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GRADIENTS OF ANATOMY AND MORPHOLOGY OF LEAVES IN THE CROWNS OF CORK OAK

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RESUM

La freqüència estomàtica, les caraterístiques anatòmiques i l'àrea foliar específica (relació pes sec, àrea^{-1}) de les «fulles de sol» i les «fulles d'ombra» de la surera, *Quercus suber* L, es van estudiar en vuit arbres adults de dues parcel·les experimentals situades a Besteiros, prop de Vedas Novas, Portugal. Les dues parcel·les corresponenien a dos models d'utilització del sòl: la parcel·la E, a una pastura de trèbol soterrani; y la parcel·la G, al model d'agricultura tradicional en la regió. En ambdues parcel·les la freqüència estomàtica és més elevada en les fulles dels estrats superiors que en les parts baixes de la copa. La longitud de les cèl·lules de guàrdia és major en els estomes situats en posició proximal en els estrats superiors i mitjos en relació a l'estrat inferior. L'àrea foliar específica no varia significativament entre els dos tipus de fulles. Els arbres creixien en espais oberts en els que l'ombrejament dels arbres entre si és mínim. Però, l'àrea foliar específica dels arbres de la parcel·la de l'agricultura tradicional és significativament més elevada que en la dels arbres de la parcel·la amb trèbol soterrani. Aquest fet és probablemet una conseqüència de les pèrdues de nitrogen que causa l'agricultura.

ABSTRACT

Stomatal frequency, anatomical characteristics and specific leaf area (ratio dry weight. area^{-1}) of «shade leaves» and «sun leaves» of *Quercus suber* L., were studied in eight adult trees in the field with two types of soil utilization, plot E with subterraneum clover pasture and plot G with tradicional agriculture in Besteiros near Vendas Novas, in Portugal. In boths plots the stomatal frequency is higher in leaves of upper stratum than in the lower part of the crown. The length of guard cells is higher in the proximal position of middle and superior stratra than in the inferior stratum. The dry weight.area^{-1} does not vary significantly between two types of leaves. The trees belong to an open stand where shading among trees is minimal. However, the dry weight.area^{-1} ratio of leaves of plot with traditional agriculture is significantly higher than that of leaves of the plot with pasture probably as a consequence of the depletion of soil nitrogen caused by agriculture.

Key words: acclimation to shade, *Quercus suber*, stomatal frequency.

INTRODUCTION

Plant productivity results from carbon assimilation by the whole canopy, which is linearly related to solar radiation intercepted by the foliage (Pereira and Tomé, 1986). However in plants capable of displaying a high foliage density in the crown (i.e., area of leaves per unit crown volume) such as cork oak, it is important to know the characteristics of leaves of in different positions in the crown. Because anatomy and biochemistry differ with shade acclimation, the quantification and modelling of canopy gas exchange requires the previous knowledge of anatomical and biochemical characterization of leaves in different parts of the crown and very little is known about this for scherophyllous vegetation (e.g. Meister et al., 1987). Therefore, as part of preliminary studies of the ecophysiology of *Quercus suber* we studied the anatomical characteristics of the stomatal apparatus for «shade leaves» and «sun leaves», i.e., from inner and outer parts of crowns as well as the ratio dry weight.area^{-1} of the same leaves.

MATERIALS AND METHODS

The study was carried out at Besteiros near Vendas Novas in central southwest Portugal, about 100Km from the Atlantic Ocean. The climate is of the Mediterranean type, soils derived from sand and sandstone are dominants. Eight adult trees of *Quercus suber* L., were sampled in the field in plots with two types of soil utilization: plot E, with subterraneum clover pasture and plot G, with traditional rainfed agriculture. Leaves were collected from proximal (closer to the trunk and shaded) and distal (away from the trunk and sunlit) locations in three strata of the crown: upper, middle and lower. The twigs were brought to the laboratory in plastic bags, inside a thermic box, after wetting the cut surface with destilled water to avoid dessication and stomatal closure.

The adult leaves of cork oak are coriaceous, small, with thick cuticle and dense and long trichomes on the abaxial face. The measurement of stomatal frequency and dimensions were doneafter the following preparation of leaf epidermis (Santos,1938): the trichomes of the leaves were scrapped with a razor blade under a magnifying glass; the leaves were then immersed in a vial filled with ethanol for 24 h after which the leaves were immersed in a vial filled with sodium hypochlorite (commercial solution) for another 24 h and after rinsing with destilled water and the epidermis was separated using a pincer and a lanceolate needle. The epidermis was stained with aquous solution of safranine (1:5000) and the dyed epidermis was set on glass slides with Balsam of Canada subsequently dried in oven at 65°C. A microscop «Carl Zeiss (Jena) Amplival» was used to determine the stomatal frequency. A microscope «Leitz SM-LUX» (micrometric ruler 0-100,1000X) was used to measure the length and width of stomatal guard cells and pores in 10 stomata per glass slide. An electronic planimeter was used to measure the areas of 4 leaves from each position and each stratum. Leaf biomass of these leaves was determined after drying at 100°C for 24 h and the dry weight.area^{-1} ratio was determined. The statistical analysis was made with a «Wang 2200 C computer». The F test was used for analysis of variance of stomatal frequency, the stomata size and dry weight.area^{-1} ratio and the means were compared using the l.s.d. test.

