

THE EFFECT OF INDIVIDUAL TREE SHELTERS IN GROWTH AND MORPHOLOGY OF CORK OAK SEEDLINGS

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RESUM

Per a estudiar l'efecte dels abrics en el creixement i la morfologia dels plaçons de surera (*Quercus suber* L.) es van utilitzar dos tipus d'abric de plàstic: abrics transparents de PVC reforçats amb malla de polièster de 75 cm d'alçada; i, abrics de propilè marró, translúcid, de doble paret, secció quadrada i 120 cm d'alçada. Els plaçons es van fer créixer en tubs de 120 cm de llargada a Évora, Portugal. El creixement dels controls i dels plaçons abrigats es va avaluar per mesura de l'alçada, la longitud de les branques, el nombre i l'àrea de les fulles i la biomassa. També es va estudiar l'anatomia de les fulles i la toleràcia a la temperatura. Els resultats mostren que els abrics estimulen el creixement en alçada. La quantitat de branques era major en les plantes dels abrics del primer tipus que en els controls, però les diferències d'aquests amb les plantes dels abrics del segon tipus no eren significatives. La relació brot/arrel era més alta en les plantes abrigades que en els controls, donat que la biomassa aèria estava incrementada mentre la biomassa soterrània es mantenia igual. Els desenvolupament foliar a l'interior dels abrics mostrava signes d'aclimatació a l'ombra i poca toleràcia a la temperatura, presentant símptomes de mort a temperatures més baixes que els controls.

ABSTRACT

To study the effects of the tree shelters in growth and morphology of cork oak (*Quercus suber* L.) seedlings two types of plastic shelters were used in this work: A, transparent PVC shelters (brown) reinforced with a white polyester net, 75 cm of height; B, translucent brown polypropylene, double walled, square cross section and 120 cm of height. The plants were grown in 120 cm long tubes in Évora, Portugal and growth of sheltered and control plants was evaluated based upon measurements of height, branch length, number and area of leaves and biomass. Additionally the anatomy and morphology and heat tolerance of leaves of sheltered and control plants was studied. The results show that growth in height was stimulated by shelters. The amount of branches was greater in seedlings with shelters A than in controls but the differences between the latter and seedlings in shelters B were not significant. The shoot/root ratio was higher in sheltered plants than in controls because of the above-ground biomass increased in the former whereas root biomass remained unchanged. Foliage developed inside the shelters showed characteristics of acclimation to shade and were less tolerant to heat being killed at lower temperatures than those of unsheltered controls.

Key Words: Acclimation to shade, heat tolerance, *Quercus suber*, tree shelters.

INTRODUCTION

The need of reforestation in cork oak areas has faced important difficulties. Among those is the destruction of young plants by herbivores coupled with slow growth of seedlings during the early years after plantation. The use of individual tree shelters may be one technique advisable to assist in reforestation programmes with this species. The use of such shelters in northern Europe with other oak species proved to be efficient not only in the protection against cattle, sheep and game but also to simulate growth in height (Tuley *et al.*, 1985). However there are very few detailed studies on the physiological basis for the responses of the plants inside the shelters and virtually nothing is known about the results of their use in regions of mediterranean climate. This work is part of a project designed to study the effects of shelters in growth and physiology of *Quercus suber* L. seedlings under conditions of mediterranean climate.

MATERIAL AND METHODS

Growth studies

The growth studies took place at Herdade da Mitra, Évora. Seedlings grown from acorns collected in Mora, Portugal grew in black plastic bags (22 x 6 cm) filled with soil between January and the end of May, 1988. In 30 May, 1988, 30 plants were transplanted into PVC tubes 120 cm long and 20 cm in diameter to facilitate the study of the root system. The tubes were filled with a mixture of 1:1 (v:v) of sand and local soil which had the following characteristics: pH (H₂O) - 7.7; Na - 0.4 meq/100 g; P₂O₅ - 345 ppm; Ca - 7.1 meq/100; NO₃ - 7 ppm; K₂O - 166 ppm. The tubes were partly buried up to 20 cm from the bottom. The tubes were installed as latin squares at a 2 x 2 m spacing. The plants were watered regularly up to 30 September after which they received only rainfall water.

