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Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions

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Abstract Sun and shade leaves of two Mediterranean *Quercus* species, *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L., were compared by measuring leaf optical properties, photosynthetic pigment composition and photosystem II efficiency. The presence of trichomes in the adaxial (upper) leaf surface of *Q. ilex* subsp. *ballota* seems to constitute an important morphological mechanism that allows this species to maintain a good photosystem II efficiency during the summer. *Q. coccifera* has almost no trichomes and seems instead to develop other physiological responses, including a smaller light-harvesting antenna size, higher concentrations of violaxanthin cycle pigments and a higher (zeaxanthin + antheraxanthin)/(violaxanthin + antheraxanthin + zeaxanthin) ratio. *Q. coccifera* was not able to maintain a good photosystem II efficiency up to the end of the summer. In *Q. ilex* subsp. *ballota* leaves, natural loss or mechanical removal of adaxial-face leaf trichomes induced short-term decreases in photosystem II efficiency. These changes were accompanied by de-epoxidation of violaxanthin cycle pigments, suggesting that the absence of trichomes would trigger physiological responses in this species. Our data have revealed different patterns of response of *Q. ilex* subsp. *ballota* and *Q. coccifera* facing the stress conditions prevailing in the Mediterranean area.

Keywords Adaxial epidermis · Photoprotection · *Quercus* · Trichomes

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Abbreviations F_o: Chl fluorescence with all PS II reaction centres open · F_{pl} and F_p: Chl fluorescence intensity at the plateau and the peak of the continuous fluorescence induction curve, respectively · F_v: variable part of Chl fluorescence (F_p–F_o)

Introduction

Many studies have focused on the ecological significance of leaf pubescence, and especially on its effects on leaf optical properties (Johnson 1975). It has been reported that the leaf absorptance of solar radiation is diminished by pubescence, mainly due to an increase in leaf reflectance (Ehleringer 1984). Such morphological variation would be accompanied by lower leaf temperatures and lower rates of water loss (Ehleringer et al. 1976, 1981; Ehleringer and Björkman 1978; Ehleringer and Mooney 1978; Ehleringer 1981; Pérez-Estrada et al. 2000). It has been suggested that all these changes may constitute an adaptive behaviour in arid environments (Billings and Morris 1951).

Theoretical reasons and empirical evidence suggest that the role of trichomes in water conservation may be less important than their protective function against excessive UV-B radiation (Grammatikopoulos et al. 1994; Karabourniotis and Bornman 1999). In fact, isolated trichomes absorb strongly UV-B light (Karabourniotis et al. 1992), possibly due to the presence of polyphenols in the trichome cell wall (Karabourniotis et al. 1998). Also, a marked reduction in the efficiency of photosystem II, analysed by chlorophyll (Chl) fluorescence induction, has been recorded after exposing artificially de-haired leaves to UV-B radiation (Karabourniotis et al. 1993, 1994).

Other mechanisms such as changes in photosynthetic pigment composition have been proposed to contribute to the adaptation of Mediterranean plants to excessive radiation (Chaves et al. 1997; García-Plazaola et al. 1999). The xanthophyll cycle pigments, particularly antheraxanthin (A) and zeaxanthin (Z), play an important role in the protection of the photosynthetic apparatus from adverse conditions, by dissipating thermally excess light. The de-epoxidation from violaxanthin (V) to A and Z and the net synthesis of Z seem to contribute to the protection of the photosynthetic apparatus in the short and long term respectively (Demmig et al. 1987; Demmig-Adams 1990; Demmig-Adams and Adams 1992; Gilmore and Yamamoto 1993). Both an increase in VAZ pigment leaf concentrations and an increased A+Z/VAZ ratio would be markers of increased excitation pressure. For instance, in *Quercus subpyrenaica* E.H. del Villar the VAZ/Chl ratio was 2-fold higher in sun than in shade leaves (Abadía et al. 1996). Also, Balaguer et al. (2001), working with *Q. coccifera* plants grown in a tree nursery, reported higher VAZ/Chl ratios in sun than in shade leaves, although changes were much less marked than in the case of *Q. subpyrenaica*.

