Differences in photosynthetic apparatus of leaves from different sides of the chestnut canopy

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Abstract

In crowns of chestnut trees the absorption of radiant energy is not homogeneous; leaves from the south (S) side are the most irradiated, but leaves from the east (E) and west (W) sides receive around 70 % and those from north (N) face less than 20 % of the S irradiation. Compared to the S leaves, those from the N side were 10 % smaller, their stomata density was 14 % smaller, and their laminae were 21 % thinner. N leaves had 0.63 g(Chl) m^{-2}, corresponding to 93 % of total chlorophyll (Chl) amount in leaves of S side. The ratios of Chl *a*/*b* were 2.9 and 3.1 and of Chl/carotenoids (Car) 5.2 and 4.8, respectively, in N and S leaves. Net photosynthetic rate (P_N) was 3.9 μmol(CO_2) m^{-2} s^{-1} in S leaves, in the E, W, and N leaves 81, 77, and 38 % of that value, respectively. Morning time (10:00 h) was the period of highest P_N in the whole crown, followed by 13:00 h (85 % of S) and 16:00 h with 59 %. Below 500 μmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD), N leaves produced the highest P_N, while at higher PPFD, the S leaves were most active. In addition, the fruits from S side were 10 % larger than those from the N side.

Additional key words: 9-amino-6-chloro-2-methoxyacridine; carotenoids; *Castanea sativa*; chlorophyll; chloroplasts; fruit; gas exchange; shade and sun leaves; water relations.

Introduction

European chestnut (*Castanea sativa* Miller) is an indicator of warm regions with oceanic climate on acidic to neutral soils (Heiniger and Conedera 1992), such as are in Terra-Fria, a sub-region of Trás-os-Montes (northeast of Portugal) dominated by mountain and sub-mountain ecosystems. In this region, chestnut is a forest tree or a cultivated tree growing between 600 and 1 000 m a.s.l., where year mean values of sunlight and precipitation are 2 400–2 600 h and 600–1 200 mm, respectively. According to Fernández-López *et al*. (2005), chestnuts have different extreme populations, probably due to their long-range distribution across the Mediterranean region with varying climate. Chestnut is moderately thermophilic, well adapted to mean year temperatures of 8–15 ºC and monthly mean temperatures of more than 10 ºC during 6 months. Nevertheless, its pollen germinates only at temperature of 27–30 ºC (Bounous 2002). Adult chestnut trees show maximal photosynthesis at 24–28 ºC, exhibiting a significant thermoinhibition for temperatures >32 ºC, which are very frequently attained during summer in south-faced foothills (Gomes-Laranjo *et al*. 2005, 2006). When chestnut trees are cultivated in north-faced orchards, they receive less radiant energy and consequently they grow under lower mean daily atmospheric temperature. According to Almeida *et al*. (2007), 90 % of maximal photosynthesis was measured at an irradiance of about 800–1 300 μmol m^{-2} s^{-1}, and the half rate was obtained at <500 μmol m^{-2} s^{-1}.

Chestnut is a large deciduous tree, reaching a height of 40 m and a 6–7 m diameter of canopy (Bounous 2002). In such canopies, it is possible to identify a deep heterogeneity in radiation availability around the crown (north, east, south, and west regions – N, E, S, and W) besides an enormous internal canopy region. The existence of shade and sun leaves is well established (Boardman 1977, Lichtenthaler *et al*. 1981, 2007, Lawlor 1993, Pearcy

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Abbreviations: A – leaf area; ACMA – 9-amino-6-chloro-methoxyacridine; ALM – area leaf mass; Car – carotenoids; Chl – chlorophyll; DM - dry mass; *E* – transpiration rate; g_s – stomatal conductance; MV – methylviologen; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; Q – ACMA fluorescence; RWC – relative water content; SLA – specific leaf area; T_L – leaf temperature; WUE – water use efficiency; Ψ_p – leaf pressure potential; Ψ_w – leaf water potential; Ψ_π – leaf osmotic potential.
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1998). These types of leaves have different strategies for available radiation resources. Shade leaves which grow under weak irradiance must catch photons as efficiently as possible. On the other hand, sun-side leaves must protect themselves against high photon fluence densitites (PPFD) which may damage their photosynthetic structures. According to Lichenthaler *et al.* (1981, 2007), the ability of leaves and chloroplasts to adapt to irradiance is the central basic growth-response. This is related to specific changes in leaf anatomy, morphology, physiology, biochemistry, and chloroplast structure.

As compared to shade leaves, sun leaves are usually thicker, smaller, with longer palisade cells, more cutin and higher dry mass per leaf area unit. Sun leaves also have higher chlorophyll (Chl) and carotenoid (Car) contents per leaf area unit, higher values of Chl *a*/*b*, and greater stomata density. Sun leaves possess sun-type chloroplasts and grana stacks are smaller than in shade-exposed ones (Lichtenthaler *et al*. 1981). Sun chloroplasts have higher saturation irradiance and they are adapted to higher photosynthetic quantum conversion.

The extensive literature (for review see Lichtenthaler and Babani 2004) shows that much work has been done with herbaceous plants or deciduous broad-leaf trees grown at high or low irradiance places or, in case of trees, with leaves grown in the inner shade or in the external sun part of the crown. This is why we tried to characterize photosynthetic apparatus around the chestnut crown under different PPFD at the level of leaf and during the day, its gas exchanges, water relations, thylakoid membrane potential, *etc*.

