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Morpho-Anatomical and Functional Modifications in Beech Leaves on the Top Ridge of the Apennines (Central Italy)

By

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Key words: *Fagus sylvatica* L. (beech), sclerophylly, tannins, water status.

Summary

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This paper examines the morpho-anatomical modifications in leaves from beech trees growing along the top ridge of the Apennines (Central Italy) and compares them to leaves from other trees growing in cooler sites and at lower altitudes. Sclerophyllous modifications were observed, as well as tannin-impregnated tissues. These modifications provide the leaves with a greater water retention capacity. The beech is an ecologically versatile species which can apply different strategies of water use according to the conditions it finds itself in.

Introduction

Fagus sylvatica L. (beech) grows naturally from the southernmost European regions (Sicily, Italy) to Scandinavia. Within its distribution area, leaf morphology variations have been observed (BAUER & al. 1997): in the central regions of the distribution area the leaf surface is largest, whereas it decreases both in the northern and the southern regions. The leaf's specific dry weight (dry weight per surface unit) shows the opposite trend. A similar gradient was also observed in relation to site altitude, with the leaf surface decreasing from lower altitudes towards ridge top conditions (BUSSOTTI & al. 1998). Specific dry weight increase is usually accompanied by morpho-anatomical modifications: increased leaf thickness, reduced water content and tissues impregnated with phenols. These

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sclerophyllous modifications are interpreted as adaptations to environmental conditions (especially as far as the duration of summer drought and the intense sunlight are concerned), but the functional implications of these changes are less self-evident. The purpose of this paper is to provide an initial evaluation of the possible effects of these morpho-anatomical modifications on water economy.

Materials and Methods

Study area. The study was carried out on beech leaves growing in natural conditions. The area studied is a beech stand at Vallombrosa (Tuscany, Italy). Altitude ranges from 1200 to 1400 m asl with a northerly exposure. The $CaCl_2$ pH level of the soil ranges from 3.3 to 3.7. The mean yearly precipitation is 1426 mm, with a Mediterranean-type regime. In this forest two sites were considered: Site A (called Cardinale): 1200 m asl, in a cool valley with a good water supply. Trees are adult (40-50 years old), 20-25 m tall. Site B (called Secchieta): 1400 m asl, on a ridge with a thin layer of soil and low water retention. Trees are the same age as those in Site A but are only 5-7 m tall. Collection of samples and ecophysiological measurements were performed in August. Samples were collected from 5 trees per site. Only sun leaves, from the upper section of the crown, were considered.

Foliar indices. From each tree samples of 10 leaves each were gathered both at predawn and midday. The following parameters were examined: leaf area (LA, measured by LI-COR LI-3100 AREA METER); fresh weight (FW) and fresh weight at saturation (SW) after immersion of leaves in demineralized water for 24 h in the dark and, subsequently, dry weight (DW, after drying in an oven at 70°C for 72 h). Leaf thickness (Lth) was measured on two sub-samples of 4 leaves from each tree, analysing cross-sections under a light microscope equipped with a calibrated micrometric grid. Calculated indices are: SDW (specific dry weight)= DW LA^{-1}; WC (percent of water content)= [1-(DW FW^{-1})]100; SWC (percent of water content in a leaf after saturation)= [1-(DW SW^{-1})]100; RWC (relative water content)= [(FW-DW)/(SW-DW)]100; Suc (Succulence: water content cm^{-2})= (FW-DW) LA^{-1}. Mean values ± standard deviation (SD) are provided; the significance of differences was calculated with t-test.

Microscopy. Four leaves per tree, from each tree sampled, were examined. From the central interveinal zone of each leaf, samples measuring 0.5x3 mm were removed. For anatomical (Light Microscope) and ultrastructural (Transmission Electron Microscope) observations of tannins, the standard procedures described by JENSEN 1962 and PARHAM & KAUSTINEN 1976 were applied.

Ecophysiological measurements. The water potential of the leaves was measured using a portable pressure chamber (Sky Instruments SKPM 1400). Measurements were recorded every three hours, starting from predawn. Each time, water potential was measured on 20 leaves chosen at random from the outer portions of the crown. Sample leaves were also gathered from each of the two sites: these sample leaves were saturated with water in order to trace the pressure-volume curves (see HINCKLEY & al. 1980, SCHULTE & HINCKLEY 1985). At midday the values of stomatal conductance (SC) and transpiration (TR) were measured on 5 trees (4 leaves from each tree at different exposures) using a Li-Cor Steady State Porometer LI 1600. At the same time, this instrument allowed us to measure the relative humidity (RH), temperature (T), leaf temperature (LT) and quantum yield (Q).