The aim of this work was to investigate the adaptation mechanisms that occur in two different Mediterranean plants, *Q. ilex* subsp. *ballota* and *Q. coccifera*, in response to high light in the field. Both oak species are typical constituents of the Mediterranean woodlands through the Iberian Peninsula, and settle in very close phyto-climatic conditions (Allué-Andrade 1990). Leaves of *Q. ilex* subsp. *ballota* are covered by a layer of trichomes on both leaf sides, which may constitute effective protection against excessive UV-B radiation (Skaltsa et al. 1994; Liakoura et al. 1997). On the other hand, leaves of *Q. coccifera* are very similar in size, shape and consistence to those of *Q. ilex* subsp. *ballota*, but they are glabrous (Franco 1990). We have compared sun and shade leaves of *Q. coccifera* and *Q. ilex* subsp. *ballota*, by investigating several leaf characteristics, including optical properties such as absorptance, reflectance and transmittance in the visible region, photosynthetic pigment composition, including pigments within the xanthophyll cycle, and photosystem II efficiency by measuring Chl fluorescence rapid kinetics at room temperature.

Materials and methods

Plant material and trichome removal

Sun and shade leaves of kermes oak (*Quercus coccifera*) and holm oak (*Quercus ilex* subsp. *ballota*), growing under natural conditions in the Mediterranean area in north-east Spain (Santa Cilia de Jaca, Huesca, Spain; 42°34′ N, 0°43′ W, 720 m a.s.l.), were used in this study. Botanical names of the species follow Franco (1990). Leaf trichomes from the adaxial face of *Q. ilex* subsp. *ballota* leaves were removed in the field on 27 August, 6 and 9 September in 1991 by gentle rubbing with a soft cotton cloth, under no pressure and with no abrasion, until leaf pubescence disappeared. Under such conditions, there was no mechanical damage to the leaf cuticle (not shown). All leaves, de-haired or not, were exposed to natural sunlight. All measurements were made in five selected individuals of each species between July and October 1991. *Q. coccifera* and *Q. ilex* subsp. *ballota* were at the same microsite (at distances of a few metres), having the same water availability and radiation.

Absorptance measurements

Absorptance was measured with a Shimadzu UV-3000 spectrophotometer equipped with an integration sphere accessory. The experimental set-up was as described in Morales et al. (1991). This device has two sample holders at the entrance of the light into the sphere, one for the reference and the other for the sample beam. At the place where the sample light beam intersects with the other side of the sphere (R) there is a third holder, where a plate (white or black) can be inserted. All measurements were made at 25-nm intervals from 700 to 400 nm, with the reference holder empty (air). First, a baseline was measured with the sample holder empty (air), and a white plate placed in R; baseline values were subtracted from any further measurements. The transmittance of a leaf piece (I_T/I_O) was measured by placing a leaf piece in the sample holder. The reflectance of the same leaf piece (I_R/I_O) was then measured, by leaving empty the sample holder and placing the leaf piece in R, with a black plate underneath it. Leaf reflectance values were corrected for any significant reflectance of the black plate. Absorptance (i.e., the fraction of incident light absorbed) at each wavelength was calculated as $a_\lambda = I_A/I_O = (I_O - I_T - I_R)/I_O = 1 - (I_T/I_O) - (I_R/I_O)$ (Morales et al. 1991). Measurements were carried out with previously sun-exposed leaves and sampling was performed at midday (12 hours solar time).