Materials and methods

Field studies were carried out in 1995–2000, from June to October in an orchard located in Carrazedo de Montenegro. This place is located in the Northeast of Portugal (41º34’36’’N and 7º26’34’’E, 770 m a.s.l.), in the centre of Trás-os-Montes region. The climate of the area is characterized by annual mean temperature of 11 ºC, total irradiation of 2 400–2 600 h, and total rainfall of 800–1 000 mm. The mean month precipitation in this region was 106, 49, 18, 26, 49, and 178 mm, for May, June, July, August, September, and October, while the mean daily temperatures were 12.3, 15.5, 19.3, 19.1, 15.2, and 10.7 ºC, respectively.

Thirty-year-old chestnut trees (*Castanea sativa* Mill.) of cv. Judia growing under similar edaphoclimatic conditions were spaced 10×10 m to avoid any irradiance interferences between canopies. Eight trees were divided into four quadrants, in accord with the cardinal points: N, S, E, and W. Incoming radiation was weekly measured with a radiometer (*Macam* model *Q102*, Livingston, Scotland) improved with minor laboratory modifications that protect the sensor from erratic radiations. Measurements were taken always at 2-m height in a position as close as possible to the external part of the canopy. Measurements were done at 10:00, 13:00, and 16:00 h in the four sectors (N, E, S, and W). Measurements were done only on cloudless days. Transmittance was determined monthly, by detaching eight leaves and putting them in front of the radiation sensor, which was directly oriented to the sunlight.

Gas exchanges were determined with an infrared gas analyser (IRGA, model *LCA-2*, *Analytical Development Co.*, Hoddesdon, UK). Relative air humidity and CO_2 concentration in leaf chamber were kept constant at 8–12 % and 330–380 μmol(CO_2) mol^{-1} s^{-1}. Two adult leaves, from the external part of each canopy, without any deficiency of nutrient symptoms, were selected up to the height of 3 m. Measurements were done fortnightly at 10:00, 13:00, and 16:00 h in July, September, and October only in days of plenty sunlight.

Selected leaves from gas exchange measurements were used for water potential (Ψ_w) measurements in a Scholander Pressure Chamber (*PMS Instrument*®, Corvallis, Oregon, USA) according to Scholander *et al.* (1965) following the recommendations of Turner (1988). After that, leaves were stored in liquid nitrogen and taken to the laboratory to determine osmotic potential (Ψ_π) using an osmometer (model *3*, *Advanced Instruments*, Needham Heights, Massachusetts, USA), according to the Van’t Hoff equation. Each leaf was pressed to obtain 100 mm^3 of fluid (from symplasm and apoplasm). The pressure potential (Ψ_p) was calculated from the difference between Ψ_w and Ψ_π (Salisbury and Ross 1992).

Leaf morphologic parameters were determined with adult leaves from the external part of the crown, which were collected in September. Leaf area (A) was measured by a leaf area meter (model *Cl-202* Area Meter, *CID*, USA) and shape factor (*f*) according to Květ and Marshall (1971).

Dry mass (DM) was determined after drying the leaves from each side at 80 ºC for 48 h. Subsequently, the relative water content (RWC), RWC = [(FM – DM)/(SM – D)]×100, specific leaf area (SLA), SLA = A/DM, and area leaf mass (ALM), ALM = DM/A) were determined (Salisbury and Ross 1992).

Four leaves from each side of each tree were frozen in liquid nitrogen and later analyzed to determine photosynthetic pigments. From each leaf, six 8-mm discs were punched out and put into test tube containing 10 cm^3 of 80 % (m/v) acetone (pH = 7.5, buffered by 25 mM Hepes) (Porra *et al*. 1989) for Chl extraction. The Chl samples were kept in the dark and incubated at 4 ºC (usually for 48 h) under regular shaking of the tubes, until all Chl was extracted (Dai *et al.* 1992). Chl and carotenoids (Car) were quantified spectrophotometrically using the equations of Lichtenthaler (1987).

Leaf mineral composition was obtained from 20 dried

leaf tissues (48 h at 70 ºC) and micro-Kjeldahl digestion using the method of Mills and Jones (1996).

For leaf anatomical studies, a 5-µm sample of lamina tissue was freshly excised from the mean region of three selected leaves in each side. Preparations were made in water for observation in an optical microscopy with a 40× objective lens.

For the fruit tests, 30 mature burs from each side were collected in order to determine the calibre, mass/volume ratio, and number of fruits per bur. Dry matter, starch, protein, fat, and fibre were determined according to Ferreira-Cardoso (2002).

Changes of membrane potential ($\Delta\Psi$) in thylakoids from N and S leaves were measured at temperatures of 12–28 ºC using the cationic probe 9-amino-6-chloro-2-methoxyacridine (ACMA) (Schuldiner *et al.* 1972, Packer *et al.* 1975, Kraayenhof 1996, Rottenberg 1997). Chloroplasts were prepared as described in Gomes-Laranjo *et al.* (2005, 2006) and incubated at a concentration of 25 kg(Chl) m^{-3} with 200 mM sorbitol, 2 mM Tricine-NaOH (pH 8.4), 150 mM KCl, 4 mM $MgCl_2$, 30 µM MV (methylviologen), and 5 µM ACMA (Torres-Pereira *et al.* 1984, Kraayenhof *et al.* 1993).

Analysis of variance and regression models were tested using the *Microsoft Excel* and *StatView 4.0* programs (*Abacus Concepts*). Comparisons were made with the Fischer test with a significance level of 0.05. Multivariate analysis was performed in *SPSS for Windows*, release *10.0.1*, standard version. It was employed in order to examine the simultaneous contribution of all studied parameters to discriminate side effects. Principal component analysis was conducted by the *Eingenvalue* matrix and 2-D scatterplot was built in the *Microsoft Excel* program using the two first principal components.

