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IMPACTS OF MULCHING AND TREE SHELTERS ON CORK OAK (*QUERCUS SUBER* L.)  
SEEDLING SURVIVAL AND GROWTH AFTER FOUR GROWING SEASONS

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RÉSUMÉ.— *Effet du paillage et des abris-serres sur la survie et la croissance de plants de Chêne-liège (Quercus suber L.) durant quatre saisons de croissance.*— Cinq modalités de paillage (Pin pignon, Lentisque, mélange de Pin pignon et de Lentisque (paillages organiques), gravier (paillage inorganique) et témoin), ainsi que trois modalités d'abris-serres (non aérés, aérés et témoins) ont été testées. Aucun des deux types d'abris-serres aéré et non aéré n'a eu d'effet significatif sur le taux de survie des plants, alors qu'ils ont significativement augmenté la croissance en hauteur moyenne des plants, par rapport au témoin, respectivement de 74-104 % et 93-106 % durant les quatre années. Contrairement à la croissance en hauteur, le diamètre moyen de la tige à la base des plants des abris-serres aéré et non-aéré a été significativement plus petit que celui du témoin, durant toute la période d'étude, respectivement de 23-31 % et 38-42 %. Les abris-serres ont significativement affecté le microclimat autour des plants par augmentation ou diminution de la température, de l'humidité relative et du déficit de pression de vapeur de l'air. Ils ont réduit, par ailleurs, le taux de photosynthèse, et donc la production en biomasse totale, en raison du faible niveau lumineux. L'augmentation de la largeur et l'aération des abris pourraient aider à diminuer la température et augmenter la transmission de la lumière, ce qui pourrait les rendre plus favorables à la croissance des plants. Le paillage n'a pas eu d'effet significatif sur la croissance des plants durant toute la période d'étude. L'utilisation combinée des abris-serres et du paillage n'a pas amélioré la croissance des plants.

SUMMARY.— We evaluated the effect of mulching and tree shelters on survival and growth of planted Cork oak (*Quercus suber* L.) seedlings during four growing seasons. Five mulch types (Italian Stone Pine, Lentisk, and a combination of Italian Stone Pine and Lentisk (organic mulches), gravel (inorganic mulch) and no mulch) and three tree shelter types (non-vented, vented and control) were tested. Tree shelters did not have a significant effect on seedling survival rate during the study period, whereas both vented and non-vented shelters significantly increased seedling mean height during the four years by 74-104 % and 93-106 %, respectively, compared to unsheltered seedlings (control). Contrary to height growth, mean basal diameter in vented and non-vented shelters was significantly lower by 23-31 % and 38-42 %, respectively, than in controls in all the years. Tree shelters significantly affected the microclimate surrounding the seedlings by increasing/decreasing temperature, relative humidity and vapour pressure deficit. Both shelters reduced photosynthesis rate, and therefore total biomass, as a result of low light availability. Increasing shelter width and aeration may help to decrease temperature and increase light transmission, which could make shelters more conducive to seedling growth. Mulching had no significant effects on seedlings growth during the study. The combined use of mulching and tree shelters did not improve seedling growth.

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Cork oak (*Quercus suber* L.) is an outstanding sclerophyllous species in Mediterranean forest ecosystems. In Tunisia, it is well represented in the north-west of the country (70 000 ha), where local inhabitants graze livestock. It plays an important ecological role, supporting a diversified floristic and faunal assemblage. It also plays a considerable socio-economic role in the life of the rural population of the area and contributes to the national economy through production and export of cork, conferring cork oak with a particular place amongst forest species in Tunisia. Unfortunately, this precious and fragile forest heritage undergoes a continual degradation under the effects of numerous factors of deterioration including poor natural regeneration, the physiological exhaustion of old trees, acorn gathering and predation, and repetitive forest fires. Fifty-seven thousand ha of cork oak forest were lost during 1934-2003, corresponding to 826 ha/yr and 39 % of its total area (Nsibi *et al.*, 2006). Early attempts with artificial regeneration of cork

oak to compensate the lack of natural regeneration have not been successful. Lack of success was mainly related to overgrazing, the inability of cork oak to compete efficiently with the surrounding vegetation (Hasnaoui, 1992), and slow juvenile growth rates.

Efforts to shield seedlings from animal browse led to the invention of individual seedling browsing protectors or tree shelters (Tuley, 1985) which are cylindrical or square, translucent, polypropylene tubes of varying heights with single or double walls. Besides their protective role from browsing, tree shelters are reported to reduce herbicide contact with protected trees (Švihra *et al.*, 1993; Bergez & Dupraz, 2000) and promote seedling survival (Potter, 1991) and height growth (Oliet *et al.*, 2005; McCreary *et al.*, 2002; Pemán *et al.*, 2010) by creating a more favourable microenvironment surrounding the plant (Tuley, 1985; Potter, 1988, 1991). While tree shelters have in most cases a positive effect on height growth, their effect on diameter growth is dependent on species and shelter type (Sharrow, 2001; Sharew & Hairston-Strang, 2005).

Regardless of protection against herbivores, the success of plantation establishment depends largely on management after planting; particularly weed control (Albouchi & Abbassi, 2000), soil preparation and technique of plantation (Varela, 2013; Varela & Amandier, 2015). Mulching is a weed control method widely used in agriculture and horticulture. More recently, the use of mulching has been studied in forestry applications, including establishment of hardwood and conifer plantations (Adams, 1997; Haywood, 2000; Green *et al.*, 2003). Mulches are beneficial to forest plantations because of their capacity to reduce vegetative competition (Green *et al.*, 2003), to conserve soil moisture, and to increase the availability of key soil resources such as nitrogen (Truax & Gagnon, 1993). Mulches can be either organic or inorganic. Organic mulches are composed of cork mulch (see Piazzetta, 2013), wood, bark, or leaves singly or in combination, while inorganic mulches include gravel, pebbles, or polyethylene film.