Chl fluorescence measurements

Continuous Chl fluorescence measurements were made with a home-made fluorometer (Morales et al. 1990) at room temperature in excised leaves in a dark room. Except when the short-term (time scale of hours; 0.5–3 h) effects of trichome removal were investigated, leaves were excised at midday (12 hours solar time), enclosed in a plastic bag and transported to the laboratory in darkness in a refrigerated case. Fluorescence induction curves were measured at the end of a 2-h period of darkness (including the transportation time) with the experimental set-up described previously (Morales et al. 1991). The measurement photosynthetic photon flux density (PPFD) was 150 μmol m^{-2} s^{-1} at the leaf level. Chl fluorescence was monitored for 2 s.

Two different Chl fluorescence parameters in dark-adapted leaves were measured: the F_v/F_p ratio, which provides an estimation of the PSII efficiency, and the $(F_{pl}-F_o)/F_v$ ratio, which measures the proportion of variable fluorescence leading to the F_{pl} point in the Kautsky induction curve (Morales et al. 1991).

Pigment analysis

Samples were collected at solar noon on clear days (except 7 September, which was cloudy). Leaf disks were taken from leaves in the field with a cork borer and immediately frozen in liquid nitrogen. Pigments were later extracted with pure acetone in the presence of sodium ascorbate, stored at –20°C and analysed by HPLC as described previously (De las Rivas et al. 1989; Abadía and Abadía 1993).

Meteorological data

Temperature, rainfall and radiation data were obtained from the nearest meteorological station to the experimental site, situated in Jaca (Huesca, Spain), approximately 8 km to the north and 100 m higher than the experimental stand. The weather during the experimental period was relatively mild (Fig. 1). At the Jaca meteorological station, minimum and maximum temperatures were approximately 15°C and 30°C respectively (Fig. 1A). At the end of the experimental period temperatures decreased (Fig. 1A), there were some days with significant rainfall (Fig. 1B) and radiation, although tending to decrease, was still high (Fig. 1C).

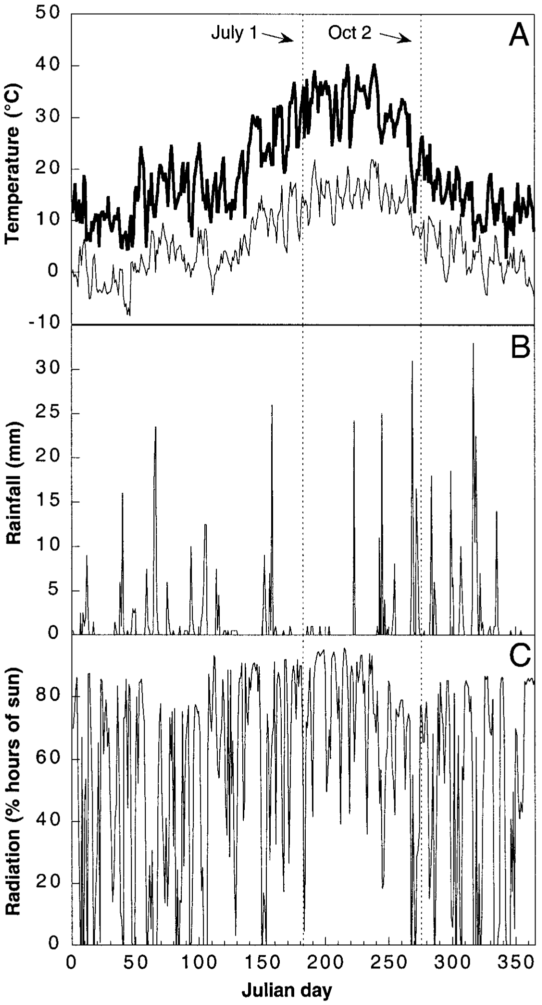

Fig. 1 Changes in maximum and minimum daily temperatures (**A**), rainfall (**B**) and radiation (**C**) during 1991 at the Jaca (Huesca, Spain) meteorological station. The experimental period (from 1 July to 2 October) is marked by two dotted lines

Results

Optical properties of kermes oak and holm oak leaves: effect of leaf trichome removal

