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Characteristics of leaf structure and photosynthetic apparatus within the crown of systematically shaded *Quercus petraea* and *Nothofagus procera* seedlings

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Abstract

Four-year-old seedlings of *Quercus petraea* (Matt.) Liebl. and *Nothofagus procera* (Poepp. et Endl.) Querst were grown outdoors in pots while subjected to full, medium and low irradiances. Shading and decrease in height of leaf attachment generally increased specific leaf area, the diameters of chloroplasts and of palisade and spongy mesophyll cells, but decreased leaf thickness, number of palisade cell layers, length of palisade and spongy mesophyll cells, number of chloroplasts per mesophyll cell and epidermal cell and cuticle thickness, stomata and hair densities per unit leaf area, hair length, maximum hair breath and cell wall thickness in the two species. However, in *N. procera* grown under full irradiance, leaves at the upper and middle positions had hairs on both upper and lower epidermes, whereas those in other treatments and all leaves in all treatments in *Q. petraea*, had theirs only on the upper epidermis.

Introduction

In a given deciduous tree species with dense foliage, sun and shade leaves of different grades can be identified. Studies on the anatomical variations in leaves of various locations in the crown of tree species are rather limited (Penfound 1931, Watson 1942, Wylie 1951, Jackson 1966, Jackson and Beakbane 1970, Šesták 1985). Such studies have not yet been carried out on *Quercus petraea* and *Nothofagus procera*.

This study assesses the anatomical variations in leaves at the upper, middle and

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lower positions of the crown of systematically shaded four-year-old seedlings of *Q. petraea* and *N. procera* as a contribution to the knowledge of their ecological relations. The two species chosen are of contrasting backgrounds, but widely distributed. *Q. petraea* is native to the British Isles and parts of Europe (temperate climate), while *N. procera* is native to Chile (mediterranean climate). Also, *Q. petraea* is shade tolerant (Jarvis 1964, Logan 1970), while *N. procera* is shade intolerant (Veblen *et al.* 1980). Their leaf anatomical properties may therefore adapt differently to irradiance and hence provide wider knowledge of anatomical variations within the crown of young deciduous trees. Such information can help studies on photosynthetic capabilities and gas exchange rates of plant canopies.

Material and methods

Plants: Four-year-old seedlings of *Nothofagus procera* (Northern beech) and *Quercus petraea* (Sessile oak) were transplanted into 23 cm diameter and 42 cm deep whale-hide pots filled with top soil. The experiments was laid out on 6 April 1983, on a flat open areas at Bilwood Park, Sunningill, Berkshire (U.K.) in a 3 x 3 factorial experiment (3 irradiances x 3 leaf insertion scales) of four replicates. Fifteen plants of each species were used in each irradiance treatment. The treatments were full daylight (H), 49 % of full daylight (M; produced by shading the plants with two layers of plastic garden netting) and 6.8 % of full daylight (L; produced by shading with a sheet of pegboard). The shading was supported by wooden frames 180 x 9 cm, with legs 120 cm high. The two layers of the plastic garden netting were allowed to extent downwards to 90 cm on all sides of the frames. Similarly, strips of pegboard 60 cm deep by 180 cm or 90 cm long were attached vertically along the lengths and breadths on the upper portions of the frames, respectively. The shading was measured with a *Licor model 185* light sensor. The seedlings were watered daily. Once a month, 2 g of an organically based fertilizer *"Garden plus" (Imperial Chemical Industries*) was added. Leaves that opened on 4 May in each treatment were selected from the upper, middle and lower parts of the foliage and tagged.

Histological measurements: These were started on 12 August when experimental leaves were fully matured (3-months-old). The taged experimental leaves were sampled at the middle portions of the top, middle and lowest branches of each seedling.

