Leaf traits variation during leaf expansion in *Quercus ilex* L.

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Abstract

The morphological, anatomical and physiological variations of leaf traits were analysed during *Quercus ilex* L. leaf expansion. The leaf water content (LWC), leaf area relative growth rate (RGR_l) and leaf dry mass relative growth rate (RGR_m) were the highest (76±2 %, 0.413 cm^2 cm^{-2} d^{-1}, 0.709 mg mg^{-1} d^{-1}, respectively) at the beginning of the leaf expansion process (7 days after bud break). Leaf expansion lasted 84±2 days when air temperature ranged from 13.3±0.8 to 27.6±0.9 °C. The net photosynthetic rate (P_N), stomatal conductance (g_s), and chlorophyll content per fresh mass (Chl) increased during leaf expansion, having the highest values [12.62±1.64 μmol (CO_2) m^{-2} s^{-1}, 0.090 mol (H_2O) m^{-2} s^{-1}, and 1.03±0.08 mg g^{-1}, respectively] 56 days after bud break. Chl was directly correlated with leaf dry mass (DM) and P_N. The thickness of palisade parenchyma contributed to the total leaf thickness (263.1±1.5 μm) by 47 %, spongy layer thickness 38 %, adaxial epidermis and cuticle thickness 9 %, and abaxial epidermis and cuticle thickness 6 %. Variation in leaf size during leaf expansion might be attributed to a combination of cells density and length, and it is confirmed by the significant (p<0.001) correlations among these traits. *Q. ilex* leaves reached 90 % of their definitive structure before the most severe drought period (beginning of June – end of August). The high leaf mass area (LMA, 15.1±0.6 mg cm^{-2}) at full leaf expansion was indicative of compact leaves (2028±100 cells mm^{-2}). Air temperature increasing might shorten the favourable period for leaf expansion, thus changing the final amount of biomass per unit leaf area of *Q. ilex*.

Additional key words: leaf anatomy; leaf expansion; leaf morphology; net photosynthetic rate; *Quercus ilex*.

Introduction

The leaf expansion process is highly dependent on environmental conditions, *i.e.*, photosynthetic photon flux density (PPFD), air temperature and soil water status (Tardieu *et al.* 1999, Cookson *et al.* 2005). The maximum leaf expansion rate may be considered a stable parameter, and it is genetically controlled (Tardieu *et al.* 1999). The genetic control functions act via various sensory systems to regulate the metabolic reactions controlling growth at cellular level (Van Volkenburgh 1999). It is not well understood how the intrinsic genetic control functions, and which developmental regulators are involved (Cookson *et al.* 2005). Leaf expansion itself is an integrating behaviour, that ultimately determines the canopy development and function, determining shoot/root volume, and the onset of reproduction (Van Volkenburgh 1999).

Differences in the rates of leaf area and dry mass increasing during leaf expansion cause changes in specific leaf mass area (LMA), this latter assuming a constant value at full leaf expansion (Květ *et al.* 1969, Gratani and Ghia 2002).

At anatomical level, variations in the leaf size have been attributed to the differences in cells number, cells size, or a combination of both these factors (Humphries and Wheeler 1963, Granier and Tardieu 1998, Van Volkenburgh 1999). Granier *et al.* (2000) suggest that cells number controls leaf size, and Green (1976) underlines that the level of leaf size control is at the tissue scale, rather than at the cells one. Different combinations of leaf size and cells number lead to different physiological outcomes in different environments (Pyankov *et al.* 1999). Nevertheless, there is a considerable controversy concerning the regulation of the leaf size during leaf expansion (Cookson *et al.* 2005).

The evolutionary and ecological importance has been attributed to the leaf structure of Mediterranean evergreen shrub species (Villar-Salvador *et al.* 2004, Quero *et al.* 2006). In Mediterranean ecosystems, drought, high

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Abbreviations: AbED – cell diameter of abaxial epidermis; AbEL – cell length of abaxial epidermis; AdED – cell diameter of adaxial epidermis; AdEL – cell length of adaxial epidermis; Chl – total chlorophyll content; DM – leaf dry mass; E – leaf transpiration rate; g_s – stomatal conductance; L – leaf lamina thickness; LA – leaf area; LCD – leaf cells density; LMA – leaf mass area; LWC – leaf water content; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; PD – cell diameter of palisade layer; PL – cell length of palisade layer; RGR_l – relative growth rate in leaf area; RGR_m – relative growth rate in leaf dry mass; SD – cell diameter of spongy layer, SL – cell length of spongy layer.