The fractions of incident light absorbed (absorptance), reflected (reflectance) and transmitted (transmittance) were wavelength-dependent (Fig. 2). Leaves of both species had absorption maxima in the blue (Chls and carotenoids) and red (Chls) part of the visible spectrum (Fig. 2A, B). Reflectance was larger throughout the whole visible spectrum in *Q. ilex* subsp. *ballota* (approximately 20% of the incident light; Fig. 2B) than in *Q. coccifera* (approximately 15% of the incident light; Fig. 2A). *Q. coccifera* had a higher transmittance (Fig. 2A) than *Q. ilex* subsp. *ballota* (Fig. 2B) with maxima at 550 and 700 nm.

Mechanical removal of the adaxial-face leaf trichomes in *Q. ilex* subsp. *ballota* caused a major decrease in reflectance, from 20% to 15% of the incident light (Fig. 2C). After trichome removal, *Q. coccifera* and *Q. ilex* subsp. *ballota* had practically identical leaf reflectance values throughout the spectrum (Fig. 2C).

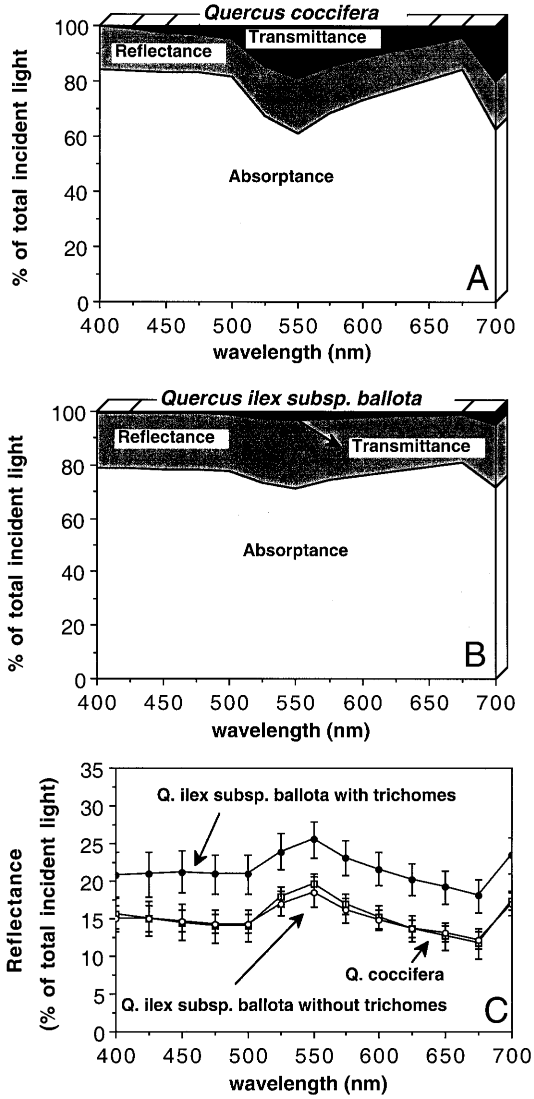

Fig. 2 Fractions of total incident light absorbed (absorptance), reflected (reflectance) and transmitted (transmittance) in *Quercus coccifera* (**A**) and *Quercus ilex* subsp. *ballota* leaves (**B**), and comparison of the reflectance in *Quercus coccifera* leaves (*open squares*) with those of *Quercus ilex* subsp. *ballota* leaves before (*solid circles*) and after (*open circles*) trichome removal (**C**; mean±SD of 5–6 measurements). Sun-exposed leaves sampled at midday (12 hours solar time) were used. See Materials and methods for experimental details

Chl fluorescence of kermes oak and holm oak leaves: effect of leaf trichome removal

The F_v/F_p ratio after 2 h of dark adaptation remained quite constant during the summer in sun leaves of *Q. ilex* subsp. *ballota*. These leaves had F_v/F_p ratios of approxi-