RESULTS AND DISCUSSION

The stomatal frequency (Tables 1 and 2) of the upper and middle strata are significantly different from the that of the lower stratum, but did not differ significantly between them. However, leaves of the upper stratum has a tendency to show higher values. The higher stomatal frequency of the upper will illuminated strata in relation

Table 1. The stomatal frequency (number of stomata.mm ^{2}) of the leaves from the upper, lower and inferior strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture). Values represent the mean+- standard deviation of 36 measurements.

	Location in Crown					
Plot	Upper		Middle		Lower	
	Distal	Proximal	Distal	Proximal	Distal	Proximal
E	635±182	649±108	599±117	599±129	455±164	476±154
G	648±70	671±105	565±113	582±105	355±190	397±125

Table 2. Analysis of variance of stomatal frequency of the leaves from different strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F	Least significant difference
Strata x Strata	307559,4976	2	153779,7488	5,6759**	117,85
Position x Position	3122,6067	1	3122,6067	0,1152 NS	
Plots x Plots	16598,2689	1	16598,2689	0,6125 NS	
Strata x Positions	3691,9173	2	1845,9587	0,0681 NS	
Strata x Plots	10990,3494	2	5495,1747	0,2028 NS	
Positions x Plots	47945,4171	1	47945,4171	1,7693 NS	
Error	1029722,9535	38	27097,9725		

** = Significantly high
NS = Not Signicant

to the lower shows clearly a certain dimorphism between the «sun leaves» and «shade leaves», which are dominant in the inferior part of the canopy. However, these anatomical differences are less intense than in the crowns of other broadleaf tree species of the temperate zone such as *Carpinus betulus*, *Acer campestre*, *Quercus sessiliflora* or *Fagus sylvatica* (Aussenac and Ducrey, 1977; Huzulak and Elias, 1975). Probably this was a result of this being a very open stand where the shading among the trees is minimal.

Tables 3 to 7 show data on stomatal dimensions. There are significant differences only for the length of the guard cells. The fact that the interaction strata x positions is significant (Table 4) results from a significant difference between the strata for the distal position whereas in the proximal position the upper and middle strata are significantly different from the lower crown stratum.

Table 3. Size of the stomatal apparatus of the leaves from upper, middle and lower strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

		Location in Crown					
Parameters	Plot	Upper		Middle		Lower	
		Distal	Proximal	Distal	Proximal	Distal	Proximal
Guard cells							
Lenght	E	20,5±2,7	22,1±1,9	21,8±1,5	21,9±2,3	22,1±2,3	21,3+1,9
Lenght	G	22,6±1,9	22,9±1,8	22,1±2,5	22,6±2,1	22,6±1,6	20,0±2,2
Width	E	16,3±1,6	15,2±1,6	16,2±2,0	16,0±2,1	15,8±1,7	15,1±1,4
Width	G	16,1±2O	15,7±1,6	15,5±2,2	15,8±1,8	16,2±1,9	15,3±1,8
Ostiole							
Lenght	E	8,4±2,7	9,5±2,1	9,2±2,O	9,6±2,1	10,3±2,7	10,0±2,1
Lenght	G	10,0±2,6	9,6±2,2	10,5±2,4	8,9±2,2	8,8±1,5	8,7±1,7
Width	E	2,6±1,7	2,5±1,1	3,0±1,0	2,8±1,3	2,7±1,3	2,8±1,3
Width	G	2,8±0,9	2,8±0,9	4,0±1,7	2,3±0,9	2,2±1,1	3,5±1,2

Table 4. Analysis of variance of the length of guard cells of the leaves from different strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F	Least significant difference
Strata x Strata	2,9809	2	1,4904	0,8895 NS	
Position x Position	0,3485	1	0,3485	0,2080 NS	
Plots x Plots	3,0755	1	3,0755	1,8356 NS	
Strata x Positions	14,5223	2	7,2612	4,3338 *	1,31
Strata x Plots	6,5215	2	3,2607	1,9461 NS	
Positions x Plots	2,0543	1	2,0543	1,2261 NS	
Error	63,6687	38	1,6755		

** = Significantly high
NS = Not Signicant

Table 5. Analysis of variance of the width stomatal guard cells of the leaves from different strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F	Least significant difference
Strata x Strata	0,7272	2	0,3636	0,3846 NS	
Position x Position	2,8812	1	2,8812	3,0479 NS	
Plots x Plots	0,0091	1	0,0091	0,0096 NS	
Strata x Positions	1,6388	2	0,8194	0,8668 NS	
Strata x Plots	1,1358	2	0,5679	0,6008 NS	
Positions x Plots	0,3267	1	0,3267	0,3456 NS	
Error	35,9196	38	0,9453		