In 17 June the test plants were enclosed in two types of shelters: shelter A consisting of a 75 cm high, nearly cylindrical (6 cm in diameter at top), made of PVC transparent «smoked» strengthened by a net of polypropilene (0.5 to 1.0 cm wide) and shelter B, made of doubled walled brown polypropilene translucent, square in cross section with 122 cm of length and 8 cm wide. Each shelter was applied to 10 seedlings chosen at random leaving 10 other seedlings as control. Measurements of stem length, number of leaves on stem axis and on branches, and number and length of branches were measured on the following dates: 17 June, 12 and 28 July, 14 September and 28 October. On 2 November the whole plants were harvested for biomass measurement after separation into stem, leaves and roots. The plant material thus obtained was dried in a oven at 80°C for 72 hours and weighted. Shoot/root (SRR), root weight (RWR), stem weight (SWR) and leaf weight (LWR) ratios were calculated.

Growth data were analysed per treatment and linear regression models were applied to each variable as a function of time. The homogeneity of the slopes of the regressions for each variable was tested ($p = 0.05$) for all treatments and the differences in slopes were tested simultaneously (Sokal and Rohlf, 1969). The

regression models were then readjusted to include all the treatments which were not significantly different ($p = 0.05$). Analysis of variance was applied to biomass data. Whenever there were significant differences ($p = 0.05$) the means were compared using the Student—Newman-Keuls method.

Leaf anatomy

The plants whose leaves were used in these studies had the same origin as the ones described above but were installed in the field with shelters A and B and unsheltered controls in Herdade da Mitra (Évora) in a complete randomized blocks design with 20 plants per plot. The plantation took place in May 1988. The leaves were collected one year after planting from 3 plants per treatment. Three leaves were taken from the middle of the seedling for analysis. Four discs (5 mm in diameter) per leaf were taken and immersed in a mixture of chromic acid (10%) and nitric acid in water (1:1). The epidermis were peeled after 30 minutes in the solution and coloured with 1% safranin in ethanol (50%) for 5 minutes.

The epidermis were mounted on glass slides with glicerol and 10 fields were observed in each leaf disc for stomata counting. Since trichomes are not very abundant in juvenile leaves, contrary to adult leaves (see Nobrega and Pereira, 1991) no further preparation was needed. The same leaves were used to determine the area per unit biomass (specific leaf area = SLA) using 5 discs per leaf dried at 80°C for 48 hours. The anatomical characteristics of leaf cross sections were measured, namely leaf thickness, thickness of the mesophyll, proportion of palissade in the whole cross sectional thickness, thickness of the epidermis + cuticule and the proportions of vascular tissue and intercellular spaces in relation to total thickness of the mesophyll. The measurements were made on 2 photomicrographs of cross sections of each of 5 leaves stained with the Foster-safranin solution. The chlorophyll content was determined in 4 leaves of the middle zone of the stem of each of 4 plants per treatment, using the Ozerol - Titus (1965) method. The analysis of variance was followed by Duncan's multiple range test for comparison of the means whenever differences were significant ($p = 0.05$).

Heat tolerance of the leaves

The plants were grown in Lisbon in large pots (10 l) filled with a sandy soil from Pegões, Portugal and were kept well watered. Only shelter B was compared to unsheltered plants. The study was conducted using leaves grown inside the shelters in comparison with leaves of the same age of the control plants. Sampling took place one month after leaf expansion inside the shelter. The technique to evaluate heat tolerance is described by Lange (1965). The detached leaves were transported inside a Petri dish with filter paper soaked in water. In the laboratory they were inserted inside a test tube with a rubber stopper and maintained inside a temperature controlled bath at temperatures ranging from 45 to 60°C for 30 minutes. After this treatment the leaves were transferred to a humid chamber for 72 hours at room temperature and dim light. The evaluation of damage was made by the quantification of the area of necrosis on the leaf. Leaves from two plants were used for each combination of temperature per treatment. The analysis of variance of the data was performed using the angular transformation of the percentage of the area of necrosis