Table 1 Photosystem II efficiency (F_v/F_p) and $(F_{pl}-F_o)/F_v$ ratios in shade and sun leaves of *Quercus coccifera* and *Q. ilex* subsp. *ballota* at different sampling dates. Samples were taken at midday (12 hours solar time). Data are mean±SD of between 2 and 30 measurements. Data in the same column followed by the same letter were not significantly different (Student's *t* test) at the $P \leq 0.05$ probability level. If shade leaves were compared to sun leaves (statistical analysis not shown in the table), significant differences were obtained only for the F_v/F_p ratios on 10 and 13 September

	Date	*Q. coccifera*		*Q. ilex* subsp. *ballota*	
		Shade	Sun	Shade	Sun
F_v/F_p	1 July	0.80±0.04 a	0.78±0.01 b	–	–
	27 August	–	–	0.72±0.01	0.75±0.05 c
	6 September	–	0.70±0.03 a	–	0.80±0.02 c
	9 September	–	–	–	0.79±0.02 c
	10 September	0.81±0.04 a	0.64±0.16 a	–	0.81±0.01 c
	13 September	0.76±0.05 a	0.66±0.09 a	–	0.74±0.01 b
	2 October	–	–	–	0.71±0.03 a
$(F_{pl}-F_o)/F_v$	1 July	0.21±0.01 b	0.23±0.06 a	–	–
	27 August	–	–	0.35±0.02	0.35±0.02 d
	6 September	–	0.29±0.05 b	–	0.16±0.02 a
	9 September	–	–	–	0.15±0.02 a
	10 September	0.20±0.05 a	0.19±0.07 ab	–	0.16±0.01 a
	13 September	0.19±0.03 a	0.20±0.03 a	–	0.20±0.02 b
	2 October	–	–	–	0.26±0.05 c

Table 2 Photosystem II efficiency (F_v/F_p) and $(F_{pl}-F_o)/F_v$ ratios in sun leaves of *Quercus ilex* subsp. *ballota* before and after trichome removal at different sampling dates. Data are mean±SD of between 2 and 10 measurements. Data in the same column followed by the same letter were not significantly different (Student's *t* test) at the $P \leq 0.05$ probability level

Date	Time after trichome removal	F_v/F_p	$(F_{pl}-F_o)/F_v$
27 August	0	0.75±0.05 a	0.35±0.02 a
	30 min	0.74±0.06 a	0.38±0.05 a
	1 h	0.64±0.04 a	0.43±0.02 a
	2 h	0.55±0.13 a	0.52±0.11 a
	3 h	0.62±0.01 a	0.47±0.05 a
6 September	0	0.80±0.02 b	0.16±0.02 a
	3 days	0.73±0.02 a	0.19±0.02 ab
	7 days	0.73±0.02 a	0.21±0.03 b
9 September	0	0.79±0.02 b	0.15±0.02 a
	3 h	0.77±0.01 b	0.18±0.04 a
	1 day	0.77±0.02 b	0.18±0.02 a
	23 days	0.67±0.04 a	0.29±0.03 b

mately 0.75–0.81 in the July-September period (Table 1). Only in October did these leaves have lower F_v/F_p ratios of 0.71 (Table 1). In sun-exposed leaves of *Q. coccifera*, however, F_v/F_p ratios decreased from 0.78 in July to 0.64–0.70 in September (Table 1). For the same sampling date, the F_v/F_p ratios of *Q. coccifera* shade leaves were always higher than those found in sun-exposed leaves (Table 1). The relative magnitude of the variable part of Chl fluorescence that corresponds to F_{pl} was similar for the same sampling date in sun and shade leaves (Table 1).