irradiance, and air temperature, for short or long periods, dramatically influence plant functioning (Filella *et al.* 1998, Mollá *et al.* 2006). Moreover, drought periods may differ in length and intensity, and it may coincide with different plant growth phases, which vary in their sensitivity to stress factors (Pereira and Chaves 1995, Gratani *et al.* 2000, Larcher 2003, Ogaya and Peñuelas 2007). Among the Mediterranean evergreen species, *Quercus ilex* L. is considered to represent the prototype of the Mediterranean sclerophyllous species. Holm oak is widely distributed in the Mediterranean Basin; it has a conservative water use, low cuticular transpiration rates, high capacity for osmotic adjustments, and xerophytic traits of leaves and canopy arrangement (Terradas and Savé 1992, Sala *et al.* 1994, Gratani *et al.* 2000).

The main objective of this paper was to study morphological, anatomical, and physiological leaf traits variation in *Q. ilex* during the leaf expansion process. Moreover, *Q. ilex* leaf expansion was analysed in order to test the following hypotheses: 1) changes in air temperature drive the leaf expansion process; 2) leaf traits at full lamina expansion reflect *Q. ilex* adaptation to drought.

Materials and methods

Study site and plants: The study was carried out in the period March – June 2007, on five *Q. ilex* shrubs (80 cm height), growing at the Botanical Garden of Rome.

The area climate was of Mediterranean type, and most of the total yearly rainfall (676 mm) was distributed in autumn-winter; dry period was from June to the end of August. The mean minimum air temperature of the coldest month (January) was 5.2±1.9 °C, the mean maximum air temperature of the hottest month (July) was 30.9±2.1 °C and the mean annual air temperature was 16.9±0.6 °C (data of the Collegio Romano Meteorological Station, for the period 1995–2006). During the study period the mean air temperature was 19.7±4.1 °C and the total rainfall 113 mm.

At the beginning of the growing season (end of March 2007) five shoots per each of the selected shrubs were labelled with nylon tape to the nearest 1mm from the top to the end of the growth tip. The selected shoots were monitored during the leaf expansion period. Measurements of leaf morphology, anatomy and physiology were carried out 7, 14, 21, 28, 56, 84, and 91 days after bud break, until the full leaf expansion was reached. Sun leaves from the south-facing outer part of the selected shrubs were considered; they represented 80 % of the total foliage of a typical *Q. ilex* shrub (Gratani *et al.* 2003).

Leaf anatomy: Leaf sections were hand-cut from the collected leaves (five leaves per shrub) on each sampling day, and they were analysed by light microscopy (Bolhar-Nordenkampf and Draxler 1993). Measurements were restricted to free-vein areas.

The following parameters were measured: leaf lamina thickness (L), palisade and spongy layer thickness, thickness of the adaxial and abaxial epidermis, thickness of the adaxial and abaxial cuticle.

Cells density was measured for each cells layer on each sampling occasion, as the cells number per unit of leaf section area (total number of cells per mm^2), according to Gratani and Bombelli (1999). Total leaf cells density (LCD, mean of epidermis, palisade and spongy layers) was calculated.

Cells diameter and the length of the abaxial epidermis (AbED and AbEL, respectively) and adaxial epidermis (AdED and AdEL, respectively), palisade layer (PD and PL, respectively) and spongy layer (SD and SL, respectively) were measured.

Leaf morphology: Measurements of leaf samples (five leaves per shrub) included: leaf area (LA), excluding the petiole, measured by *Image Analysis System* (*Delta-T Devices*, England); leaf dry mass (DM), oven-dried at 90 °C to constant mass; leaf mass area (LMA), *i.e.* the ratio of leaf dry mass to leaf area.

Relative growth rate (RGR) was defined as the rate of the increase in leaf dry mass (RGR_m, Fisher 1920), and in leaf area (RGR_l, Bazzaz and Harper 1977), at any instant in time (during the leaf expansion period).