Results

The N, E, S, and W sides of chestnut tree crowns absorbed different amounts of radiation at 10:00, 13:00, and 16:00 h (Fig. 1). The E-S half of the canopy was the sunny side during morning and during afternoon it was the S-W half of canopy. The N side was always a shade side with only 15 % of the S radiation availability. Mean incoming PPFD in whole canopy increased from morning to midday and then decreased in the afternoon, being 534, 806, and 562 µmol m^{-2} s^{-1}. Temperature continuously increased from 10:00 to 13:00 and 16:00 h from of 22.8 to 26.3 to 26.6 ºC, respectively.

Maximal values of g_s and E (Fig. 1) were found in the E side at 10:00 h [g_s = 226 mmol m^{-2} s^{-1}; E = 3.8 mmol (H_2O) m^{-2} s^{-1}] and then decreased from 10:00 to 16:00 in opposition to variations in the W side. Nevertheless, the impact of irradiance and temperature changes in net photosynthetic rate (P_N) was very strong. Although the maximal irradiance was found on S side at 13:00 h, the highest P_N [6.9 µmol(CO_2) m^{-2} s^{-1}] was determined in E side at 10:00 (80 % of maximal irradiance). At this time, P_N in S side was 4.3 µmol(CO_2) m^{-2} s^{-1}, being maximal at 13:00 with 5.1 µmol(CO_2) m^{-2} s^{-1} which was close to P_N in W side at 16:00 [4.9 µmol(CO_2) m^{-2} s^{-1}]. Irradiance at this time was similar to that measured in E side at 10:00. Meanwhile, overall P_N of the crown decreased from 10:00 and further to 16:00 from 3.6, 2.8, and 2.2 µmol(CO_2) m^{-2} s^{-1}. In consequence of this variation, photosynthetic quantum efficiency (P_N/PPFD) ranged between 0.0065 and to 0.0052 µmol(CO_2) µmol^{-1}. Variation of WUE (Fig. 1) was very close to variation of P_N.

Contrary to gas exchanges, all maximal values of Ψ_w were found at 10:00, varying between –0.94 MPa in N and –1.15 MPa in E (Fig. 2*A*). Minimal values of Ψ_w for each side were found when the side was under maximal irradiance, being –1.29 MPa in S (13:00) and –1.24 MPa in W (16:00). Highest Ψ_π values were obtained in leaves collected during morning, being between –1.36 MPa (W) and –1.42 MPa (E) (Fig. 2*B*). These values diminished between –1.43 MPa (N, 13:00–16:00), –1.50 MPa (S, 16:00), and –1.46 MPa (W, 16:00). Highest values of Ψ_p were found at 10:00 in N (0.40 MPa), W (0.42 MPa), and S (0.33 MPa), but in the E side maximal value was found at 16:00 (0.38 MPa). RWC oscillated between 74.7 (N, 10:00) and 76.3 % (S, 13:00).

As concerns mean values (10:00, 13:00, and 16:00) of PPFD and gas exchange around the crown, leaves from the S side (the side with the smallest variation) were exposed to the highest mean PPFD, 912 µmol m^{-2} s^{-1}, but the N side received only less than 20 % of the S irradiance (Table 1). The balance of irradiance also partially influenced variation of leaf temperature (T_L). The highest values, 25.1 ºC, were observed on the side of highest intercepted radiation (S), where the shift between for the shady side (N) in the canopy was about 0.7 ºC. The highest mean P_N around the crown was also found in the S side [3.9 µmol(CO_2) m^{-2} s^{-1}], in the E and W sides being decreased by about 20 % and in the N side by 60 %. Important variations were also detected in photosynthetic quantum efficiency, that was the highest in N leaves, 0.0064 µmol(CO_2) µmol^{-1}, which was 39 % more than in the S leaves. Contrarily, differences in mean E and intercellular CO_2 concentration (C_i) were less than 5 % (in E and W sides) and in g_s less than 3 % in these sides.

Leaves exposed to the highest irradiance showed the lowest values of Ψ_w, Ψ_π, and Ψ_p,, in contrast to those from the N face (Table 1). Between S and N leaves there was a difference of 0.11 MPa (p<0.05), while it was only 0.01 MPa between E and W sides. Overall variation for Ψ_π and Ψ_p between the highest (N) and lowest (S) was only 0.05 and 0.06 MPa, respectively, and these differences were significant. Nevertheless, no significant

differences were detected in RWC (Table 1).

Differences in P_N were related to leaf position. According to Fig. 3, 90 % of maximal photosynthesis [P_{N90} = 6.0 μmol(CO_2) m^{-2} s^{-1}] in N leaves was found at PPFD of 1 300 μmol m^{-2} s^{-1}, while in leaves from the S side at 1 420 μmol m^{-2} s^{-1} [P_{N90} = 6.9 μmol(CO_2) m^{-2} s^{-1}]. Half rates (P_{N50}) were obtained at 250 and 400 μmol m^{-2} s^{-1} in N and S leaves, respectively.