Experiments examining the effect of tree shelters are often of a short duration not exceeding generally one (Navarro Cerrillo *et al.*, 2005; Taylor *et al.*, 2009; Mechergui *et al.*, 2012) or two growing seasons (Leroy & Caraglio, 2003; Jiménez *et al.*, 2005; Mariotti *et al.*, 2015). Less information is available for longer durations. In addition, the effects of tree shelters and mulching have frequently been examined in separate experiments, but rarely in concert. Furthermore, classically measured parameters in studying the effect of treatments on oak growth (i.e., height, basal diameter, and biomass growth (Costello *et al.*, 1996; McCreary & Tecklin, 1997)) do not account for the typically rhythmic pattern of height growth in oak species (Reich *et al.*, 1980; Champagnat *et al.*, 1986; Harmer 1990; Mechergui *et al.*, 2012). The study of this growth pattern involves the division of the main stem into growth units, and provides useful information on this growth response characterization.

The goal of this study was 1) to evaluate the effectiveness of tree shelters and mulching in establishing of an autochthonous species, cork oak (*Quercus suber* L.), and 2) to determine *i*) the most appropriate mulch and/or tree shelter type(s), *ii*) if increased height growth by tree shelters, often cited in the literature, is temporary or persistent and *iii*) if tree shelters require improvements in their design based on their effect on photosynthetic activity of plant. This paper presents 4-year results of a study of tree shelters and mulching established in north-western Tunisia.

## MATERIALS AND METHODS

### STUDY AREA

The study was undertaken at the M'hibeus national forest (9°07'52"N, 37°06'05"E, 200 m a.s.l.) in north-western Tunisia (Sejnane forest subdivision), from February 2009 to December 2012. The climate is Mediterranean with an annual average temperature of 18.2 °C (1975–2004). Average maximum and minimum temperatures are 34.4 °C and 5.6 °C, respectively. The average annual rainfall is 911.1 mm, with 77 % of the total rainfall falling in winter and autumn and only 4 % in summer. Soil organic matter content was 5.74 % in A-horizon, 2.43 % in B-horizon and 0.78 % in C-horizon. The

site was cleared of maquis vegetation mainly dominated by *Calicotome villosa* (Poir.) Link, *Cistus monspeliensis* L., *Myrtus communis* L., and *Pistacia lentiscus* L.

## PLANT MATERIAL

Three hundred 1-year-old seedlings of cork oak (*Quercus suber* L.) were hand planted on the site, in February 2009, at 4 m × 4 m spacing in an area of 4800 m<sup>2</sup>. Seedlings had been grown in a local Sejnane nursery, in containers of a diameter of 12 cm and 30 cm of depth, filled with a mixture of 50–50 % (v/v) blue-leaved wattle (*Acacia saligna* (Labill.) Wendl. f.) and Italian stone pine (*Pinus pinea* L.) bark composts. Prior to planting, all vegetation line was cut close to ground level. Manual hoeing around each planted seedling prepared a surface of 1 m<sup>2</sup> for the mulch sheeting. The prepared surface around each seedling was 3 cm deep to fix the mulch and avoid removal by wind or runoff. Seedlings were watered after planting with 5 L per seedling. The experimental site was fenced to restrict herbivore access. Initial values measured just after plantation varied between 28.6 to 105 cm for height growth, with an overall mean of 65.1 ± 0.71 cm (± S.E) and between 3.1 to 11.6 mm for basal diameter, with an overall mean 7.5 ± 0.08 mm. They were not significantly different among the two controlled factors (mulch and shelter types) or interactions.

## EXPERIMENTAL DESIGN AND TREATMENTS

The trial was a split-plot design with main plots in randomized blocks with four replications (or blocks). Each block had 75 seedlings allocated between the following treatments: five mulch and three tree shelter types (5 mulch types × 3 tree shelter types × 5 seedlings). Seedlings were planted in a rectangular block (60 × 20 m). Mulch types were the main plots and tree shelter types were the subplots. Each subplot was a five-seedling row. Rows were adjacent. Tested mulches were: 1) Italian Stone Pine (*Pinus pinea* L.); 2) Lentisk (*Pistacia lentiscus* L.); 3) combined mulch of Italian Stone Pine and Lentisk (50–50%) (organic mulches); 4) gravel (inorganic mulch) and 5) untreated control (no mulch). Organic mulches consisted of prunings, 20 to 40 cm long and 5 to 20 mm thick. Mulches were not oven-dried before use due to the risk of rapid disintegration during the major rainfall season, which may lead to an accelerated rate of decomposition (Oelbermann *et al.*, 2004). Moreover, the use of fresh biomass is also more representative of natural decay processes when these mulching materials are applied to the soil surface (Fang *et al.*, 2008). Inorganic mulch consisted of gravel (calibration: 4–16 mm) from a quarry in Bizerte. All mulches were applied in an approximately 3-cm thick layer to an area of 1 × 1 m square around individual seedlings (see Mechergui *et al.*, 2012). The three tree shelter types were: (1) ‘Non-vented’ tree shelter, (2) ‘Vented’ tree shelter, and (3) Control with no shelters. The non-vented tree shelters (Tubex ‘L’ Standard®) were translucent green (Tubex Co., South Wales, UK). The wall of the tree shelter is totally airtight and the entrance of fresh air is only possible through the top of the tree shelter. The vented tree shelters (Tubex ‘E’ Equilibre®) were ventilated by ten 1-cm-wide round holes at their base, creating a “chimney effect”. Both vented and non-vented tree shelters are circular, 1.8-m tall, 8.0–12.0 cm wide, UV stabilized polypropylene with twin-walls. They were buried 5 cm into the soil immediately after planting to prevent air movement through the shelter bottom that could cause desiccation. They were then secured to a 1.8 m untreated eucalyptus stakes anchored 20 cm into the soil. Mesh caps were placed over them to keep birds out.

## GROWTH MEASUREMENTS

Measurements were made in the 300 seedlings. Height and basal stem diameter were measured and recorded at the time of planting (February 2009) and at the end of each growing season (December, 2009, 2010, 2011 and 2012). As survival was very high (97 %, only 9 of 300 seedlings died), treatments were not analysed for survival differences. Heights were recorded from the base of the seedling to the end of the longest shoot held straight. Diameter measurements were taken at the base of the seedling, approximately 2 cm above the ground, using electronic callipers.