Leaf gas exchange and leaf water content (LWC): Field measurements were carried out *in situ* on five sun leaves per shrub on each sampling occasion, under natural conditions, on cloud-free days [PAR > 1000 μmol(photon) m^{-2} s^{-1}]. Leaves were retained in their natural orientation during measurements. PAR [μmol(photon) m^{-2} s^{-1}], net photosynthetic rate [P_N, μmol(CO_2) m^{-2} s^{-1}], leaf transpiration rate [E, mmol(H_2O) m^{-2} s^{-1}] and stomatal conductance [g_s, mol(H_2O) m^{-2} s^{-1}] were monitored by an infrared gas analyser, *LCA-4* (*ADC Bioscientific*, Hoddesdon, UK) open system.

LWC was calculated as (leaf fresh mass – leaf dry mass)/ leaf fresh mass × 100 (Cappelletti 1954).

Chl was measured by a *SPAD-502* (*Konica Minolta Sensing, Inc*., Osaka, Japan). Measurements were carried out *in situ*, after cleaning adaxial surface dust from each leaf (three leaves per shrub), and three readings per leaf were averaged to account for within-leaf variation (Sadras *et al.* 2000).

Chl SPAD readings were converted to Chl per fresh mass by the equation regression for the same species (Gratani 1992).

Statistical analysis: All statistical tests were performed using a statistical software package (*Statistica, Statsoft*, Tulsa, USA). Differences in the morphological and physiological leaf variables were determined by the analysis of variance (*ANOVA*) and Tuckey test for multiple comparisons. The data presented in the text are means ± SD.

The correlation analysis among the considered variables was carried out. Correlations between T_m and both RGR_l and RGR_m were also carried out.

Moreover, physiological (Chl, g_s, P_N), morphological (DM, LA), and anatomical (AbED, AbEL, AdED, AdEL, L, LCD, PD, PL, SD, SL) leaf traits, measured at 7, 14, 21, 28, 56, 84 and 91 days after bud break were subjected to step-wise discriminant analysis. At each step, all traits were reviewed in order to evaluate which one contributed most to the discrimination during the leaf expansion process, according to Nevo *et al.* (2000), and Menalled and Kelty (2001).

Results

Leaf growth dynamics and leaf morphology: Bud break occurred on the 26th March, and the beginning of shoot elongation was immediately followed by leaf expansion.

LA and DM increased significantly (p<0.001) until full leaf expansion was reached (11.0±1.6 cm^2 and 162.4±29.4 mg, LA and DM, respectively) (Fig. 1).

LMA increased from bud break to full leaf expansion (15.1±0.6 mg cm^{-2}) (Fig. 1).

The highest RGR_l and RGR_m were measured 7 days after bud break (0.413 cm^2 cm^{-2} d^{-1} and 0.709 mg mg^{-1} d^{-1}, respectively) (Fig. 2).

The leaf expansion duration was 84±2 days, and it took place within the range of the air temperature 13.3±0.8 to 27.6±0.9 °C.

Leaf anatomy: The leaf anatomical traits of *Q. ilex* during leaf expansion are shown in Table 2. L increased from the beginning of the process to 56 days after bud break (262.4±1.9 μm). Palisade parenchyma thickness, and adaxial and abaxial epidermis thickness increased from bud break to full leaf expansion (124.0±3.1, 21.2±1.2 and 13.8±0.8 μm, respectively), while spongy parenchyma did until 28 days after bud break (134.7±4.9 μm), then it decreased by 18 % up to 56 days after bud break.

Palisade and spongy parenchyma cells density attained the highest values 7 days after bud break (4190±381 and 8094±522 cells mm^{-2}, respectively), decreasing by 61 % and 77 %, respectively, until full leaf expansion was reached.

The highest cell density of the adaxial and abaxial epidermis (7011±527 and 10647±819 cells mm^{-2}, respectively) was measured 7 days after bud break, then it decreased by 56 % until full leaf expansion.

PL and SL varied during leaf expansion, showing the maximum values (67.2±0.9 and 25.3±2.3 μm, respectively, p<0.05) 56 days after bud break.

