351.
- Oñoro, F., Villar-Salvador, P., Domínguez Lerena, S., Nicolás, J.L., Peñuelas, J.L., 2001. Influencia de la siembra y plantación con dos tipos de tubos protectores en el desarrollo de una repoblación de *Quercus faginea* Lam. *Actas III Congreso Forestal Español*. Mesa 3, 137–142.
- Pesoli, P., Gratani, L., Larcher, W., 2003. Responses of *Quercus ilex* from different provenances to experimentally imposed water stress. *Biologia Plant.* 46 (4), 577–581.
- Peterson, J.A., Groninger, J.W., Seiler, J.R., Will, R.E., 1995. Tree shelter alteration of seedling microenvironment. In: Boyd, E.M. (Comp.), *Proceedings of the Eighth Biennial Southern Silvicultural*

- tural Research Conference, 1994 November 1–3, Auburn, AL. USDA Forest Service Southern Research Station Gen. Tech. Rep. SRS-1, Asheville, NC, pp. 305–310.
- Potter, M.J., 1988. Tree shelters improve survival and increase early growth rates. *J. For.* 86, 39–41.
- Potter, M.J., 1991. Tree shelters. Forestry Commission. Handbook 7, 48 pp.
- Retana, J., Espelta, J.M., Gracia, M., Riba, M., 1999. Seedling recruitment. In: Rodà, F., Retana, J., Gracia, C., Bellot, J. (Eds.), *Ecology of Mediterranean evergreen oak forests*. Ecological Studies. Springer, Berlin, pp. 89–103.
- Rey Benayas, J.M., Camacho-Cruz, A., 2004. Performance of *Quercus ilex* saplings planted in abandoned Mediterranean cropland after long-term interruption of their management. *For. Ecol. Manage.* 194, 223–233.
- Rodà, F., Retana, J., Gracia, C., Bellot, J. (Eds.), 1999. *Ecology of Mediterranean evergreen oak forests*. Ecological Studies. Springer, Berlin, 373 pp.
- Sala, A., 1999. Modelling canopy gas exchange during summer drought. In: Rodà, F., Retana, J., Gracia, C., Bellot, J. (Eds.), *Ecology of Mediterranean evergreen oak forests*. Ecological Studies. Springer, Berlin, pp. 149–161.
- Savé, R., Castell, C., Terradas, J., 1999. Gas exchange and water relations. In: Rodà, F., Retana, J., Gracia, C., Bellot, J. (Eds.), *Ecology of Mediterranean evergreen oak forests*. Ecological Studies. Springer, Berlin, pp. 135–147.
- Sharew, H., Hairston-Strang, A., 2005. A comparison of seedling growth and light transmission among tree shelters. *Northern J. Appl. For.* 22 (2), 102–110.
- Sharpe, W.E., Swistock, B.R., Mecum, K.E., Demchik, M.C., 1999. Greenhouse and field growth of northern red oak seedlings inside different types of tree shelters. *J. Arboric.* 25 (5), 249–257.
- Spiro, R.G., 1966. Analysis of sugars found in glycoproteins. In: Neufeld, E.F., Ginsburg, V. (Eds.), *Methods in Enzymology*, vol. VIII. Academic Press, New York, pp. 3–36.
- Swistock, B.R., Mecum, K.A., Sharpe, W.E., 1999. Summer temperatures inside ventilated and unventilated brown plastic tree shelters in Pennsylvania. *Northern J. Appl. For.* 16, 7–10.
- Tuley, G., 1983. Shelters improve the growth of young trees in the forest. *Quart. J. For.* 77, 77–87.
- Tuley, G., 1985. The growth of young oak trees in shelters. *Forestry* 58, 181–195.
- Valladares, F., Martínez-Ferri, E., Balaguer, L., Pérez-Corona, E., Manrique, E., 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol.* 148, 79–91.
- Valladares, F., Dobarro, I., Sánchez-Gómez, D., Pearcy, R.W., 2005. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. *J. Exp. Bot.* 56 (411), 483–494.
- Vallejo, R., Alloza, J.A., 1999. The restoration of burned lands: the case of eastern Spain. In: Vallejo, R. (Ed.), *Large Forest Fires*. Backhuys, Leiden, pp. 91–108.
- Villar-Salvador, P., Planelles, R., Oliet, J., Peñuelas-Rubira, J., Jacobs, D.F., González De Chavez, M., 2004. Drought tolerance and transplanting performance of holm oak (*Quercus ilex* L.) after drought hardening in the nursery. *Tree Physiol.* 24, 1147–1155.