After tree shelters were removed, the ability of a seedling to support itself without a stake was evaluated by determining whether the seedling would stand on its own or bend and touch the ground.

Oak height growth occurs in a rhythmic pattern with periods of uninterrupted terminal bud growth (flushes) alternating with periods of bud development and apparent rest (Harmer, 1990; Mechergui *et al.*, 2012). During each growth flush, a distinct portion of the stem called the growth unit (GU) is established. It is composed of nodes, on which are inserted basal scales or foliage leaves, and internodes (Chaar *et al.*, 1997). GUs occurred during a given growth season form a portion of the stem named annual shoot. The following growth components were recorded at the end of the growing season: length and number of internodes per GU, number of GUs established by the main stem, and length of the GU and annual shoot.

At the end of year 4, 2 seedlings per block were randomly selected from each treatment (total of 120 seedlings) and harvested to determine seedling biomass. Leaves were removed from stems, stems were severed at the root-collar, and all roots were then rinsed free of soil. Weight of the above- and below-ground biomass were then determined after drying at 70 °C for 6 days. Shoot : root ratio was calculated for each seedling from dry weights of the biomass components.

The effect of the tree shelters on the number branches produced was assessed in 4 seedlings, grown with and without tree shelters, randomly selected.

Measurements of photosynthesis rate, as detailed by Oliet & Jacobs (2007), were performed in spring of the fourth year (2012) on a total of 4 plants randomly chosen from each shelter treatment (1 plant/block/shelter type) on a fully

expanded, recently mature leaf, using a portable photosynthesis system (LI-6400XT, LI-COR Biosciences, Lincoln, NE, USA).

## MICROCLIMATE MEASUREMENTS

The microclimatic conditions inside the tree shelters were studied, in the course of the fifth year (2013), by measuring air temperature, relative humidity, light transmission, CO<sub>2</sub> concentration and vapour pressure deficit which were then compared with measurements made on control treatment (outside). Temperature and relative humidity were measured using digital thermo-hygrometers MAX-MIN (TFA®, Kat.Nr.30.5015) suspended by monofilament fishing line at 50 cm height above soil level. The measurement period was from 1-3-2013 to 30-11-2013. Light intensity was measured using a LIGHT METER (Model: DVM1300), while CO<sub>2</sub> was measured using a CO<sub>2</sub> METER (Model: GC-2028). To measure the light intensity and CO<sub>2</sub> concentration in the shelter windows were cut in the wall of the shelter and hermetically taped during the measurements. Measurements of light intensity were made without seedlings inside tree shelters, at 40, 80, 120 and 160 cm above the ground, in order to make a better characterization of the light ambient inside tree shelter during the growing period (Pemán *et al.*, 2010). Data were recorded in lux, and then transformed in μmol of photons m<sup>-2</sup>s<sup>-1</sup> to quantify photosynthetically active radiation (PAR) according to the method of Thimijan & Heins (1985). Measurements of CO<sub>2</sub> concentration were made at 40, 80, and 120 cm above the ground. Data were recorded on the spring, summer and autumn from 8h to 18h each 2 hours (three times per season). Vapour pressure deficit (VPD) was calculated using air temperature ( $T_{air}$ , °C), dew point temperature ( $T_{dew}$ , °C), and relative humidity (RH) (%) according to the procedures of Murray (1967).

VPD =  $e_s - e_a$  (kPa), where:

$$e_s \text{ (saturation vapour pressure)} = 0.611 \exp(17.27 T_{air} / 237.3 + T_{air})$$

$$e_a \text{ (actual vapour pressure)} = \frac{0.611 \exp(17.27 T_{dew} / 237.3 + T_{dew})}{RH}$$

$$T_{dew} = \frac{\left( \frac{1}{\left( \frac{\ln \left( \frac{RH}{100} \right)}{17.27} \right) + \left( \frac{T_{air}}{237.3 + T_{air}} \right)} - 1 \right)}{17.27}$$

## DATA ANALYSIS

Quantitative variables such as biomass, height growth, basal diameter, height/diameter ratio, annual shoot, growth unit (GU), and length and number of internodes were analysed as a split-plot arrangement with Whole plots in Randomized Blocks using PROC MIXED of Statistical Analysis System (SAS Institute Inc., Cary, NC, Version 9.2). The two factors Mulch type and Tree shelter type and their interaction were modulated as fixed effects, and Block, Block × Mulch type as random effects. To eliminate browsing effect on seedlings growth, browsed seedlings were excluded from statistical analyses. For height, diameter and height-to-diameter ratio, the initial value of the variable-measured just after planting was treated as a covariate measured on the small-size experimental unit (seedling), and an analysis of covariance (ANCOVA) was then conducted (Littell *et al.*, 2006).

Quantitative variables associated with temperature, relative humidity, light intensity, CO<sub>2</sub> concentration, vapour pressure deficit, photosynthesis rate and number of branches were subjected to a one-way ANOVA (tree shelter type). For each analysis, when the ANCOVA or ANOVA was significant, statistically significant differences between means were identified using Tukey–Kramer multiple comparison test, at  $p \leq 0.05$ .

Seedling posture (qualitative variable) was expressed in proportions (%). A comparison of mean proportions was then performed thanks to  $\chi^2$ -test by using the PROC FREQ procedure. Differences were considered significant at  $p \leq 0.05$ .

## RESULTS

### MICROCLIMATIC CHARACTERISTICS

As shown in Tab. I, maximum temperature (diurnal temperature) was, on average, significantly higher inside tree shelters, while minimum temperature (night temperature) was significantly higher outside. This trend was observed during all the growing season (spring, summer and autumn). Inside tree shelters maximum temperature was always significantly higher in non-vented than in vented tree shelters, while minimum temperature was similar in both shelter types throughout the growing season. Average maximum RH (night RH) did not show clear tendencies differences between inside and outside tree shelters, while average minimum RH (diurnal RH) was significantly lower inside tree shelters during the entire growing season (Tab. I). Excepting summer, the period for which differences between shelter treatments were not

significant, maximum relative humidity was significantly higher in vented than in non-vented tree shelters. This trend was observed for minimum RH throughout the growing season.