PD and SD were 8.9±0.9 and 10.2±0.2 μm, respectively, 7 days after bud break, increasing by 32 and 62 %,

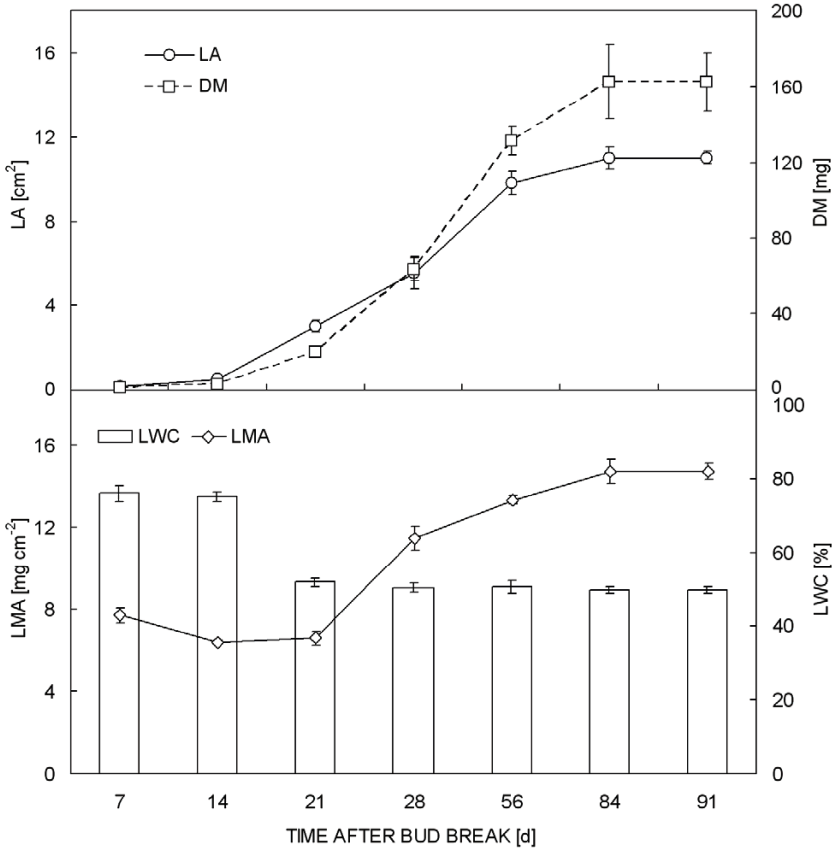

Fig. 1. Time course of the leaf area (LA), leaf dry mass (DM), leaf mass area (LMA), and leaf water content (LWC) during *Quercus ilex* leaf expansion. Means ±SD , n = 25.

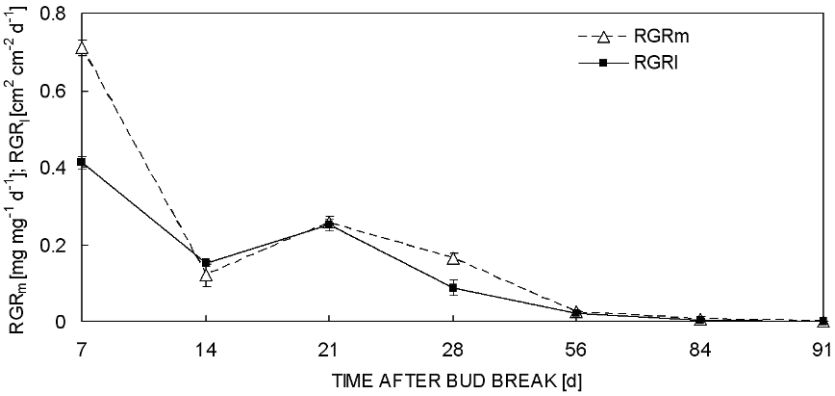

Fig. 2. Time course of the relative growth rate in leaf dry mass (RGR_m) and in leaf area (RGR_l) during *Quercus ilex* the leaf expansion. Means ±SD, $n = 25$.

respectively ($p<0.05$), until full leaf expansion. AdED and AbED were 8.2±0.8 and 5.9±0.4 μm, respectively, 7 days after bud break, increasing by 101 and 109 % respectively, until full leaf expansion.

At full leaf expansion, the palisade tissue comprised 2 layers. The total mesophyll thickness was 86 % of the total leaf thickness, and the ratio of palisade to mesophyll thickness was 0.55±0.02.