The effect of leaf position on temperature of chloroplast acclimation was also studied. The energization of thylakoids led to an extra increase of net negative charge at their stroma surface. This led to an extra

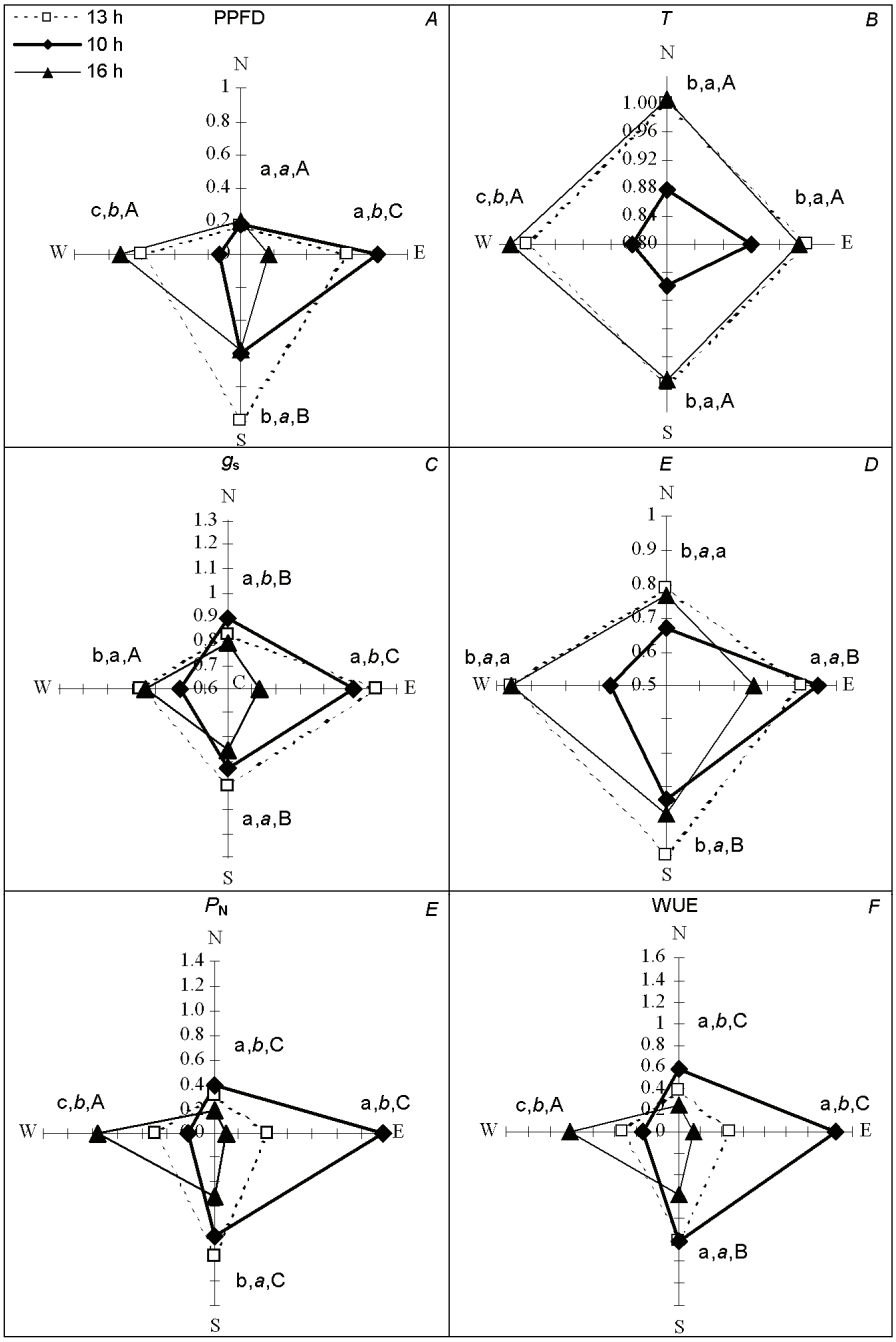

Fig. 1. Irradiance, PPFD (*A*), temperature, *T* (*B*), stomatal conductance, g_s (*C*), transpiration rate, *E* (*D*), net photosynthetic rate, P_N (*E*), and water use efficiency, WUE (*F*) variation at 10:00, 13:00, and 16:00 h in north (N), east (E), south (S), and west (W) sides of the canopy. Irradiance (n = 352) was measured with a radiometer and leaf temperature (n = 184) was taken simultaneously with the gas exchange parameters (n = 184) using the *LCA-2* IRGA. *For each side, letters* represent the comparison between 10:00 (*upright letters*), 13:00 (*italic letters*), and 16:00 (*caps*) h. *Values with the same letters* are not significantly different according to the Fisher test, 5 %.

Table 1. Mean values of absorbed radiation (PPFD) [μmol m^{-2} s^{-1}], leaf temperature (T_L) [°C], stomatal conductance (g_s) [mmol m^{-2} s^{-1}], net photosynthetic rate (P_N) [μmol(CO_2) m^{-2} s^{-1}], transpiration rate (E) [mmol(H_2O) m^{-2} s^{-1}], water use efficiency (WUE) [mol(CO_2) mol^{-1}(H_2O)], internal CO_2 concentration (C_i) [g m^{-3}], water potential (Ψ_w), osmotic potential (Ψ_π), and pressure potential (Ψ_p) [MPa], and relative water content (RWC, n = 88) in the north (N), east (E), south (S), and west (W) sides of canopy. Values of absorbed radiation (n = 1270) were fortnightly taken by a radiometer. Data on gas exchange (n = 552) and water relations (n = 620) were simultaneously taken monthly, all of them at 10:00, 13:00, and 16:00 h, between June and October. Deviations were calculated as a percentage of the control results from the south. Comparisons were made inside each parameter, between the sides of canopies. Values with the same letters were not significantly different according to the Fisher test, 5 %.