Transverse sections 25 µm thick were prepared from the middle portion of these leaves by means of a *Cambridge* rocker microtome fitted with a freezing stage. Temporary slides were prepared with the sections mounted in glycerol jelly. The thicknesses of leaves, cuticle, upper and lower epidermal cells were measured with a light microscope. The number and dimensions of cells in the upper and lower palisade and mesophyll cells, including the chloroplast number per cell, chloroplast diameter, hair density, length and breadth, and wall thickness were similarly measured.

Stomatal densities on the adaxial and abaxial leaf surfaces were measured from imprints with nail varnish lacker.

Specific leaf area (SLA) was calculated as leaf area divided by leaf dry mass.

Statistical analyses: Analysis of variance on main effects and first order interactions, the Student-Newman-Neul (S.N.K.) multiple range test and the *t*-test were done according to Zar (1974).

Results and discussion

Q. petraea and *N. procera* seedlings produced leaves with structures much affected by different irradiances (Tables 1 to 3). The extreme forms were considered to be "sun" and "shade" leaves. In this study, sun leaves were those borne at the upper and middle positions under full irradiance and shade leaves those at all positions under medium irradiance. Those at upper positions under medium irradiance and lower branches under full irradiance produced leaves intermediate in the variable anatomical characteristics studied (Tables 2 and 3). Leaves in different parts of plant canopies formed at different times were initiated and developed under different irradiances due to self-shading. Since all these leaves contribute to the overall economy of the whole plant, their structural differences and how they relate to physiological processes as reported in literature should be discussed.

Table 1. Changes in mean specific area (SLA) and stomatal density of leaves born at different positions in the crown of *Quercus petraea* and *Nothofagus procera* seedlings grown at high (H), medium (M) and low (L) irradiances (± S.E. xt; $P = 0.05$). Adaxial stomatal density was 0 for all irradiances and leaf positions. In each character, between irradiances in each species means followed by the same letter were not significantly different at 5 % level.

Species	Irradiance	SLA [cm^2 g^{-1}]			Abaxial stomatal density [mm^{-2}]		
		Upper	Middle	Lower	Upper	Middle	Lower
Quercus petraea							
	H	11538.9	12285.6	13069.7	115.2	97.5	87.3
		±581.0	±490.4	±618.2	±6.3	±3.5	±4.7
	M	14887.1	15947.3	16325.6	93.7	86.1	74.7a
		±506.7	±455.8	±620.3	±6.2	3.3	±2.8
	L	17101.2	15859.3	16955.8	78.5	73.1	69.6a
		±907.8	±625.9	±577.8	±3.8	±1.9	±2.7
Nothofagus procera							
	H	14427.9	15859.3	16955.8	41.8b	35.4d	30.5e
		±869.3	±647.0	±636.2	±3.0	±3.7	±2.7
	M	18811.1	20865.8	25726.5	24.2c	30.4d	26.6a
		±484.6	±528.5	±493.7	±2.4	±1.2	±2.7
	L	40279.9	42194.9	42725.1	34.2c	40.4d	26.6a
		±894.8	±976.4	±647.3	±2.4	±1.2	±1.8

Table 2. Changes in mean anatomical characters of leaves borne at different positions in the crown of *Quercus petraea* and *Nothofagus procera* seedlings grown at high (H), middle (M) and low (L) irradiances. Means followed by the same letter are not significantly different at 5 % level.