LA during leaf expansion was significantly ($p<0.001$) and negatively correlated to LCD. L was significantly ($p<0.001$) and positively correlated to LA, and it was negatively correlated to LCD (Table 1).

Leaf gas exchange: P_N and g_s rates varied during leaf expansion, increasing from 1.03±0.28 μmol(CO_2) m^{-2} s^{-1} and 0.001 mol(H_2O) m^{-2} s^{-1}, respectively (7 days after bud break), to 12.62±1.65 μmol(CO_2) m^{-2} s^{-1} and 0.090 mol(H_2O) m^{-2} s^{-1}, respectively (56 days after bud break).

E showed the same trend as P_N, with the highest value [3.68 mmol(H_2O) m^{-2} s^{-1}] 56 days after bud break.

LWC had the significantly highest values (76±2) at the beginning of the process.

There were significant ($p<0.001$) correlations between P_N and g_s, and between *E* and g_s (Table 1).

Chl content increased linearly from bud break up to a maximum (1.03±0.08 mg g^{-1}) 56 days after (Fig. 3). Chl was directly correlated to P_N and DM ($p<0.001$) (Table 1).

Discriminant analysis: The discriminant analysis showed that DM, P_N, LCD, LA, PL, and L were the most discriminating ($p<0.05$) traits of *Q. ilex* leaf expansion process (Table 3).

Table 1. Summary of the regression analysis among the considered *Quercus ilex* leaf traits (n=150), collected during the leaf expansion period. LA – leaf area [cm^2]; DM – [dry mass, mg]; LCD – leaf cells density [cells mm^{-2}]; L – leaf lamina thickness [μm]; P_N – net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; g_s – stomatal conductance [mol(H_2O) m^{-2} s^{-1}]; Chl – total chlorophyll content [mg g^{-1} of fresh mass]; *E* – leaf transpiration rate [mmol(H_2O) m^{-2} s^{-1}]. The correlations were significant at $p<0.001$. Time – days after bud break.

y – x	Relationship	*r*
LA – DM	$y = 0.069 x + 0.779$	0.98
LA – LCD	$y = 5E + 10 x^{-2.9}$	0.97
L – LA	$y = 152.2 x^{0.246}$	0.98
L – LCD	$y = -0.028 x + 318.4$	0.97
P_N – g_s	$y = 116.0 x + 0.742$	0.95
P_N – Chl	$y = 11.86 x - 1.404$	0.93
Chl – DM	$y = 0.005 x + 0.213$	0.94
E – g_s	$y = 31.10 x + 0.431$	0.91

Discussion

Air temperature is one of the most important environmental factors acting on the leaf expansion process (Van Volkenburgh 1999, Sun *et al.* 2006). *Q. ilex* bud break occurs at the end of March, when the air temperature is 13.3±0.8 °C, and leaf expansion finishes when the air temperature is 27.6±0.9 °C.

The highest LWC (76±2%) drives the beginning of leaf expansion, presumably mediated by turgor changes (Frensch 1997, Hsiao *et al.* 1998), and according to the results of Sobrado (2008). RGR_l and RGR_m are the highest (0.413 cm^2 cm^{-2} d^{-1} and 0.709 mg mg^{-1} d^{-1}, respectively) at the beginning of the process, according to Gratani and Ghia (2002) for other Mediterranean species.

P_N and g_s increase from the beginning of the process to 56 days after bud break [12.62±1.65 μmol (CO_2) m^{-2} s^{-1} and 0.090 mol (H_2O) m^{-2} s^{-1}, respectively], according to the results of Zhang et al. (2008) for other species. *E* has the highest value [3.68 mmol (H_2O) m^{-2} s^{-1}] in the same period, confirmed also by the significant ($p<0.001$) correlation between *E* and g_s. Chl has the same trend as P_N, as in most dicotyledonous species, according to the results of Choinski *et al.* (2003), and it is confirmed by the significant ($p<0.001$) correlation between the two variables.