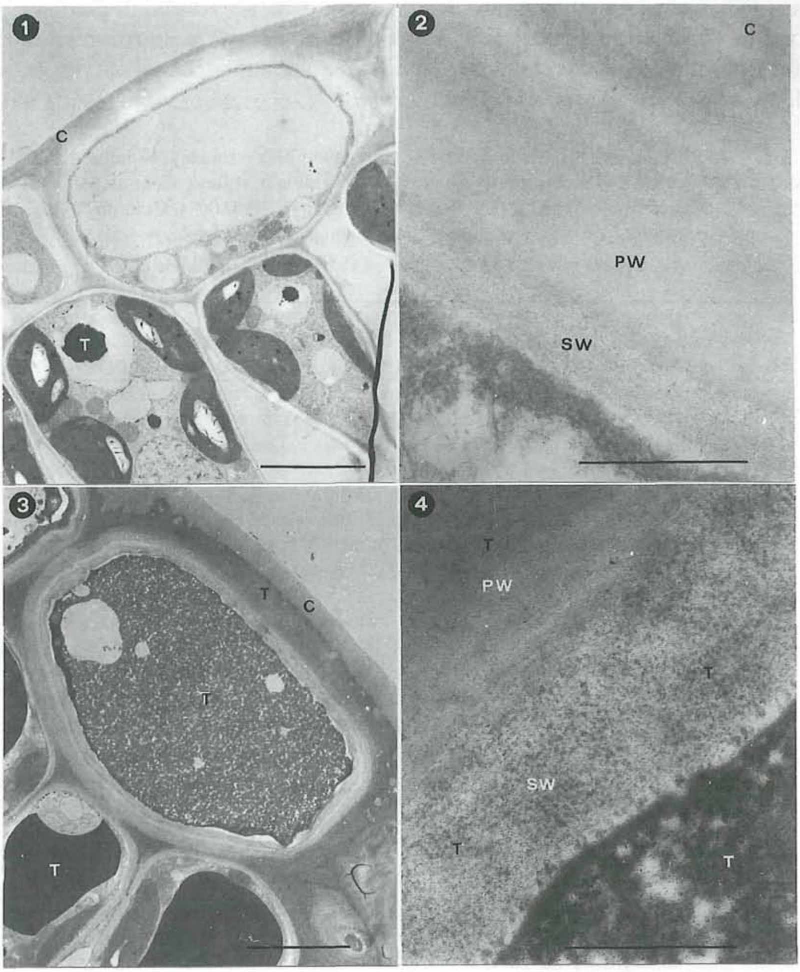
Results

The values relating to leaf indices are given in Table 1. The findings show clearly that the leaves from top ridge trees have a smaller LA and a higher degree of SDW as compared to the leaves from trees growing at lower altitudes; this appears to be due to their increased Lth. As far as the water status parameter is concerned, WC and SWC are both lower in top ridge leaves; while Suc levels are, conversely, higher in top ridge leaves. No differences have been recorded in RWC. Water status parameters show no significant variations between predawn and midday.

Table 1. Leaf indices, Mean ± SD. PD = predawn; MD = midday. Column 4 and 5: values per site, n = 10 trees (SE = Secchieta, top ridge; CA = Cardinale, valley). Column 6: significance of differences between column 4 and 5 (* P<0.05; ** P<0.01; *** P<0.001). Columns 7-10: values of water status parameters, predawn and midday, at Secchieta and Cardinale, n = 5 trees. Explication of acronyms in column 1 in Materials and Methods. LA and DW refer to a singular leaf.

1	2	3	4 SE PD+MD	5 CA PD+MD	6 P	7 SE PD	8 SE MD	9 CA PD	10 CA MD
LA	cm^2	M	15.95	30.71	***				
		sd	1.82	3.50					
DW	mg	M	135	116	***				
		sd	16.12	25.06					
SDW	mg cm^{-2}	M	8.61	3.83	***				
		sd	1.56	0.55					
Lth	µm	M	210	104	***				
		sd	25.05	9.11					
WC	%	M	58.71	64.70	***	58.23	59.20	65.57	63.85
		sd	1.35	2.57		1.80	0.54	1.32	3.36
SWC	%	M	61.11	66.82	***	61.88	60.35	67.29	66.36
		sd	1.94	2.63		1.74	2.01	2.32	3.12
RWC	%	M	90.88	91.33		85.93	91.59	92.75	89.91
		sd	8.30	8.43		3.57	9.57	8.26	9.32
Suc	mg cm^{-2}	M	12.18	6.89	***	11.46	12.89	7.11	6.69
		sd	1.72	0.86		0.90	2.14	0.40	1.19

The main histological modifications, illustrated in Figs. 1-4, occur primarily in the cells of the upper epidermis and in their outer wall. Compare Fig. 1 (valley leaves) and Fig. 3 (top ridge leaves): notice that in the former there is practically no content at all in the vacuoles of the epidermal cells, whereas in the latter the vacuoles of the epidermal cells and of the palisade mesophyll are full of electron-dense substances, identified as tannins. Also very evident are the differences in the outer tangential wall of the cells of upper epidermis. Fig. 2 (valley leaves) shows that the secondary wall, the primary wall and the cuticular layer are not clearly separated, but rather appear to blend into each other, while in Fig. 4 (top ridge leaves) these layers are very clearly defined. A very evident electron-dense strip between the primary wall and the cuticular layer is also visible in Fig. 3. Lastly, in Fig. 4 one can observe the presence of amorphous droplets of electron-dense substances (tannins) between the cellulose fibrils of the wall.