Table 1. Mean values of leaf anatomy characteristics

Characteristics	Treatments		
	Shelter A	Shelter B	Control
Stomatal frequency (number of stomata per mm ²)	397 a *	427 b	629 c
Specific leaf area (mm ² mg)	15.938 a	20.019 b	13.159 c
Leaf thickness (μ m)	198.4 a	169.6 b	245.4 c
Mesophyll thickness (μ m)	156.8 a	161.7 a	185.7 b
Proportion of palisade in the whole cross sectional thickness (%)	53.7 a	36.2 b	50.4 c
Epidermis + cuticle thickness (μ m)	41.6 a	7.9 b	59.7 c
Proportion of intercellular spaces in the whole cross sectional thickness (%)	7.5 a	5.4 b	5.4 b
Proportion of vascular tissue in the whole cross sectional thickness (%)	15.2 a	17.2 b	11.6 c
Chlorophyll content (mg g ⁻¹)	5.038 a	5.217 b	4.751 c
Chlorophyll content (mg d m ⁻²)	3.174 a	2.608 b	3.563 c
Chlorophyll a / Chlorophyll b ratio	3.121 a	8.310 b	3.690 c

* In each variable values with the same letter do not differ statistically ($p = 0.05$)

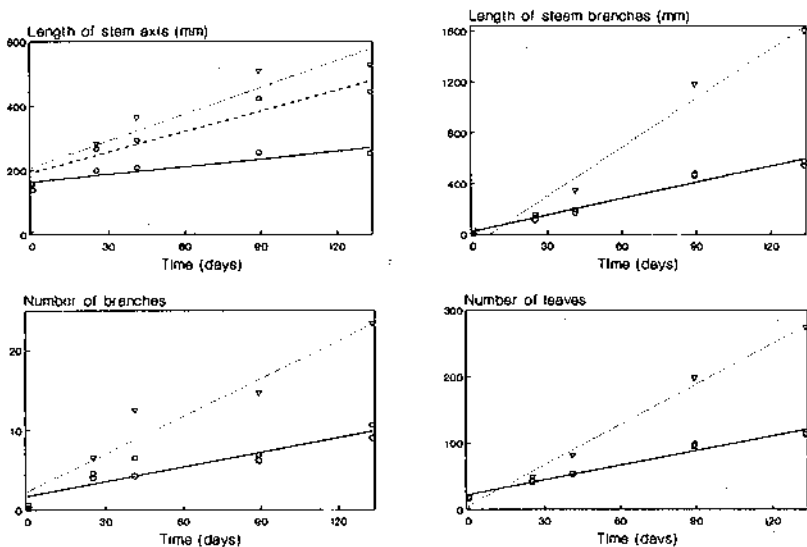


Figure 1 - Mean values and regression lines for the length of stem axis and stem branches, the number of stem branches and the number of leaves in control (○), shelter A (Δ) and shelter B (⊗).

on the leaf considering treatment (shelter B and control), temperature and leaf position (acropetal numbering).

RESULTS AND DISCUSSION

The rate of elongation of stem axis was significantly different among the treatments, being highest in shelter A and higher in shelter B than in the control. The number and length of branches as well as the number of leaves were, however, higher in shelter A than in shelter B or in the control, which were not significantly different between them (Fig. 1). A greater number of branches in shelter A compared with the shelter B and control seems to be the explanation for a larger leaf number in the former because the average number of leaves per unit of branch length was the same in all treatments (Fig.