TABLE I

Maximum (Max) and minimum (Min) temperatures ( $T$  (°C)), vapour pressure deficit (VPD (kPa)), maximum (Max) and minimum (Min) relative humidity (RH (%)), and  $CO_2$  concentration inside (NV, non-vented; V, vented) and outside (C, control) tree shelters recorded in spring, summer and autumn

Season	Tree shelter type	Max T (°C)	Min T (°C)	Max RH (%)	Min RH (%)	VPD (kPa)	$CO_2$ (ppm)
Spring	NV	31.8a	10.0a	82.8a	54.4a	0.82a	425.8a
	V	28.8b	10.1a	85.2b	59.7b	0.64b	418.1a
	C	20.8c	12.4b	86.2b	69.3c	0.45c	407.9b
Summer	NV	42.9a	17.0a	79.5a	30.0a	1.98a	430.7a
	V	41b	17.1a	79.6a	33.2b	1.82a	407.1b
	C	32.2c	20.4b	76.7a	47.6c	1.37b	396.7c
Autumn	NV	37.5a	15.2a	84.2a	46.6a	1.3a	428.2a
	V	33.6b	15.2a	87.3b	57.0b	0.92b	412.6b
	C	24.7c	18.0b	83.8a	70.5c	0.64c	402.3c

For a given season, means marked with different letters were significantly different according to the Tukey–Kramer multiple comparison test, at  $p \leq 0.05$  level.  $CO_2$  concentration was measured from 8h to 18h each 2 hours at 40, 80, and 120 cm above the ground where each value is the mean of 72 replicates taken in spring, summer and autumn of the fifth year (2013), while the other variables were measured each 24 h, from 1 March to 30 November from the same year.

Average VPD was significantly higher inside than outside tree shelters, during the entire growing season (Tab. I). Inside tree shelters, VPD was always higher inside non-vented than inside vented tree shelters with significant differences during spring and autumn.

Mean  $CO_2$  concentration was significantly higher inside than outside tree shelters, across all measurement periods (Table I).  $CO_2$  concentration inside tree shelters was always higher for non-vented than for vented tree shelters, with significant differences during summer and autumn.

Radiation was significantly reduced by tree shelters during all periods of measurement ( $P < 0.0001$ ); the fitted curve of the Michaelis-Menten model between total radiation outside and inside the shelters using data from all the periods of measurement (spring, summer and autumn) showed that photosynthetically active radiation (PAR) received by sheltered seedlings was always below 238 and 350  $\mu\text{mol}$  of photons/ $\text{m}^2/\text{s}$  in non-vented and vented tree shelters, respectively, even when environmental PAR reached 2063.6  $\mu\text{mol}$  of photons/ $\text{m}^2/\text{s}$  (Fig. 1). Inside tree shelters, there were no significant differences in PAR in both shelter types during the entire periods of measurement.

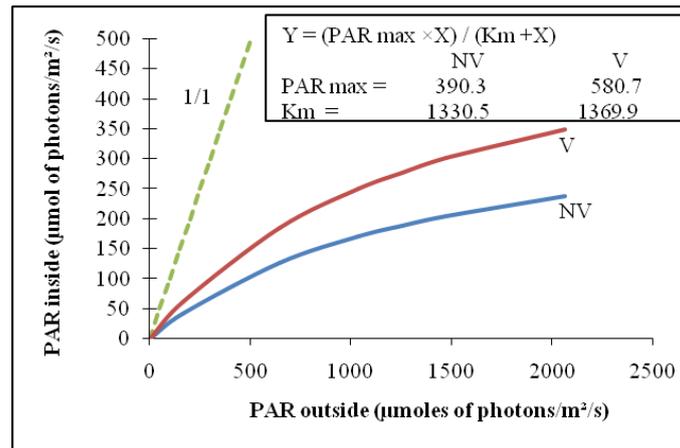


Figure 1.— PAR inside non-vented (NV) and vented (V) tree shelters according the PAR recorded in the control treatment (C). The fitted function corresponds to Michaelis-Menten model ( $PAR_{in} = (PAR_{max} \times PAR_{ext}) / (K_m + PAR_{ext})$ ), where  $PAR_{in}$  = PAR inside shelters,  $PAR_{ext}$  = PAR outside shelters,  $PAR_{max}$  = maximum inside PAR,  $K_m$  = outside PAR value which produces half of the  $PAR_{max}$  inside.

## GROWTH

All quantitative variables measured at stem level (height, diameter, height-to-diameter ratio, annual shoot, growth unit and length and number of internodes) were significantly affected by tree shelter type (Tab. II). However, they were independent of mulch type. Mulch  $\times$  tree shelter interaction had a significant effect on diameter growth and annual shoot.

TABLE II

Tests of fixed effects ( $P > F$ ) on height growth (H), diameter (D), Height-to-diameter ratio (H/D), annual shoot (AS), growth unit (GU), length (LI) and number (NI) of internodes. M, T = types of mulches and tree shelters, respectively

Year	Fixed effects	H	D	H/D	AS	GU	LI	NI
1	M	0.2890	0.1011	0.2954	0.1528	0.2550	0.3043	0.1879
	T	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	M $\times$ T	0.1607	0.0005*	0.5787	0.0035*	0.2201	0.3816	0.5821
2	M	0.4072	0.0573	0.6457	0.8340	0.9279	0.5225	0.9036
	T	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	M $\times$ T	0.8837	0.0065*	0.4436	0.9911	0.9862	0.8916	0.8867
3	M	0.3137	0.1229	0.1951	0.2911	0.2312	0.8554	0.1398
	T	<0.0001*	<0.0001*	<0.0001*	0.0289*	0.0062*	0.2125	0.0004*
	M $\times$ T	0.3774	0.2599	0.5261	0.5647	0.3469	0.1384	0.2816
4	M	0.2769	0.1484	0.2679	0.4395	0.4209	0.8757	0.3993
	T	<0.0001*	<0.0001*	<0.0001*	0.0926	0.1216	0.7039	0.1100
	M $\times$ T	0.1639	0.2233	0.1988	0.4728	0.2654	0.1341	0.4038

Values marked with an asterisk indicate the presence of a significant effect at the  $p \leq 0.05$  level.