At the anatomical level, the palisade parenchyma thickness increases from the beginning of the process up to full leaf expansion (124.0±3.1 μm), while the spongy parenchyma one up to 28 days after bud break. Moreover, the results underline that leaf area variations during leaf expansion can be attributed mostly to differences in the

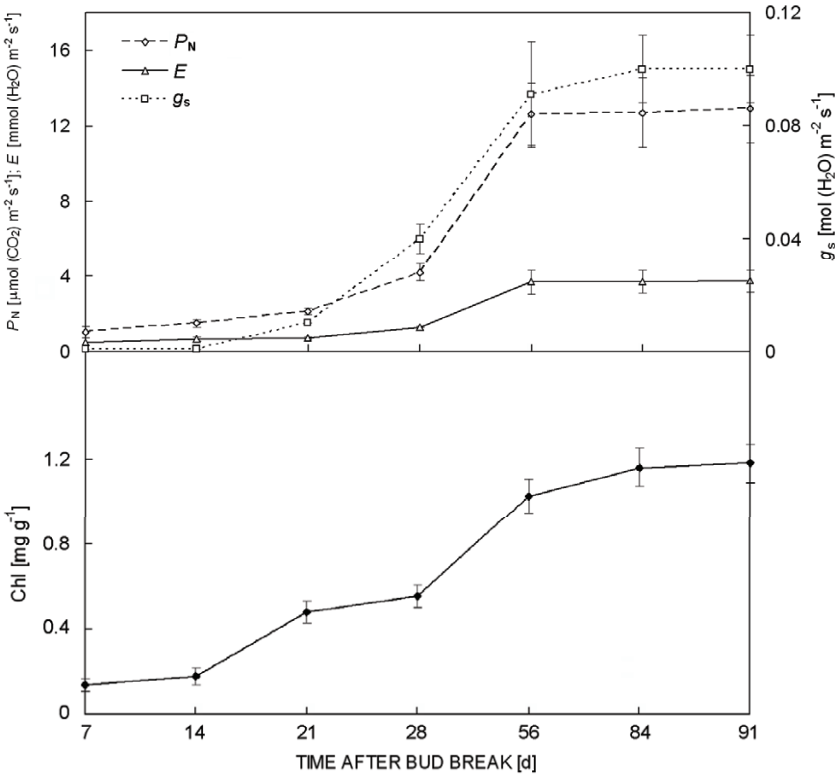

Fig. 3. Time course of the net photosynthetic rate (P_N), leaf transpiration rate (E), stomatal conductance (g_s), and total chlorophyll content (Chl) during *Quercus ilex* leaf expansion. Means ±SD, n = 25.

Table 2. Anatomical leaf traits of *Quercus ilex* during the leaf expansion process. Means ±SD are shown, n=25. In each row, means with the same letters are not significantly different (*ANOVA*, p>0.05). * = at full leaf expansion, means of adaxial and abaxial cuticle are significantly different (*ANOVA*, p<0.001).** = adaxial and abaxial epidermis thickness correspond to the adaxial and abaxial epidermis cells length, respectively.