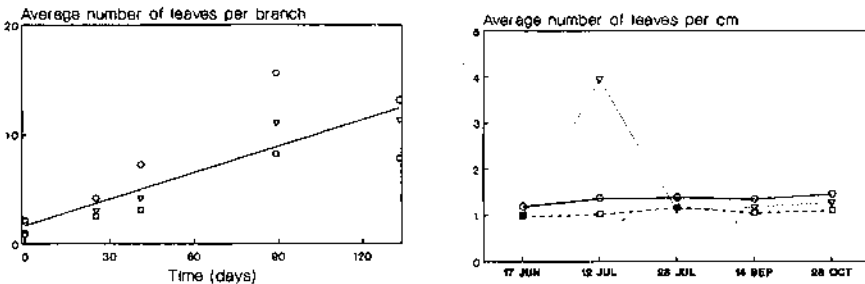


Figure 2 - Mean values and regression line for the number of leaves per branch and average number of leaves per unit of stem length (control (°), shelter A (Δ), shelter B (□)).

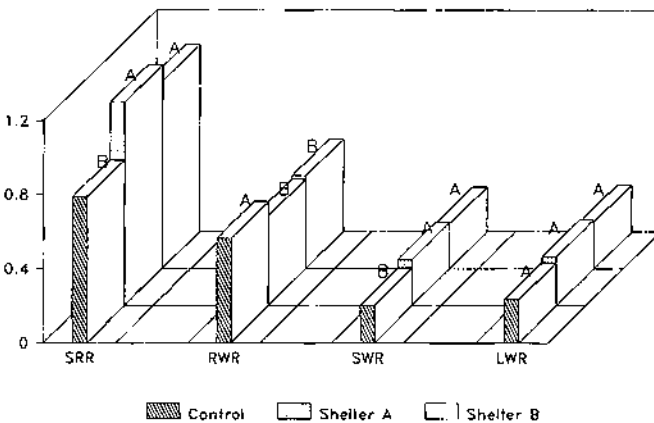


Figure 3 - Mean values of total, root, above ground, stem and leaf biomass for control, shelter A and shelter B. (For each variable values with the same letter do not differ statistically ($p = 0.05$)).

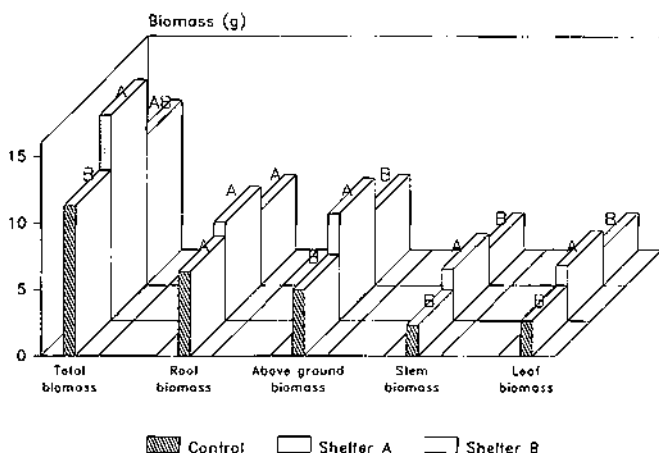


Figure 4 - Mean values for the shoot/root ratio (SRR), biomass partition to roots (RWR), biomass partition to stem (SWR) and leaf weight ratio (LWR). (For each variable values with the same letter do not differ statistically ($p = 0.05$)).

2). The average length of one branch was not significantly different among treatments. The use of shelter B only influenced the length of stem axis when compared to the control. On the contrary, shelter A resulted in the increase of the whole crown growth in comparison with shelter B and control. Likewise total biomass produced by the plants in shelter A was greater than in shelter B or the difference in biomass in shelter B and control were not significant nor was the difference between shelters B and A. This resulted from increased production of all above ground components in shelter A (Fig. 3). There were not significant differences among treatments concerning root biomass. The shoot/root ratio (SRR) was not significantly different between the two shelter treatments but was higher in sheltered than in control plants (Fig. 4). Apparently biomass partition to roots (RWR) was higher in the control than in sheltered plants. The opposite happened with partition to stem (SWR) whereas there were not significant differences among treatments concerning leaf weight ratio (LWR) as shown in figure 4.