NS = Not Signicant

Table 6. Analysis of variance of the length of stomatal pore of the leaves from upper, middle and lower strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F	Least significant difference
Strata x Strata	0,4999	2	0,2500	0,1249 NS	
Position x Position	0,3169	1	0,3169	0,1583 NS	
Plots x Plots	0,1825	1	0,1825	0,0912 NS	
Strata x Positions	1,5663	2	0,7832	0,3913 NS	
Strata x Plots	8,5584	2	4,2792	2,1378 NS	
Positions x Plots	3,2448	1	3,2448	1,6210 NS	
Error	76,0657	38	2,0017		

NS = Not Signicant

The dry weight.area^{-1} (Tables 8 and 9) was not significantly different among the several positions in the crown in the same tree, contrary to the expectation. That reinforces the idea there is only a moderate dimorphism between «sun leaves» and «shade leaves» in the open stands of cork oak. The dry weight.area^{-1} from plot G (traditional agriculture) is significantliy higher than the plot E (subterraneum clover pasture). Probably the cause for that is the predominance of higher water and mineral deficits in plot G as a result of soil degradation caused by traditional agriculture. On the other hand the soil of plot E could have higher capacity of water retention and higher nitrogen available released by the legumes in the pasture.

Table 7. Analysis of variance of the width of stomatal pore of the leaves from upper, middle and lower strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F	Least significant difference
Strata x Strata	0,9898	2	0,4949	0,6612 NS	
Position x Position	0,1728	1	0,1728	0,2309 NS	
Plots x Plots	0,4033	1	0,4033	0,5388 NS	
Strata x Positions	4,9334	2	2,4667	3,2955 NS	
Strata x Plots	0,0855	2	0,0428	0,0572 NS	
Positions x Plots	0,0040	1	0,0040	0,0053 NS	
Error	28,4422	38	0,7485		

NS = Not Signicant

Table 8. Dry weight.area-1 (mg.mm-²) of the leaves from upper, middle and lower strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

	Location in Crown					
Plot	Upper		Middle		Lower	
	Distal	Proximal	Distal	Proximal	Distal	Proximal
E	0,7±0,2	0,7±0,2	0,7±0,2	0,5±0,1	0,5±0,2	0,5±0,2
G	0,9±0,3	0,8±0,3	0,7±0,1	0,7±0,3	0,7±0,2	0,7±0,3

Table 9. Analysis of variance of the dry weight.area-¹ of the leaves from different strata and distal and proximal positions of the branches of the crowns of cork oak from plot E (subterraneum clover pasture) and plot G (traditional agriculture).

Interactions	Sum squares	Degrees of freedom	Means squares	F
Leaves positions	0,0626	5	0,0125	2,4510 NS
Plots	0,0566	1	0,0566	11,0980 *
Error	0,0257	5	0,0051	

* = Significant

NS = Not Signicant

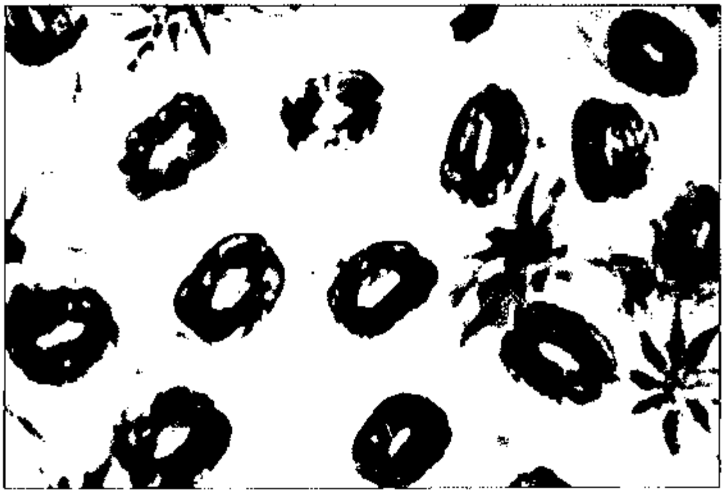

Figure l. Stomata distribition in the epidermis of a cork oak leaf, 71OX.

The results suggest that even fairly dense crowns of open stands of the «montado/ dehesa» systems may have only a moderate degree of acclimation to shade in the inner part of the crown as a consequence of easy penetration og light through the outer leaf layers. We may speculate that this moderate acclimation to shade may unable trees to have a high efficiency of use of intercepted solar radiation for biomass production. A more intense acclimation might result in lower photosynthetic capacity and thus in a reduced ability to exploit the available photosynthetically active radiation (Meister et al., 1987).

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