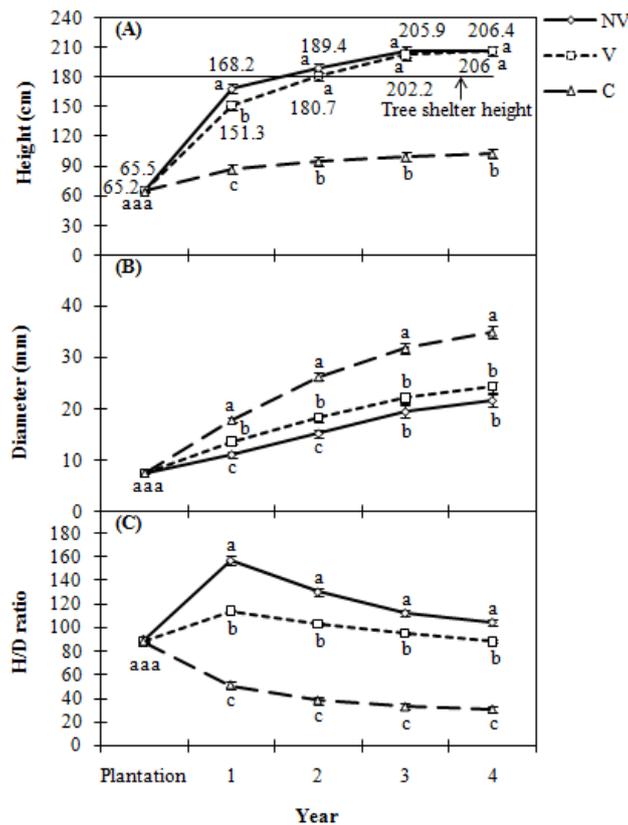


Figure 2.— Height (A), diameter (B) and H/D ratio (C) according to date of measurement (just after plantation and at the end of each year) and tree shelter type (NV, non-vented; V, vented; C, control): adjusted mean  $\pm$  S.E (standard error). For a given year, means marked with different letters are significantly different according to the Tukey–Kramer multiple comparison test, at  $p \leq 0.05$  level.

Over the four years of the study, the average height in sheltered seedlings was always significantly higher than that in the unsheltered control (Fig. 2A). The gain in height growth induced by non-vented and vented tree shelters, during the four years, varied from 93 to 106 % and 74 to 104 %, respectively. The average height reached by seedlings in non-vented and vented tree shelters after only one year of plantation was significantly higher than that reached by the control after four years of the plantation, by 63 and 46.6 %, respectively. In sheltered seedlings, those in non-vented tree shelters were significantly taller than those in vented tree shelters during the first year; for the other years, differences were not significant.

Diameter growth followed an opposite trend to that described for height growth for every year; thus, the average diameter in sheltered seedlings was significantly smaller than that in the unsheltered control (Fig. 2B). The reduction of the basal diameter due to non-vented and vented tree shelters, during the four years, varied from 38 to 42 % and 23 to 31 %, respectively. In sheltered seedlings basal diameter was, each year, smaller in non-vented than in vented tree shelters, but differences were significant only for the first two years.

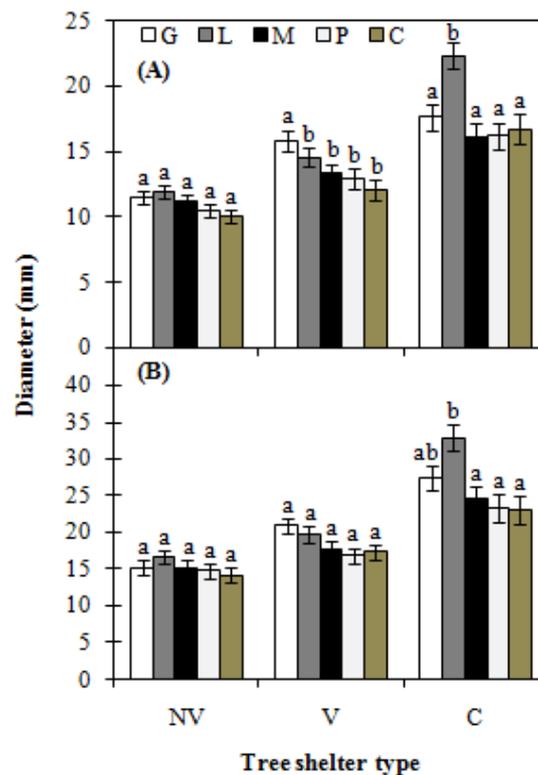


Figure 3.— Diameter during the first (A) and second (B) years, after planting, according to mulch (G, gravel; L, lentisk; L+P, lentisk + Italian stone pine; P, Italian stone pine; C, control) and tree shelter (NV, non-vented; V, vented; C, control) types: adjusted mean  $\pm$  S.E. For a given year, means marked with different letters are significantly different according to the Tukey–Kramer multiple comparison test, at  $p \leq 0.05$  level.

One year after planting, basal diameter in non-vented tree shelters was similar under the different mulches (Fig. 3). In vented tree shelters basal diameter was significantly improved under gravel mulch, while that in the control treatment (unsheltered seedlings) was significantly improved under lentisk mulch compared to the non-mulched control. The following year basal diameter in each of both shelter types (vented and non-vented shelters) was similar under the different mulches, while that in the control treatment was significantly improved under lentisk

mulch. In the course of the last two years, the interaction mulch  $\times$  tree shelter was not significant; thus, seedling diameter in each shelter type was similar for the different mulch types (data not shown).