A decrease in apical dominance in plants of shelter A in comparison to shelter B seems to be a deviation from the known tendency of acclimation to shade (Grime, 1979). The only explanation available to us is the possibility that increased temperatures in shelter A (Dias *et al.*, 1990) might compensate for shade because higher temperatures are known to increase branching (Charles-Edwards *et al.*, 1986). A higher leaf area per plant may explain the greater productivity observed in shelter A. As shown in table 1, leaf anatomy reflected the shade acclimation inside the shelters, when compared to unsheltered plants, with lower stomatal frequency (number of stomata per mm^2), larger specific leaf area, lower thickness of the whole leaf and of the cuticle, as well as a greater chlorophyll content per unit of dry weight but a lower chlorophyll content per unit of leaf area. These differences in leaf anatomy were significant in all the treatments. The exceptions were leaf pore space and thickness of the mesophyll. Regarding pore space in leaf cross section shelter B is not significantly different from

the control, whereas leaves from shelter A differ from the other treatments. Total thickness of the mesophyll is not different in either shelter type but leaves from the control were significantly different from sheltered plants.

Even though these suggest acclimation to shade inside shelters some results end to contradict that. For example even though it might be expected a lower ratio chlorophyll a / chlorophyll b in sheltered plants than in the unshaded controls, this occurred only in shelter A. The percentage of vascular tissue in the whole of cross section was also lower in the controls than in sheltered plants. The percentage of palisade was substantially lower in shelter B than in shelter A or in the control. Shade leaves show normally less palisade and less vascular tissue, than sun leaves. It seems clear that shelter A not only induced little acclimation to shade, but also resulted in more xerophytic leaves than those grown in shelter B. This may be the result of higher temperatures in shelter A than in shelter B as detected in some periods of the year (Dias *et al.*, 1990). On the other hand, shelter B resulted in more typically shade acclimated leaves possibly as a consequence of a greater radiation interception by this than by shelter A.

Although tolerance to high temperatures was evaluated only in shelter B in comparison to unsheltered controls the results demonstrated a decrease in tolerance in leaves from sheltered plants. Between 45 and 49°C there were not significant differences between treatments, even though there was some damage at temperatures above 47°C. At 60°C all leaves had 100% necrosis. It is between 49°C and 59°C that significant differences occur between treatments. These differences, however, depend upon leaf age. The youngest leaves (with the highest numbers acropetally) were least tolerant and significant differences between shelter and control occur at lowest temperatures (52°C). As temperatures increased, differences between treatments occur at increasing low leaf numbers whereas in younger leaves the results for the sheltered and control plants tend to equalize.

The method used, i.e. exposure to heat stress for a restrict time may be, as stressed by Kappen (1981), characteristic of the natural environment with short periods of overheating during the middle of the day. This is strongly characteristic of the microenvironment in the shelters which have been shown to increase temperature oscillations in comparison to external, well ventilated environment (Dias *et al.*, 1990). In this case the decrease in heat tolerance in sheltered plants may explain in part the increase in summer defoliation in sheltered plants in comparison with unsheltered controls in the field (J. Tomé, A. Dias, A. Oliveira and J. Pereira, unpublished). The decrease in heat tolerance inside shelters may be related to shade acclimation of the leaves. Repeated periods of high temperatures alternating with periods of moderate temperatures are typical of the environment inside the shelters. This type of environment has been shown to induce an increase in heat tolerance (Kappen, 1981). However the structural (and eventually biochemical) lack of heat tolerance in shade leaves was not apparently counteracted by the exposure to higher temperatures during growth inside the shelter as compared to unsheltered plants.

CONCLUSIONS

Young plants of cork oak responded positively to protection with shelters not only in terms of stem elongation but also, in the case of shelter A, an increase in biomass

production. This may be explained by the microenvironment created by the shelters: increase in average temperature of the air and shading (Dias *et al.*, 1990). The leaves grown inside shelters show clear signs of acclimation to shade. These leaves have also less tolerance to high temperatures. The occurrence of short periods of heat stress inside the shelters together with the eventual occurrence of water deficits in the summer, may lead to defoliation of sheltered plants especially as a result of decreased heat tolerance in these plants. More research is needed to develop tree shelters effective under Mediterranean type of climates.

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