Removal of the adaxial-face leaf trichomes in sun-exposed *Q. ilex* subsp. *ballota* leaves decreased the F_v/F_p ratio after 2 h of dark adaptation both in the short and long term (Table 2). Leaves de-haired in August and then exposed to full sunlight for 3 h showed decreases in F_v/F_p ratio from 0.75 to 0.5–0.6. In September, however, the same treatment did not induce major changes in F_v/F_p ratio in the short term, although in the long term F_v/F_p ratios decreased to 0.67 (Table 2). Also, trichome removal increased $(F_{pl}-F_o)/F_v$ ratios from 0.35 to approximately 0.5 in 2–3 h in August, whereas in September $(F_{pl}-F_o)/F_v$ ratio increases were only from 0.16 to 0.2–0.3 (Table 2). Long-term effects on F_v/F_p ratios might be also independent of trichome removal, since the F_v/F_p ratios decreased gradually during the summer (see Discussion).

Photosynthetic pigment composition of kermes oak and holm oak leaves: effect of leaf trichome removal

The photosynthetic pigment composition of *Q. coccifera* and *Q. ilex* subsp. *ballota* leaves was found to be remarkably different in shade- and sun-exposed leaves (Table 3). Typical molar stoichiometries were, for neoxanthin: VAZ pigments: lutein: β-carotene: Chl *b*: Chl *a*, 44: 93: 209: 154: 303: 1,000 and 31: 203: 205: 168: 260: 1,000 in shade and sun leaves of *Q. coccifera*, respectively (Table 3). In the case of *Q. ilex* subsp. *ballota* stoichiometries were 57: 88: 211: 150: 321: 1,000 and 50: 125: 244: 161: 287: 1,000 in shade and sun leaves, respectively (Table 3). Shade leaves in both species had similar VAZ concentrations and Chl *a*/Chl *b* ratios, and the (Z+A)/VAZ ratios were relatively low (Table 3). Sun-exposed leaves, however, had remarkably lower VAZ concentrations and (Z+A)/VAZ and Chl *a*/Chl *b* ratios in *Q. ilex* subsp. *ballota* than in *Q. coccifera* (Table 3).

Removing trichomes from the *Q. ilex* subsp. *ballota* adaxial leaf surface did not induce significant changes in

Table 3 Photosynthetic pigment composition (mmol pigment/mol Chl *a*, Chl *a*/Chl *b* and Z+A/VAZ ratios) in shade and sun leaves of *Quercus coccifera* and *Q. ilex* subsp. *ballota*. Sampling was at the beginning of July. Data are mean±SD of 3 measurements. Statistical analysis compares pairs of data (sun vs shade) within species. Data followed by the same letter were not significantly different (Student's *t* test) at the $P \le 0.05$ probability level. *ND* Not detected.

	Q. coccifera		*Q. ilex* subsp. *ballota*	
	Shade	Sun	Shade	Sun
Neoxanthin	44±8 a	31±2 a	57±3 b	50±1 a
Violaxanthin	54±4 a	18±5 a	66±6 a	61±10 a
Antheraxanthin	18±1 a	36±3 b	23±4 a	38±2 b
Zeaxanthin	22±1 a	148±8 b	ND a	26±1 b
V+A+Z	93±3 a	203±1 b	88±4 a	125±9 b
Lutein	209±1 a	205±14 a	211±7 a	244±12 b
Chl *b*	303±13 b	260±8 a	321±9 b	287±8 a
β-carotene	154±3 a	168±3 b	150±2 a	161±2 b
Chl *a*/Chl *b*	3.3±0.1 a	3.9±0.1 b	3.1±0.1 a	3.5±0.1 b
Z+A/VAZ	0.42±0.03 a	0.91±0.03 b	0.25±0.04 a	0.51±0.06 b

Table 4 Photosynthetic pigment composition (total Chl in µmol m^{-2}, Chl *a*/ Chl *b* ratio, mmol VAZ/mol Chl *a* and Z+A/VAZ ratios) in sun leaves of *Quercus ilex* subsp. *ballota* before and after trichome removal at different sampling dates. Time 0 corresponds to trichome removal at the beginning of September in 1991. Data are mean±SD of 3 measurements. Data in the same column followed by the same letter were not significantly different (Student's *t* test) at the $P \le 0.05$ probability level