Differences in height and diameter growth between sheltered and unsheltered seedlings affected H/D ratio. This ratio was, on average, significantly greater in sheltered than in unsheltered seedlings in each year of the study (Fig. 2C). In sheltered seedlings, the H/D ratio was significantly greater in non-vented than in vented tree shelters throughout the study.

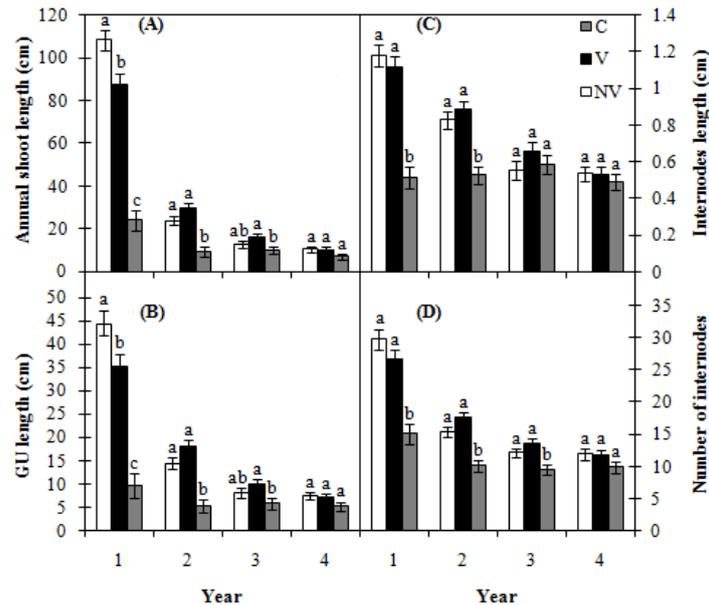


Figure 4.— Length of annual shoot (A) and growth unit (GU) (B) and length (C) and number (D) of internodes according to date of measurement (year) and tree shelter type (NV, non-vented; V, vented; C, control): adjusted mean  $\pm$  S.E. For a given year, means marked with different letters are significantly different according to the Tukey–Kramer multiple comparison test, at  $p \leq 0.05$  level.

Annual shoots were, on average, significantly longer in sheltered than in unsheltered seedlings during the first two years for non-vented tree shelters, and during the three years for vented tree shelters (Fig. 4A). There were no differences for the remaining years (year 3 and 4 for the non-vented shelters, and year 4 for the vented ones). In sheltered seedlings, annual shoot length was significantly greater in non-vented than in vented tree shelters during the first year; the other years, differences were not significant. The same trends were observed for the GU (Fig. 4B).

Figure 5 represents annual shoot length in sheltered and unsheltered seedlings under the different mulches, in the course of the first year. In sheltered seedlings with non-vented tree shelters as in unsheltered ones mean length of annual shoots was significantly increased under lentisk mulch, while that of annual shoots in vented tree shelters was significantly increased under gravel mulch compared to the unmulched control. In the following years, annual shoot length in each shelter type (non-vented, vented and control) was similar under the different mulch types (data not shown).

One and two years after planting length of internodes was significantly greater in sheltered than in unsheltered seedlings, while no significant differences were noted during the following years (Fig. 4C). With regard to the number of internodes, it was significantly higher in sheltered than in unsheltered seedlings until the third year; the following year, differences were not

significant (Fig. 4D). In sheltered seedlings, both length and number of internodes were similar in both shelter types during the four years.

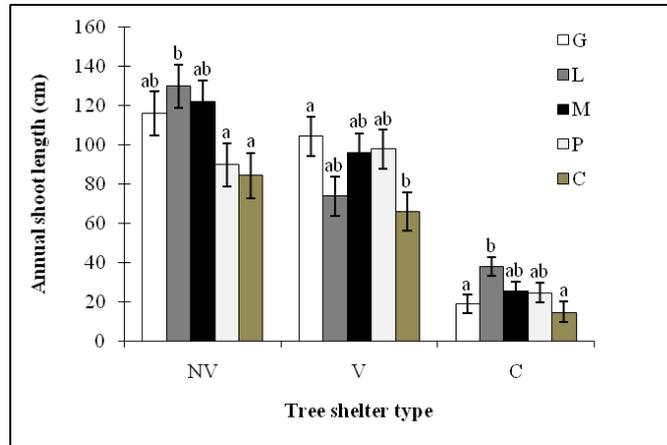


Figure 5.— Annual shoot length during the first year, after planting, according to mulch (G, gravel; L, lentisk; L+P, lentisk + Italian stone pine; P, Italian stone pine; C, control) and tree shelter (NV, non-vented; V, vented; C, control) types: adjusted mean  $\pm$  S.E. For a given shelter type, means marked with different letters are significantly different according to the Tukey-Kramer multiple comparison test, at  $p \leq 0.05$  level.

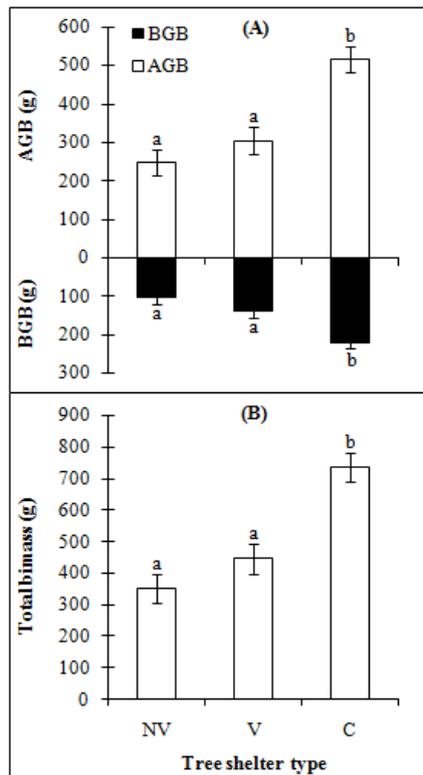


Figure 6. Above-ground biomass (AGB) and below-ground biomass (BGB) (A) and total biomass (B) of seedlings belonging to non-vented tree shelters (NV), vented tree shelters (V) and control (C) treatments, four years after plantation. Mean  $\pm$  S.E. Means marked with different letters were significantly different according to the Tukey-Kramer multiple comparison test, at  $p \leq 0.05$  level.