	Time after bud break [d]					
Anatomical leaf traits	7	14	21	28	56	84
Total lamina thickness [µm]	100.7±6.4 a	106.6±7.8 a	232.4±13.7 b	255.3±4.7 c	262.4±1.9 d	263.1±1.5 d
Adaxial cuticle thickness [µm]	–	–	–	2.4±0.4 a	3.6±0.3 b	3.8±0.2 b *
Adaxial epidermis thickness** [µm]	13.6±1.3 a	14.0±0.5 a	15.1±0.8 ab	16.0±0.2 b	18.5±0.5 c	21.2±1.2 d
Palisade parenchyma thickness [µm]	26.3±0.7 a	26.8±0.9 a	85.6±5.1 b	88.7±4.9 b	116.4±3.6 c	124.0±3.1 d
Number of palisade cells layers	1	1	2	2	2	2
Spongy parenchhyma thickness [µm]	50.1±5.4 a	53.4±5.1 a	119.8±5.7 b	134.7±4.9 c	108.8±4.4 d	101.2±4.8 d
Mesophyll thickness [µm]	76.4±6.2 a	80.2±5.9 a	205.4±10.1 b	223.4±11.2 bc	225.2±7.2 c	225.1±8.7 c
Ratio palisade parenchyma/mesophyll	0.34±0.02 a	0.33±0.03 a	0.42±0.02 b	0.40±0.02 b	0.52±0.03 c	0.55±0.02 c
Ratio palisade/spongy parenchyma	0.52±0.02 a	0.50±0.02 a	0.71±0.03 c	0.66±0.03 c	1.07±0.02 d	1.23±0.03 e
Abaxial epidermis thickness** [µm]	9.9±0.5 a	10.1±0.4 a	11.1±0.5 b	11.5±0.3 b	12.5±0.3 c	13.8±0.8 d
Abaxial cuticle thickness [µm]	–	–	–	1.8±0.3 a	2.4±0.2 b	2.6±0.2 b *
Ratio adaxial/abaxial epidermis thickness	1.37±0.06 a	1.39±0.05 a	1.36±0.06 a	1.39±0.03 a	1.48±0.04 b	1.54±0.08 b
Palisade parenchyma cells length [µm]	26.3±0.7 a	26.8±0.9 a	45.6±2.3 b	57.8±2.3 c	67.2±0.9 d	68.1±1.6 d
Spongy parenchyma cells length [µm]	13.7±1.1 a	15.1±1.7 a	20.1±1.3 b	23.4±0.8 c	25.3±2.3 cd	27.9±1.7 d
Adaxial epidermis cells diameter [µm]	8.2±0.8 a	8.8±0.6 a	12.8±0.7 b	13.6±1.1 b	14.1±1.3 bc	16.5±1.3 c
Palisade parenchyma cells diameter [µm]	8.9±0.9 a	9.6±1.1 ab	9.7±0.8 ab	9.9±0.7 ab	10.7±0.8 bc	11.7±0.7 c
Spongy parenchyma cells diameter [µm]	10.2±0.2 a	10.9±0.9 ab	11.7±1.4 abc	12.5±1.1 bc	13.8±1.3 cd	16.5±2.1 d
Abaxial epidermis cells diameter [µm]	5.9±0.4 a	6.1±0.9 a	9.4±1.8 b	10.0±1.1 b	11.1±0.3 bc	12.3±0.9 c
Adaxial epidermis cells density [cells mm^{-2}]	7 011±527 a	6 064±505 a	4 134±459 b	3 784±270 b	3 679±255 b	3 079±154 c
Palisade parenchyma cells density [cells mm^{-2}]	4 190±381 a	3 827±383 a	2 394±83 b	2 050±46 c	1 863±60 d	1 625±54 e
Spongy parenchyma cells density [cells mm^{-2}]	8 094±522a	7 719±429 a	2 917±142 b	2 488±88 c	2 317±177 c	1 858±52 d
Abaxial epidermis cells density [cells mm^{-2}]	10 647±819 a	10 000±1000 a	7 765±480 b	7 098±789 b	6 522±725 b	4 688±212 c
Leaf cells density [cells mm^{-2}]	7 801±213 a	7 160±181 b	3 087±142 c	2 707±110 d	2 555±100 d	2 028±100 e

Table 3. Results of the stepwise discriminant analysis based on the considered *Quercus ilex* leaf traits: DM – dry mass [mg]; P_N –net photosynthetic rate [μmol (CO_2) $m^{-2}s^{-1}$]; LCD – leaf cells density [cells mm^{-2}]; LA – leaf area [cm^2]; PL – palisade cells length [μm]; L – leaf lamina thickness [μm]; SL – spongy cells length [μm]; AdEL – adaxial epidermis cells length [μm]; AbED – abaxial epidermis cells diameter [μm]; Chl – total chlorophyll content [mg g^{-1} of fresh mass]; AbEL – abaxial epidermis cells length [μm]; g_s – stomatal conductance, [mol(H_2O) m^{-2} s^{-1}]; SD – spongy cells diameter [μm]. AdED – adaxial epidermis cells diameter [μm] PD – palisade cells diameter [μm]. N = number in the model.