	Time	Control	De-haired
Total Chl (µmol m^{-2})	0	504±52 a	504±52 a
	3 h	504±52 a	478±32 a
	1 day	561±37 a	500±50 a
	3 days	504±52 a	538±26 a
	7 days	518±5 a	521±19 a
	23 days	553±55 a	529±54 a
Chl *a*/Chl *b*	0	3.9±0.1 a	3.9±0.1 a
	3 h	3.9±0.1 a	3.7±0.2 a
	1 days	4.0±0.1 a	3.7±0.3 a
	3 days	3.9±0.1 a	3.6±0.2 a
	7 days	3.6±0.1 a	3.7±0.1 a
	23 days	3.7±0.1 a	4.0±0.2 a
mmol VAZ/mol Chl *a*	0	104±15 a	104±15 a
	3 h	104±15 a	122±26 a
	1 day	99±11 a	104±14 a
	3 days	104±15 a	108±9 a
	7 days	104±14 a	132±23 a
	23 days	100±22 a	114±13 a
Z+A/VAZ	0	0.57±0.06 a	0.57±0.06 a
	3 h	0.57±0.06 a	0.80±0.08 b
	1 day	0.14±0.01 a	0.52±0.05 b
	3 days	0.57±0.06 a	0.73±0.07 b
	7 days	0.65±0.07 a	0.76±0.08 a
	23 days	0.57±0.06 a	0.66±0.07 a

leaf total Chl concentration, Chl *a*/Chl *b* ratio and VAZ pigment/Chl *a* ratio (Table 4). However, leaves with trichomes removed always had higher (Z+A)/VAZ ratios than control leaves (Table 4).

Discussion

Leaves from *Q. coccifera* had almost no trichomes in their adaxial side and reflected 15% of the incident visible light. In contrast, leaves from *Q. ilex* subsp. *ballota* had trichomes in their adaxial side and reflected 20% of the incident light. When trichomes were mechanically removed, reflectance values of *Q. ilex* subsp. *ballota* leaves became practically identical to those of *Q. coccifera* leaves. This is in good agreement with other studies indicating that reflectance is higher in pubescent than in non-pubescent leaves (Billings and Morris 1951; Pearman 1966; Sinclair and Thomas 1970), and also that leaf trichomes could reflect 5–20% of the incident visible light in olive trees (Karabourniotis et al. 1992).

Shade leaves of *Q. coccifera* and *Q. ilex* subsp. *ballota* were similar in many ways. Typical pigment stoichiometries were similar in both cases, with VAZ/Chl *a* ratios of 0.093 and 0.088 in *Q. coccifera* and *Q. ilex* subsp. *ballota* respectively. Also, PSII efficiency was similar in both species and did not change significantly during the summer. Sun-exposed leaves of *Q. coccifera* and *Q. ilex* subsp. *ballota* had larger Chl *a*/Chl *b* ratios than those of shade leaves, suggesting a reduction in light-harvesting antenna size. This has been observed previously in other species (Thayer and Björkman 1990; Demmig-Adams and Adams 1992). Also, sun leaves of both species had higher VAZ concentrations and higher Z+A/VAZ ratios than those found in shade leaves. Similar xanthophyll cycle shade-sun responses have been reported recently in *Q. coccifera* plants, grown in a nursery from acorns collected in three contrasting environments on the Iberian Peninsula (Balaguer et al. 2001).