Both above- and below-ground biomass were significantly ( $P < 0.0001$ ) affected by tree shelter type. By contrast, neither mulch type nor its interaction with tree shelter type had a significant effect on above-ground biomass ( $P = 0.3373, 0.4087$ , respectively) or below-ground biomass ( $P = 0.2021, 0.4770$ , respectively).

The shoot : root ratio was not significantly affected neither by the mulch type ( $P = 0.5483$ ) or the tree shelter type ( $P = 0.2058$ ) nor by the interaction of these two factors ( $P = 0.3423$ ).

The effect of mulch type ( $P = 0.2888$ ) and its interaction with tree shelter type ( $P = 0.4104$ ) on total biomass was not significant, but the effect of tree shelter type was ( $P < 0.0001$ ).

After four years, both above and below-ground biomasses were significantly lower in sheltered than in unsheltered seedlings (Fig. 6). There were no significant differences between sheltered seedlings for both above- and below-ground biomass. The same trends were observed for total biomass growth (Fig. 6).

Total number of branches per seedling was significantly ( $P = 0.0006$ ) reduced in both vented ( $770.4 \pm 342.2$ ) and non-vented ( $497 \pm 342.2$ ) tree shelters, compared to unsheltered seedlings ( $2885.8 \pm 342.2$ ). The difference between the two types of tree shelters was not significant.

#### PHOTOSYNTHESIS RATE

Photosynthesis rate was significantly affected by tree shelter type ( $P = 0.0021$ ). Unsheltered seedlings had the highest value ( $10 \pm 3.7 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ), followed by those in vented tree shelters ( $6.4 \pm 1.7 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ); the lowest value was found in non-vented tree shelters ( $4.3 \pm 3.3 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ). The difference between sheltered seedlings was not significant.

#### SEEDLING POSTURE AND BROWSING

Only sheltered seedlings suffered stability problems; all unsheltered seedlings were able to support themselves during the entire experiment (Tab. III). The percentage of seedlings unable to support themselves after removal of the tree shelter was significantly higher in non-vented than in vented tree shelters during the first two years; no significant differences were detected in following years.

TABLE III

*Percentage (%) of plants unable to support themselves after the removal of the tree shelter at the end of each growing season.*

Tree shelter type	Year 1	Year 2	Year 3	Year 4
	% of plants			
Non-vented	79.5a	94.1a	67.1a	34.1a
Vented	57b	73b	59a	53a
Control	0c	0c	0b	0b

For a given year, percentages marked with different letters were significantly different according to the  $\chi^2$ -test, at the  $p \leq 0.05$  level.

While more than half (52.5 %) of the sheltered seedlings emerged from the top of tree shelters after the first year only unsheltered seedlings were injured by animal browsing.

#### DISCUSSION

Our results for microclimate inside tree shelters confirmed reports from previous studies, showing increased diurnal temperature (maximum temperature) (Dupraz, 1997; Bergez & Dupraz, 2009), VPD (Bellot *et al.*, 2002),  $\text{CO}_2$  concentration (Oliet & Jacobs, 2007), and reduced night temperature (minimum temperature) (Dupraz, 1997; Bergez & Dupraz, 2009) and light intensity (Famiani *et al.*, 2007). In contrast with these studies, however, we did not find that tree shelters

increased air relative humidity; maximum relative humidity (night humidity) did not show clear tendencies differences between inside and outside tree shelters, and minimum relative humidity (diurnal humidity) was significantly lower inside tree shelters during the entire growing season. Navarro Cerrillo *et al.* (2005) found that tree shelters had no significant effect on air relative humidity. These discrepancies suggest variable influence of tree shelters on air relative humidity.

The first photomorphological response to shade is shoot elongation (Smith, 1994). This could explain the increase in height growth inside tree shelters, where the light intensity was severely reduced. Under shaded conditions seedlings are forced to grow more in height to reach available light (Jacobs & Steinbeck, 2001), which could explain why the stimulation of shoot elongation was more important before rather than after emergence from the shelters (see fig. 2A). Light level was similar for both tree shelter types (vented and non-vented tree shelters). For that reason perhaps sheltered seedlings did not express differences in height growth. Mariotti *et al.* (2015) reported that tree shelters inhibited the apparition of branches in *Quercus robur*, which was attributed to a higher frequency of apical dominance and could explain the inhibition of branches for sheltered seedlings in our study. Oak is characterized by weak apical dominance, as noted for unsheltered seedlings, which gives rise to form defects (fork of the main axis, multiple lateral branches on the main axis, stems), and making pruning advisable. As reported by Mariotti *et al.* (2015), the use of tree shelters reduces the need to prune in early stages after establishment which is an advantage not offered by other types of protection against animal browsing (i.e., fence, tree guards, repulsive). Finally, it must be noted that the differences in apical dominance between sheltered and unsheltered seedlings is due to differences in light availability. Once seedlings emerged from the top of tree shelters, branch production recovered (personal observation).

Contrary to height growth sheltered seedlings consistently showed a significantly smaller basal diameter than unsheltered counterparts, in agreement with others (McCreary *et al.*, 2002; Jiménez *et al.*, 2005; Pemán *et al.*, 2010). The smaller diameter under low light was a result of forced directional growth and absence of wind-induced trunk movement to stimulate diameter growth (Harris *et al.*, 1976). Peterson *et al.* (1994) noted that seedlings grown in shelters with ventilation holes exhibited increased diameters compared to seedlings grown in shelters without ventilation holes. In our study, this trend was observed during the first two years after planting. However, there were no significant differences between the two types of tree shelters (non-vented and vented tree shelters) during the last two years of study although diameter growth was always greater in vented tree shelters. This suggests that the positive effect of ventilation in tree shelters on diameter growth compared to non-ventilated tree shelters is temporary. After seedlings emerged from the tree shelters (in year 3, see Fig. 2A) diameter growth seems to become more dependent on wind movement of main stem than on tree shelter type, which could explain differences in diameter growth observed between the two shelter types before seedlings emergence.