Traits	N	Wilk's partial	r^2	F	*p*-level
DM	1	0.091	0.97	27.83	$8.4\ 10^{-7}$
P_N	2	0.102	0.67	24.70	$1.8\ 10^{-6}$
LCD	3	0.138	0.47	17.42	$1.4\ 10^{-5}$
LA	4	0.166	0.97	14.03	$5.0\ 10^{-5}$
PL	5	0.419	0.52	3.87	0.0205
L	6	0.472	0.50	3.13	0.0420
SL	7	0.516	0.70	2.63	0.0707
AdEL	8	0.628	0.62	1.66	0.2088
AbED	9	0.697	0.68	1.22	0.3517
Chl	10	0.746	0.57	0.95	0.4792
AbEL	11	0.749	0.68	0.94	0.4871
g_s	12	0.769	0.33	0.84	0.5439
SD	13	0.780	0.35	0.79	0.5728
AdED	14	0.860	0.63	0.46	0.8016
PD	15	0.862	0.50	0.45	0.8065

leaf cells density and cells length, and it is confirmed by the significant (p<0.001) correlation between the two variables. In particular, cells density has its maximum value at the beginning of the process (7801±213 cells mm^{-2}), decreasing by 74 % until full leaf expansion, when the leaf lamina thickness increases to 161 % of its initial value.

Considering *Q. ilex* fully expanded leaves, the well developed palisade parenchyma (two layers), and the ratio palisade/spongy parenchyma (1.23±0.03) are the typical ones of sun leaves, according to the results of Dengler (1980). The ratio of the upper to the lower epidermis thickness (1.54±0.08), and the total leaf lamina thickness (263.1±1.5 μm) are within the range monitored for Mediterranean evergreen species (Gratani and Bombelli 1999, Gratani and Varone 2004, Ogaya and Peñuelas 2006).

The ratio of palisade parenchyma thickness to mesophyll thickness (0.55) reveals a xeromorphic habitus, according to Christodoulakis and Mitrakos (1987).

Q. ilex LMA (15.1±0.6 mg cm^{-2}) at full leaf expansion is in the range of Mediterranean species (Oliveira and Penuelas 2004, Gratani and Varone 2006, Ogaya and Peñuelas 2007), and it is indicative of compact leaves (2028±100 cells per mm^2, leaf cells density at full leaf expansion). LMA is picked up as a useful integrator of thickness and density – a measure of investment per unit of leaf area (Aranda *et al*. 2004). High LMA is a recurrent plant trait in the Mediterranean region, having a protective function useful in facing drought stress, and it results from the selection for increased leaf longevity (three years in *Q. ilex*, Gratani and Crescente 1997), under situations of resource shortage (Turner 1994, Salleo and Nardini 2000).

The high LMA (*i.e*., high biomass allocation to leaves) accounts for the lower RGR of evergreen species compared to deciduous ones (Cornelissen *et al.* 1998, Antúnez *et al.* 2001). For plants growing in an environment where water is available for limited periods of the year. It is important to be able to take full advantage of favourable conditions for leaf expansion (Gratani and Ghia 2002), maximizing CO_2 assimilation and minimising leaf loss (Westoby *et al.*, 2002; Sun *et al.*, 2006). The long leaf expansion period (84 days) of *Q. ilex* as to other Mediterranean species (Gratani and Bombelli 1999, Gratani and Ghia 2002) is justified by the high leaf consistency, allowing the convection of excess heat under condition of water stress and high irradiance, and limiting water loss by transpiration. Moreover, newly initiated leaves are often exposed to full irradiance (Jiang *et al.* 2006), particularly in the Mediterranean climate, where high irradiance is associated with high air temperatures in spring and summer. *Q. ilex* leaf expansion reaches 90 % of its definitive structure before the most severe drought period (beginning of June – end of August). Moreover, photoprotective mechanisms are very active during early stages of the leaf expansion allowing the leaf to cope with high irradiance (Yoo *et al.* 2003, Jiang *et al.* 2006); leaf production of monoterpenes in *Q. ilex* (Delwiche and Sharkey 1993, Loreto *et al.* 1996) and leaf polyphenols deposition (Karabourniotis *et al.* 1998) serve as a sink for the excess of photochemical energy (Osmond *et al.* 1982), preventing photoinhibition damage to the photosynthetic system (Werner *et al.* 1999). *Q. ilex* steep leaf inclination angle (Gratani and Bombelli 1999) is an additional preventive mechanism against the potential photoinhibition.

In the light of the above considerations, the correlation among physiological, morphological and anatomical leaf traits during leaf expansion, reflects *Q. ilex* adaptability to the Mediterranean climate. Global climate effects on the Mediterranean Basin are likely to provide more and stronger drought periods. Air temperature increase might shorten the favourable period for leaf expansion, thus changing the final amount of biomass per unit leaf area of *Q. ilex*.

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