Leaf position	Irradi-ance	Thickness of leaf [µm]	cuticle [µm]	epidermis [µm] upper	lower	Palisade cell number [mm^{-2}] upper	lower	length [µm] upper	lower	breadth [µm] upper	lower	Spongy cell number [mm^{-2}]	length [µm]	breadth [µm]
Quercus petraea														
Upper	H	173.4	5.8	23.3	14.8	1500	120	60.9	26.9	7.6	11.0	1200d	18.8	12.3
	M	149.7	3.2	18.7	11.2	1200a	--	46.7	--	9.5	--	1100d	15.6	14.9g
	L	113.7	2.5h	16.3i	9.9i	1200	--	34.7	--	11.0	--	700	13.9f	20.0
Middle	H	157.5	4.8	21.6	13.5	1100b	100	56.0	22.3	9.1	11.5	1000d	18.3	13.9
	M	134.2	2.7j	17.6	10.5	1060b	--	35.2	--	10.7	--	1000d	14.3	15.8
	L	88.4	2.3h	15.3	10.0k	90c	--	28.4	--	11.2	--	600	12.1	21.5
Lower	H	139.1	3.9	19.4	12.1	1000	100h	34.1	--	9.4	--	800e	17.7	14.9g
	M	119.2	2.6hj	16.8i	10.2k	90c	--	30.1	--	12.1	--	800e	13.7f	17.3
	L	75.9	1.9	14.5	9.7i	90c	--	22.4	--	12.5	--	600	11.6	23.4
Nothofagus procera														
Upper	H	167.9	2.7	12.2	11.3	200	100h	57.8	38.7	7.1	11.5	100k	18.3	13.6
	M	114.7	2.4e	11.2	9.8f	90f	--	42.5	--	12.0r	--	80j	15.9l	18.3
	L	67.1	1.7n	10.5p	8.7r	80f	--	27.4	--	12.8s	--	60t	12.0	20.4v
Middle	H	146.1	2.4e	11.9	10.7	100g	100h	53.3	30.6	7.9	11.9	100k	17.8	14.3m
	M	101.3	2.0	10.6p	9.1	80f	--	33.7	--	12.9r	--	80j	15.3l	19.7
	L	58.4	1.6n	9.3q	8.2	80f	--	24.3	--	13.3s	--	70t	11.4	21.2
Lower	H	108.5	2.3e	11.6	9.9f	100g	--	31.7	--	10.3	--	100k	17.0	14.5m
	M	95.4	1.7n	9.3q	8.8r	80f	--	29.6	--	13.4s	--	70k	13.1	20.4v
	L	54.9	1.3	9.1q	7.7	60	--	18.4	--	13.9s	--	60	10.4	23.5

Table 3. Changes in mean anatomical characters of leaves borne at different positions in the crown of *Quercus petraea* and *Nothofagus procera* seedlings grown at high (H), middle (M) and low (L) irradiances. In each character, between height, irradiances, upper and lower palisade and epidermal hairs, means followed by the same letter are not significantly different at 5 % level.

Leaf position	Irradiance	Chloroplast per palisade cell: number, upper	Chloroplast per palisade cell: number, lower	Chloroplast per palisade cell: diameter [μm], upper	Chloroplast per palisade cell: diameter [μm], lower	Chloroplast per spongy cells: number	Chloroplast per spongy cells: diameter [μm]		Hairs: number per mm²	Hairs: length [μm], upper	Hairs: length [μm], lower	Hairs: maximum breadth [μm], upper	Hairs: maximum breadth [μm], lower
Quercus petraea													
Upper	H	23.1	10.8b	2.7	3.1f**	10.7	3.3		2300	215.1		19.8	
	M	16.8	--	3.3c	--	9.1g	3.9j		1600	196.3		13.4r	
	L	10.2	--	4.3d	--	8.6hi	4.3i		600n	163.1		10.3s	
Middle	H	20.1	10.3b	3.3ct	3.4ft	9.2g	3.7j		1100	207.5		17.7	
	M	15.5	--	3.8e	--	8.9h	4.0k		500n	186.7		13.2r	
	L	9.5a	--	4.3d	--	8.4i	4.5m		300p	152.0		10.2s	
Lower	H	17.7	--	3.7e	--	8.6hi	4.0k		300p	176.2		15.5	
	M	13.9	--	4.1d	--	7.5	4.4lm		200	170.5q		12.8	
	L	9.3a	--	4.6	--	7.1	5.0		100	117.6		10.0s	
Nothofagus procera										upper	lower	upper	lower
Upper	H	20.5	13.3b	2.4	3.2	12.7	3.3	700	500	222.8	89.9	20.4n	15.7
	M	15.4A	--	3.5	--	7.9	4.2g	500	--	195.71	--	17.4p	--
	L	6.2	--	4.6d	--	4.0	5.2	100i	--	174.5m	--	18.5p	--
Middle	H	18.8	12.7b	2.8	3.7	11.7f	4.0g	300s	300s	192.91	53.1	20.0n	14.5r
	M	14.9	--	4.0b	--	6.7	4.4h	200k	--	179.0m	--	15.2q	--
	L	5.6	--	4.8cd	--	3.5	5.5	80	--	150.2	--	14.8q	--
Lower	H	15.2a	--	4.0b	--	11.7f	4.0g	200k	--	175.0mn	--	15.0q	--
	M	11.3	--	4.2b	--	6.1e	4.6h	100j	--	134.2	--	14.2q	--
	L	5.0	--	4.9c	--	6.0e	5.8	50	--	120.0	--	8.8	--