Parameter	N		E		S		W	
	value	deviation	value	deviation	value	deviation	value	deviation
PPFD	137 d	15	673 b	74	912 a	100	611 c	67
T_L	24.4 b	97	25.1 a	100	25.1 a	100	24.8 ab	99
g_s	169 b	90	190 a	101	188 a	100	183 a	97
P_N	1.47 c	38	3.14 b	81	3.89 a	100	3.02 b	77
E	3.26 c	87	3.54 b	95	3.74 a	100	3.56 b	95
WUE	0.49 c	47	0.84 b	80	1.05 a	100	0.75 b	71
P_N/PPFD	0.0064 c	139	0.0037 b	80	0.0044 a	100	0.0048 b	104
C_i	292 a	109	281 b	105	268 d	100	275 c	102
Ψ_w	–1.08 a	91	–1.13 b	95	–1.19 c	100	–1.14 b	96
Ψ_π	–1.41 a	97	–1.45 b	99	–1.46 b	100	–1.42 a	97
Ψ_p	0.27 a	129	0.24 a	114	0.21 b	100	0.23 a	110
Water content	59.7	103	59.9	103	58.1	100	58.7	101
RWC	69.7 a	100	71.5 a	102	69.8 a	100	70.3 a	101

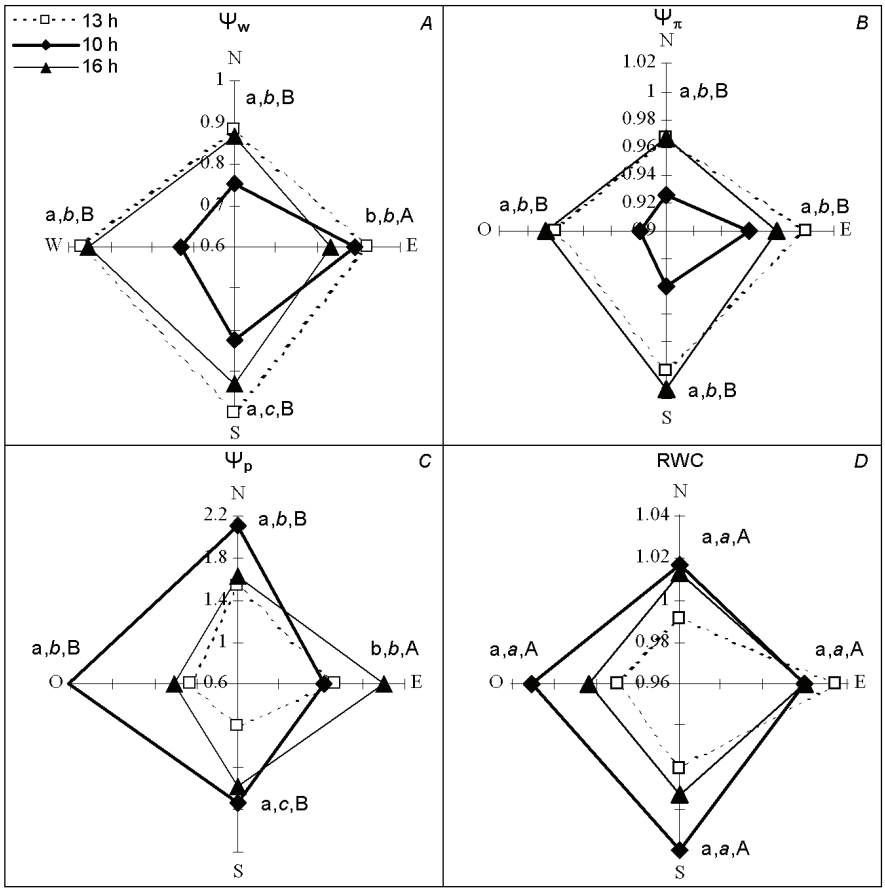

Fig. 2. Variation of water potential (Ψ_w; *A*), osmotic potential (Ψ_π; *B*), pressure potential (Ψ_p; *C*) (n = 205), and relative water content (RWC; *D*) (n = 88) in leaves from north (N), east (E), south (S), and west (W) side of canopy at 10:00, 13:00, and 16:00 h. *For each side, letters* represent the result of the comparison between 10:00 (*upright letters*), 13:00 (*italic letters*), and 16:00 (*caps*) h. *Values with the same letters* are not significantly different according to the Fisher test, 5 %.

Table 2. Leaf and fruit characteristics. Leaf biometrics, histology, and contents of photosynthetic pigments (n = 432) and mineral nutrients (n = 76) were compared between canopy sides (N – north, E – east, S – south, W – west). For fruits, biometrics (n = 150) and biochemistry (n = 20) were also determined. *Letters* represent the comparison between the sides of canopy for each parameter according to the Fisher test, 5 %.