Data obtained for sun leaves suggest that the existence of trichomes in *Q. ilex* subsp. *ballota* leaves may constitute a protective light-filtering mechanism for the photosynthetic machinery. The Chl *a*/Chl *b* ratios of sun leaves were larger in *Q. coccifera* than in *Q. ilex* subsp. *ballota*, suggesting that the former has a smaller antenna size. Sun leaves also had higher VAZ concentrations and Z+A/VAZ ratios in *Q. coccifera* than in *Q. ilex* subsp. *ballota*, suggesting that at full sunlight *Q. coccifera* requires more xanthophyll-based photoprotection than *Q. ilex* subsp. *ballota*. Both species were also different when examining Chl fluorescence signatures, since sun leaves of the trichome-less *Q. coccifera* had marked decreases in PSII efficiency in mid-summer, whereas similar leaves of trichome-covered *Q. ilex* subsp. *ballota* had decreases in PSII efficiency only when they lost trichomes in October. These data are in line with those recently reported for two subspecies of *Lotus creticus* (Savé et al. 2000). *L. creticus* subsp. *creticus* had a higher density of trichomes on the adaxial leaf surface than *L. creticus* subsp. *cytisoides*, and this was accompanied by a decreased PSII efficiency in the less pubescent subspecies.

Removal of trichomes in *Q. ilex* subsp. *ballota* by gentle rubbing confirms the protective role of trichomes. Trichome removal did not change Chl and VAZ leaf concentrations per area and Chl *a*/Chl *b* ratios, but led to higher (Z+A)/VAZ ratios. This suggests that when trichomes are removed excitation pressure increases in the short term and the need for xanthophyll-based photoprotection increases. The xanthophyll cycle, particularly A and Z, plays an important role in the protection of the photosynthetic apparatus, by dissipating excess light thermally and regulating PSII efficiency (Horton and Ruban 1992; Ruban et al. 1994, 1997; Gilmore et al. 1995; Formaggio et al. 2001; Polivka et al. 2002). In fact, trichome removal was associated with a short-term decrease in PSII efficiency, as indicated by decreases in the F_v/F_p ratio. However, in the long term effects on F_v/F_p ratios may be independent of trichome removal, since the F_v/F_p ratios decreased in sun leaves during the summer similarly when trichomes were (Table 2) or were not (Table 1) removed (compare data of 13 September in Tables 1 and 2). Long-term decreases in the F_v/F_p ratios can be related to decreases in temperature with still relatively high levels of radiation at the end of the experimental period. Trichome removal may result in other changes in the photosynthetic apparatus, and for instance a marked (up to 50%) decrease in photosynthesis has been reported after de-hairing olive tree leaves (Grammatikopoulos et al. 1994).

We observed some trichome loss during the life span of *Q. ilex* subsp. *ballota* leaves (not shown). A natural loss of trichomes with leaf age has been reported previously in *Olea europaea*, *Cydonia oblonga* and *Eriobotrya japonica* (Karabourniotis et al. 1994). Also, trichome density and type were affected by environmental conditions in developing leaves of different *Quercus* species (Hardin 1979) and in *Wigandia urens* (Pérez-Estrada et al. 2000). This type of natural loss of trichomes is relatively slow and the protective role against excessive radiation could be taken over progressively by other mechanisms of avoidance or reparation (Karabourniotis et al. 1994; Karabourniotis and Fasseas 1996).

In summary, the presence of trichomes in the adaxial leaf surface of *Q. ilex* subsp. *ballota* could be an important mechanism to decrease susceptibility to photodamage when compared to other *Quercus* species, preserving a good PSII efficiency during the whole summer. This may be especially important in summer conditions in areas such as the Mediterranean basin, where high levels of irradiance (higher than 2,000 µmol photons m^{-2} s^{-1}) may occur and lead to an excess of light energy input. When trichomes are absent, such as in *Q. coccifera*, other physiological responses to light excess are enhanced, including a smaller light-harvesting antenna size, higher leaf VAZ pigments concentrations and high (Z+A)/VAZ ratios. This species, however, was not able to maintain a good PSII efficiency until the end of the summer. We believe that *Quercus* may be used as a model system to investigate the possible adaptive significance of pubescence and photosynthetic pigment changes in species growing under stress in Mediterranean conditions.

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