Figs. 1 - 4. Ultrastructural features of the upper epidermis in beech leaves gathered in the valley site (Cardinale, Figs. 1-2) and on the top ridge site (Secchieta, Figs. 3-4). Figs. 1-3 (bar = 5 μ m) show the different filling pattern of tannins in vacuoles of the epidermis and palizade cells in both sites. Figs 2-4 (bar = 0.5 μm) show the shape of the wall-cuticle complex in the upper epidermis in both sites. Notice the different impregnation in tannins. (from BUSSOTTI & al. 1998, modified). C = Cuticle; T = Tannins; PW = Primary Wall; SW = Secondary Wall.

Fig. 5. illustrates the leaf water potential (Ψ_l) in the two considered sites on a typical summer day: it is interesting to note that even in the predawn measurement the top ridge leaves display very low values. Fig. 6. compares two typical pressure-volume curves for top ridge leaves and valley leaves. Based on these data, we calculated the osmotic potential at saturation (π_{sat}:Secchieta = -1.7

MPa; Cardinale = -1.3 MPa) and at the turgor loss point (π_{tlp}: Secchieta = -3.3 MPa; Cardinale = -1.8 MPa). In both cases π_{tlp} values are close to the lowest levels of water potential (Ψ_{min}). Table 2 shows the measurements obtained with the porometer: top ridge leaves display lower values of SC and TR than valley leaves. Alongside this reduced stomatal function we also observed a higher LT, an increased Q and a reduced RH. High SD of Q is due to different exposures of leaves.

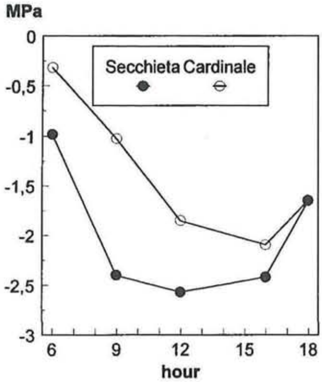


Fig. 5. Daily course of leaf water potential in the ridge (Secchieta) and the valley (Cardinale) site.

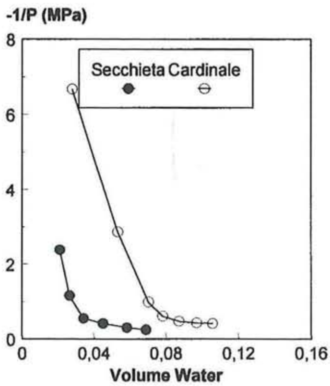


Fig. 6. Typical pressure-volume curves of leaves in both sites (Y-axis = applied pressure; X-axis = Water volume loss at the applied pressure).

Table 2. Parameters measured with porometer; n=20. Mean ± SD (abbreviations in Materials and Methods).

Site	LT °C	RH %	Q $\mu mol\ s^{-1}\ m^{-2}$	SC $cm\ s^{-1}$	TR $\mu g\ cm^{-2}\ s^{-1}$
Secchieta	29.83 ± 0.86	39.67 ± 2.65	1599 ± 233	0.10 ± 0.03	1.80 ± 0.61
Cardinale	26.87 ± 1.08	47.33 ± 2.64	1109 ± 415	0.30 ± 0.05	4.01 ± 0.58

Discussion and Conclusions

The increased sclerophylly in the top ridge leaves of beech trees is a response to stress and has already been described by BUSSOTTI & al. 1998. It seems to indicate a great phenotypic plasticity of this species. The sclerophylly is also expressed with the values of π_{sat} and π_{tlp}. They appear to be regulated in function of Ψ_{max}, to prevent leaves from wilting. Despite the fact that during the course of the day the leaves from both sites reach very low levels of Ψ_l, no significant variations in water status parameters (as WC, SWC and RWC) were observed, likely thanks to compensative mechanisms and osmoregulation (cf. MORGAN 1984). WC and SWC are greater in the valley site; the accumulation of tannins in the vacuoles is probably co-responsible for the reduced WC and SWC observed in top ridge leaves. Likely that tannin accumulation is part of the compensative mechanisms to maintain a similar value of RWC in both sites. The succulence of the top ridge leaves (Suc) is markedly higher than that of the valley leaves. The modifications above described suggest that in difficult ecological conditions beech trees are able to optimize water usage, assuming a "water-saving" behaviour. This aspect highlights a marked ecological versatility of this species.

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