Organ		Parameter	N	E	S	W
Leaf	Biometrics	Area [cm^2]	73.7 b	82.6 a	81.9 a	75.5 b
		Length [cm]	18.9 a	18.9 a	18.6 a	18.0 b
		Width [cm]	5.88 b	5.98 b	6.52 a	5.83 b
		Length/width	3.20 a	2.93 b	2.84 b	2.89 b
		SLA [m^2 kg^{-1}]	1319 a	1233 b	1196 b	1237 b
		ALM [kg m^{-2}]	7.85 b	8.47 a	8.79 a	8.63 a
		Stomata [mm^{-2}]	254.8 c	270.5 b	297.4 a	292.0 a
		f	0.645 b	0.734 a	0.662 b	0.720 a
	Histology [μm]	Thickness	188.7 a	194.6 c	239.2 a	208.7 b
		Upper epidermis	22.0 c	23.8 bc	29.1 a	24.4 b
		Lower epidermis	15.2 a	15.0 a	16.2 a	15.4 a
		Palisade mesophyll	82.4 c	87.8 b	110.8 a	86.8 b
		Spongy mesophyll	73.4 b	66.6 c	82.2 a	83.6 b
		Palisade/spongy	1.19 b	1.38 a	1.39 a	1.07 c
		Transmittance [%]	5.44 b	5.25 a	5.24 a	5.36 a
	Pigments	Chlorophyll (a+b) [g m^{-2}]	0.63 b	0.64 b	0.68 a	0.67 a
		Chlorophyll (a+b) [g kg^{-1}]	2.83 a	2.67 c	2.78 b	2.87 a
		Chl a/b	2.91 b	3.09 a	3.14 a	3.08 a
		Car [g m^{-2}]	0.12 a	0.13 a	0.14 a	0.15 a
		Car [g kg^{-2}]	0.47 a	0.45 b	0.45 b	0.46 a
		Chl/Car	5.23 a	4.92 b	4.76 c	4.62 c
	Nutrients [g kg^{-1}(DM)]	N	287.9 a	299.4 a	296.0 a	292.6 a
		P	4.96 a	4.40 b	4.97 a	4.66 ab
		K	2.59 a	1.98 c	2.42 ab	2.28 b
		Ca	7.94 a	6.69 b	6.73 b	7.62 a
		Mg	1.81 a	1.76 ab	1.35 c	1.55 bc
		Mn	4.39 a	4.00 ab	3.59 b	3.94 ab
Fruit	Biometrics	Calibre [number per kg]	81.2 a	77.7 ab	73.5 b	82.8 a
		Number per bur	1.47 b	1.63 a	1.51 b	1.59 a
		Global calibre [number per kg]	153.8 a	126.6 c	140.8 bc	142.9 ab
		Mass/volume [kg m^{-3}]	1044 a	1053 a	1052 a	1045 a
	Biochemistry	Dry mass [%]	46.2 b	47.4 a	47.7 a	47.4 a
		Starch [%DM]	64.1 b	66.8 a c	66.8 a	64.8 a
		Protein [%DM]	6.31 b	6.41 ab	6.66 a	6.43 ab
		Fat [%DM]	2.08 a	1.95 a	1.91 a	2.06 a
		Fibre [%DM]	21.1 b	23.6 a	23.3 a	22.5 ab

absorption of cationic monoamine molecules (ACMA), which further led to a loss of its fluorescence (Q) (Fig. 4). This measurement determines indirectly the thylakoid membrane potential. According to Fig. 4, where a three-phase curve is considered, maximal potential was obtained at 17 and 19 ºC, respectively, in the N and S sides.

Leaves from the N and W were significantly smaller than those from E and S, which had a similar area (Table 2). N leaves also had the lowest width in contrast to S leaves, but the longest leaves were from N and E. The leaf length/width ratio was lowest in the S side and the highest one in the N side. SLA was lower in leaves from the three sun sides than in the N leaves. In contrast, ALM was the highest in the three better irradiated sides. The highest stomata densities were found in S and W leaves. After leaf area determination, shape factor (f) was also calculated and significant differences were also detected (Table 2).

Histological studies revealed that N leaves were significantly thinner, due to thinner upper epidermis and palisade and spongy mesophylls (Table 2). Optical microscopy (Gomes-Laranjo 2001) showed two layers of these cell types in N leaves and three layers of palisade cell type in S leaves. No significant differences were detected in leaf transmittance.

Leaves from S and W contained the highest amount of total Chl per leaf area unit, which was 7 % higher than in N leaves, which showed the highest Chl content per FM, 2 % more than in S leaves. Typical differences between sun and shade leaves in Chl a/b and Chl/Car were also

found comparing leaves from different canopy sides. Leaves from E, S, and W had identical Chl *a/b* ratio which was higher than that in N leaves. This suggests a more heliophilic characteristic of these leaves. The characteristic is also sustained by the highest Car content per leaf area unit in the leaves from S and W and the lowest content per leaf FM in the E and S leaves. As expected, leaves from N exhibited the highest Chl/Car ratio (5.23) in contrast to those from the S and W sides (4.76 and 4.62, respectively). Leaves from the N side had higher contents of P, K, Ca, Mg, and Mn than the other leaf types (Table 2). The N/P ratios were 63.9, 68.0, 69.5, and 68.5 for the N, E, S, and W leaves, respectively.