Leaf thickness and its components (thicknesses of cuticle and the upper and lower epidermal cells, the length of palisade and spongy mesophyll layers), including the number of spongy and palisade cells per unit leaf area, decreased with decreasing irradiances with leaf position were principally consequences of both variations in irradiance and leaf age (*cf.* Šesták 1985). These observations are in general accord with those of Penfound (1931), Wyle (1951), Jackson (1966) and Evans (1972). These findings are best summarized by the results of specific leaf area, which increased with shading and with decrease in leaf insertion level (Table 1). Hence, the amount of photrosynthates and other dry matter produced per unit leaf surface was larger with increasing irradiance. These characteristics are usually associated with greater leaf cell wall mass (Pearce *et al.* 1969, Anake *et al.* 1977) and greater tissue rigidity (Jones and Turner 1978) with increasing irradiance. These qualities affect water relations of sun and shade leaves (Hellkvist *et al.* 1974): As plants transpire, the more rigid sun leaves approach their zero pressure potential (turgor) much faster than the more pliable shade leaves, and hence, exposed sun leaves are better adapted to draw water up than the shade leaves within the canopy (Igboanugo 1988).

Number of chloroplasts per palisade and spongy cells, chloroplast diameter, palisade and spongy cell diameters, decreased also with increase in irradiance (Table 3). These findings are of adaptive significance to ensure maximum use of radiation on or within the crown. These observations generally agree with those of Priestley (1925) and Kirk and Tilney-Basset (1967). Wilson and Cooper (1967) demonstrated that under high irradiance the increased in leaf thickness usually increased the rate of carbon dioxide assimilation due to larger exposed mesophyll cell surfaces and higher number of chloroplasts. Similarly, Chonan (19654) showed that under optimum irradiance, the increases in leaf thickness, palisade layer thickness and cell length were associated with increased photosynthetic rates due to increased mesophyll conductance for carbon dioxide transfer. Under low irradiance, thinner shade leaves would be relatively better irradiated than thicker sun leaves and hence, their photosynthetic rates under low irradiance become higher than those of sun leaves (Böhning and Burnside 1956).

Density, maximum breadth, length and wall thickness of hairs also increased with irradiance and leaf insertion level (Table 3). In all treatments and height in oak, hairs were borne on the upper epidermis only, but in Northern beech, only leaves at the upper and middle positions under full irradiance had hairs on both epidermes, whereas other leaves bore hairs only on the upper epidermis (Table 3). Baker and Myre (1969) showed that the boundary layer resistance was directly related to the frequency of hairs. Hence, boundary leyer resistance might be higher in sun than shade leaves in the two species studied and therefore decrease with stomatal density with the decrease in irradiance and insertion level (Table 1). Similar findings have been reported for tobacco and sunflower (Rawson and Craven 1975). Under the same air humidity, Smith and Nobel (1977) observed higher stomatal resistance in sun leaves than in shade leaves of *Hyptis emoryi.* Similar responses have been reported for several plant species (Lösch and Tenhunen 1981).

Thus, all active leaves in the canopy, whether exposed or shaded, can be well adapted anatomically to exploit the irradiation environment in which they exist and thus contribute effectively to the overall economy of the whole plant.

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