The difference in height-to-diameter ratio between sheltered and unsheltered seedlings indicates that tree shelters adversely affected the balance between height and diameter growth. The increase of this ratio in sheltered compared to unsheltered seedlings suggests that increased height growth by tree shelters is due to the detriment of diameter growth. Unbalanced growth in sheltered seedlings is due to increased height growth and decreased diameter growth (sheltered seedlings had greater height and a smaller diameter, compared to the unsheltered control), in agreement with results published by IML & CRPF (2005). Inside tree shelters, seedlings in vented tree shelters had lower values of height-to-diameter ratio compared to those in non-vented tree shelters, during the entire period of study, and consequently had a more balanced height and diameter growth.

While height growth was significantly greater inside rather than outside tree shelters during the study, the main stem division into annual shoots and GUs showed that growth stimulus of tree shelters was lost as of the third year for non-vented tree shelters, and one year later for the vented ones. This indicates that the positive effect of tree shelters on height growth is temporary. This

means that the decrease in diameter growth in sheltered seedlings to benefit height growth is also temporary. The improvement of height growth during the first year in non-vented compared to vented tree shelters is due to an increase in both length of annual shoots and GU. Subsequently, there were no significant differences between the two shelter types in both length of annual shoots and GU, and consequently there were no significant differences in seedling final height.

There were no significant differences between sheltered and unsheltered seedlings in shoot : root ratio after 4 years, suggesting that shelters do not adversely affect the balance between seedling shoot and root. This result is in agreement with McCreary *et al.* (2002) but contrasting with previous work in zeen oak (Mechergui, 2016), suggesting a species-dependent response in shoot : root with tree shelters. Lower total biomass in sheltered vs. unsheltered seedlings indicates that increased height growth inside tree shelters was not due to increases in dry matter production. Similar results were reported by Famiani *et al.* (2007) and Mechergui (2016). Poor biomass growth (total biomass) is a function of low photosynthesis rate (Dupraz, 1997), which could explain lower values of total biomass in sheltered seedlings where photosynthesis rate was negatively affected by tree shelters. The limiting factors of photosynthetic activity are CO<sub>2</sub> air concentration, light, and temperature. We did not observe an increase in photosynthetic activity for cork oak under the decreased temperatures in vented compared to non-vented tree shelters, as was previously observed for zeen oak (Mechergui, 2016). Cork oak is known to be generally more tolerant to high temperatures than zeen oak (Vignes *et al.*, 1985 in Alatou, 1990). This suggests that the reduction of photosynthetic activity for cork oak inside vented and even non-vented tree shelters was not linked to high temperatures or to CO<sub>2</sub> concentration (which was higher inside than outside tree shelters), but rather to the reduced light levels inside these tree shelters.

Tree shelters provided effective protection against browse damage, even after seedling emergence. By contrast, fencing to limit herbivore access was not efficient to protect seedlings against animals and browse damage was noted only in unsheltered seedlings. This indicates that seedling protection with tree shelters is more effective than use of a collective fence.

During the four years, only sheltered seedlings suffered stability problem. Analogous results were reported by Sharpe *et al.* (1999) and Mechergui (2016). This problem may be more dependent on sheltered species than on shelter type (Mechergui, 2016), which may explain the lack of differences in stability between the two tree shelter types. On the other hand, its duration seems to vary between species; for example, only during the first year for zeen oak (Mechergui, 2016).

Mulching has been reported to be more beneficial on sites of poorer quality (Green *et al.*, 2003). In this study, mulching was applied on a fertile site (soil organic matter content on horizons A and B was 5.7 and 2.4 %, respectively), which probably led to no effects on seedlings growth during the study. With regard to the combined use of mulching and tree shelters, none of the mulches appeared more favourable than others to improve seedlings growth in both shelter types (vented and non-vented tree shelters).

## CONCLUSION

The slow height growth of oak seedlings during early development (Taylor *et al.*, 2006) extends the period of establishment and makes seedlings vulnerable to animal browsing. Our results show that tree shelters can greatly enhance seedling height growth and provide efficient protection from herbivores. Unsheltered seedlings were injured by goats, cows and Barbary red deer (*Cervus elephus barbarus*). The use of 1.8-m tall tree shelters effectively shields seedlings against these types of animals.

Comparison of total biomass between sheltered and unsheltered seedlings showed that increased height growth inside tree shelters is not due to an increase in the amount of dry matter

produced (biomass growth was significantly lower in sheltered than in unsheltered seedlings). The increased height growth was also not at the expense of root growth (shoot : root ratio was not affected by shelters), but rather to a reallocation of growth from branches and stem diameter to the terminal leader. Branches result in knots within the stem that constitute a defect in both appearance and structural timber. The use of tree shelters could be, therefore, helpful to reduce the apparition of these knots on the main stem by inhibiting branch development. In addition, the production inside tree shelters of a straight and vertical stem may enhance cork quality by reducing the cork waste and extraction costs.

Both non-vented and vented tree shelters were effective in protecting seedlings against animal browsing and stimulating height growth. However, vented shelters were superior to non-vented shelters for producing seedlings with balanced height and diameter growth. Positive effects of vented tree shelters on annual shoot and GU elongation were longer lasting than that of non-vented tree shelters (3 years vs. 2 years), but final height was similar in both shelter types.

Reduction of photosynthesis rate (and therefore of the total biomass) in sheltered seedlings could be related to low light level as found in this study or to high temperatures as reported by Mechergui (2016) for zeen oak grown with non-vented tree shelters. The improvement of tree shelters design by increasing width and aeration (to decrease temperature and increase light transmission) could make them more conducive to seedling growth.

The use of mulching alone or in combination with shelters appeared useless in this study probably because of their application on a fertile site. Future research should study the effect of mulch on poorer quality sites, where the control of vegetative competition could more favour seedling growth.

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