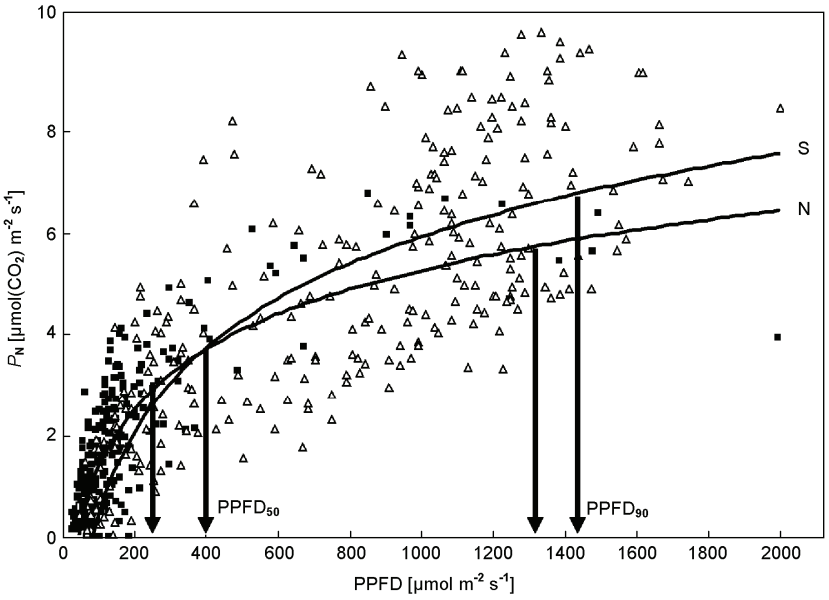

Fig. 3. Correlation between photosynthetic photon flux density (PPFD) and net photosynthetic rate (P_N) in north (N) and south (S) sides of canopy. Data were obtained between 20 and 30 °C in July, August, and September. *Arrows* represent the PPFD for 90 and 50 % of maximal P_N in S and N sides. Logarithmic equation analysis was used to determine the equation of the best-fitting line. The values of r^2 were (N) 0.64 and (S) 0.68, respectively.

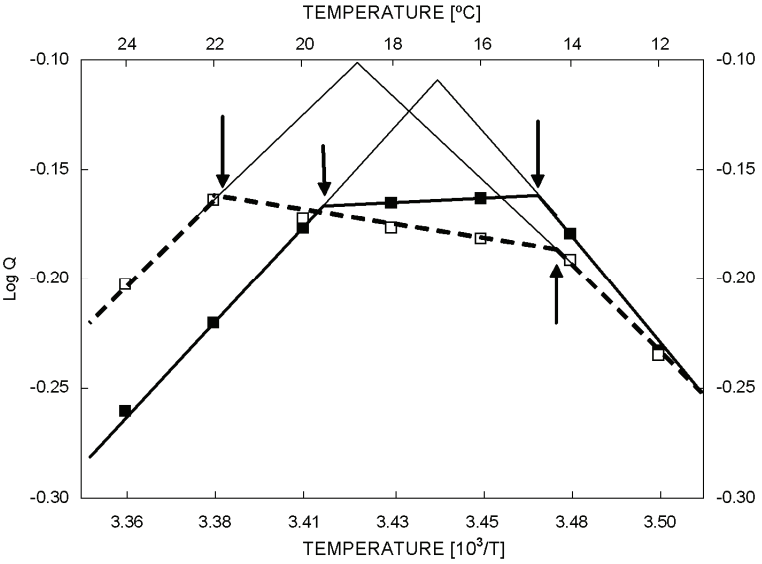

Fig. 4. Arrhenius plot of the membrane potential variation as a function of temperature in north, N (■) and south, S (□) chloroplasts. Measurements were made with the cationic probe ACMA. Exchange of ACMA fluorescence (Q) was determined in chloroplast extracts at equivalent of 25 g(Chl) m^{-3} which were incubated in 200 mM sorbitol, 2 mM tricine-NaOH (pH = 8.4), 4 mM $MgCl_2$, 150 mM KCl, 30 µM methylviologen, and 5 µM ACMA. Results from a single assay, but similar results were seen in three different experiments.

The S side produced also the biggest fruits, 10 % larger than in the N (Table 2). The crown side is important for polination of flowers. In E and W there was the highest amount of fruits per bur; the difference between N and E was about 10 %, but there was no difference in their density (mass/volume). Fruits from S side showed highest DM percentage. As concerns the main nutrients, the fruits from E, S, and W contained more starch, protein, and fiber than fruits from the N side. No differences were observed in contents of fat.

Total variation was explained using three principal components which were extracted according the eingenvalues. Relative percentages were 64.7, 22.9, and 12.4 %, respectively for Factors 1, 2, and 3. The first two components explained 87.6 % of total variation (Fig. 5). The first component separates S and N sides, locating the first one in the positive end of the first principal axis and the north point in the negative part of the axis. E and W

are positioned in the centre of the first principal axis, being separated by the second principal component, leaving W side in the positive part and E side in the negative part of the axis.

Discussion

Leaves in the shade have structural and functional features which distinguish them from those in the sun (Boardman 1977, Anderson *et al.* 1988, Thompson *et al.* 1992a,b). The same is true for leaves located around the crown where irradiation is not homogeneous during the daylight. In our experiments, the S side was most irradiated, followed by the E and W sides. The least irradiated was the N side, always a shady side. The W and E sides were intermediate regions since during the daylight they could be sun or shade sides (Fig. 1) depending on the orientation of canopy in relation to the sunlight. These daily variations in PPFD in each side were followed by correspondent variations in g_s, E, P_N, and WUE. Then, during daylight, almost half part of the canopy was active in photosynthetic productivity, decreasing its mean value from 10:00 to 13:00 and further to 16:00 h.

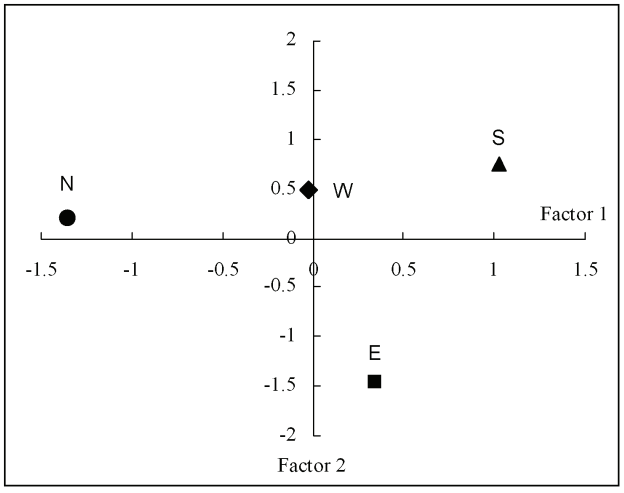

Fig. 5. Ordination of the north (N), east (E), south (E), and west (W) in a 2D-scatterplot, using the two principal components extracted from all the studied parameters which explain 64.7 % (Factor 1) and 22.9 % (Factor 2) of the variance between sides, according initial Eingenvalues.

Leaves located in the N side received less than 20 % of the radiant energy received by the S side (Table 1), which made the S part of the canopy hotter than the opposite part. The E and W sides had between 74 and 67 % of the S irradiation. These facts induce a cascade of occurrence at many levels of biological organization (Boardman 1977, Lichtenthaler *et al.* 1981, Osmond and Chow 1988, Thompson *et al.* 1992a).

In comparison to S side, the leaves from N had lower g_s, P_N, and E, as opposed to the three leaf potentials which had higher values. Such features of leaves from the shade region of the crown lead to a general leaf shade tolerance, even in the context of the same plant, which is valid mainly for vigorous species such as chestnut tree.

Because of all these features, the fruit was not totally homogeneous around the crown. Fruits from the S side were bigger by 11 % than the ones from N. According to Taiz and Zeiger (2002), sources and sinks are primarily located on the same side of the canopy, so the heterogeneity of fruits is mainly due to the observed differences in P_N around the crown.

Saturation irradiance for P_N (Fig. 3) was the highest in S leaves, which agrees with well known differences between sun and shade leaves of other broadleaf species (Lichtenthaler 1985, Osmond and Chow 1988, Kubiske and Pregitzer 1997, Lichtenthaler *et al.* 2007). In leaves from top and bottom of the crown of *Acer*, *Fagus*, *Tilia*, and *Abies*, Lichtenthaler *et al.* (2007) found P_{max} at much higher saturation irradiance than in our experiments. Additionally, for PPFD lower then 500 μmol m^{-2} s^{-1}, the N leaves showed the highest P_N of the crown; this value is ascribed to compensation irradiance between the N and S leaves.

Heterogeneity in chloroplast energization between N and S was also demonstrated: chloroplasts from S leaves showed a higher temperature than those from N leaves. This acclimation to high temperature is normally associated with a greater saturation of fatty acids in membrane lipids which turns membranes less fluid (Taiz and Zeiger 2002, Gomes-Laranjo *et al.* 2005b). Gomes-Laranjo (2001) using the ACMA methodology (Torres-Pereira *et al.* 1984) demonstrated that chloroplasts from N side showed approximately 5 % larger thylakoid surface than S chloroplasts. Chloroplasts from shade show larger stacking than sun chloroplasts (72 : 56; Lichtenthaler *et al.* 1981, 1984, Lichtenthaler 1985).

The N leaves were 10 % smaller than the S leaves, but they were longer (Table 2). Additionally, S leaves had lower SLA (by 10 %) and stomatal density (by 14 %), but higher ALM than N leaves. We found smaller differences than Lichtenthaler *et al.* (2007) in *Fagus* crown. Nevertheless, for *Abies* they found differences between shade and sun leaves of about 33 %. According to Lichtenthaler *et al.* (2007), SLA values characterize leaf structure, thickness, and morphological difference between sun and shade leaves. In fact, leaves from N were 21 % thinner, which was mainly a result of thinner palisade (25 %) and spongy (11 %) mesophylls containing less Chl per leaf area and lower Chl *a/b* ratio. The difference in the thickness was also lower than that found by other authors (differences of 39–50 % between beech sun and shade leaves).

Higher P_N saturated by radiant energy in sun (south) leaves is mainly related to a greater amount of nitrogen

per leaf area unit in sun leaves as compared to shade (N) leaves (see Table 2). They had consequently greater content of Chl per leaf area unit and larger content of RuBPCO enzyme (not determined) and subsequently higher capacity of CO_2 uptake at high irradiances (Lichtenthaler *et al*. 2007). Per FM, shade leaves contained significantly more Chl than sun leaves.

The N leaves possessed lower Chl *a*/*b* and higher Chl/Car than the S leaves. These differences were in the range of typical values for sun and shade leaves (Lichtenthaler *et al*. 2007). These authors stated that the significantly lower Chl/Car in S leaves than in N leaves is primarily caused by their content of the xanthophyll cycle Cars, which constitute part of the adaptation mechanism to high irradiance with fewer light-harvesting Chl proteins and a larger number of reaction centre pigment proteins (*e.g*. CPa, CPI) on a total Chl basis as compared to shade leaves, as well as a greater number of electron transport chains.

Finally, fruit characteristics were significantly influenced by canopy position: those from S and E sides were the biggest ones (Table 2). But, when global calibre was studied, a difference of almost 13 fruits per kilogram between E and S was found indicating that polination in E side was more efficient than in S side, possible due to the daily hotter and dryer atmospheric conditions.

In conclusion, the significant range of the PPFD which was measured around the canopy in N, E, S, and W did not influence the photosynthetic attributes of leaves more than was ever referred in the literature for typical shade and sun leaves.

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