Photoprotection mechanisms in *Quercus ilex* under contrasting climatic conditions

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A R T I C L E   I N F O

A B S T R A C T

Acclimation to local conditions may produce adaptive responses in plants subjected to diverse climatic stresses. However, it has not been assessed how local adaptation and phenotypic plasticity contribute to photoprotection mechanisms in response to contrasting climatic conditions in Mediterranean tree species.

We analyzed photoprotection mechanisms in mature trees of the Mediterranean evergreen oak *Quercus ilex* at three sites with contrasting climatic conditions, i.e. xeric, continental and mesic sites. We studied morphological and physiological parameters indicative of photoprotection in adult trees in the field. In order to establish whether these parameters were genetically determined we compared adults with seedlings germinated from acorns of the three sites and grown under common greenhouse conditions.

In the field we found no significant differences in most of the physiological parameters in summer, but in winter the adult trees from the continental site were photoinhibited. In contrast, there were significant differences between seedlings in most photoprotective parameters evaluated. Morphological traits such as trichome density and leaf reflectance differed between populations, both in field-grown trees and in greenhouse-grown seedlings, being higher in all cases in plants from the xeric site than elsewhere. Our findings suggest the existence of constitutive differences in leaf photoprotection mechanisms among *Q. ilex* populations. These divergences may represent an inherent source for more stress tolerant ecotypes in the face of changing climatic conditions.

Introduction

The Mediterranean climate is characterized by the presence of a summer drought and the occurrence of cold or mild winter conditions in inland or coastal sites, respectively, leading to a growing seasonal split in two separated periods, namely spring and autumn (Camarero et al., 2010; Mitrakos, 1980). As a consequence, leaves must adapt to contrasting conditions in summer and winter. High irradiance and temperature together with drought stress also strongly affect leaf growth and development in evergreen trees (Bréda et al., 2006; Gratani, 1996; Ogaya and Peñuelas, 2003). On the other hand, low winter temperatures also reduce photosynthetic activity enhancing the damaging effects of high irradiance (Corcuera et al., 2005). Facing the stresses associated with both conditions requires not only a great stress tolerance, but also good acclimation ability in functional features including morphological and ecophysiological traits (Nicotra et al., 2010).

One of the main effects of unfavorable climatic conditions on photosynthetic organs is the generation of photo-oxidative stress, which is induced by the imbalance between energy absorption by chlorophyll and its metabolic use. Photosynthetic tissues are protected through photoprotection mechanisms which reduce the efficiency of light energy absorption, increase the rate of energy dissipation and enhance the detoxification of oxidative species (Niyogi, 2000). A reduction of the absorption of solar radiation can be achieved through an increase in leaf pubescence or in cuticle waxes or by changing leaf inclination and curling (Ehleringer, 1984; Karabourniotis et al., 1992). The photoprotection mechanisms that contribute to the thermal dissipation of excess energy involve the movement of chloroplasts and, most importantly, the synthesis of xanthophyll-cycle pigments (V, violaxanthin; A, antheraxanthin; Z, zeaxanthin – Demmig-Adams and Adams, 2006). When energy excess exceeds the capacity for thermal dissipation, antioxidants represent the third and last line of defence. Among these substances, the antioxidant α-tocopherol has been considered

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especially relevant for the acclimation of Mediterranean trees to temperature extremes (García-Plazaola et al., 2003). This molecule participates in the detoxification of active oxygen, and its concentration responds to the magnitude of the stress and the tree species' sensitivity (Munné-Bosch, 2005). The combined action of the aforementioned photoprotection mechanisms shelter leaf cells against damage and maintain the photosynthetic function, playing a key role for the carbon uptake of evergreen Mediterranean trees facing both summer drought and winter coldness (Oliveira and Peñuelas, 2004).

Quercus ilex L. (Holm oak) is the dominant evergreen oak species in the Western Mediterranean Basin, occupying a wide array of Mediterranean climate-types from continental to mild conditions (Barbero et al., 1992). Its acclimation to face diverse climatic stressors such as very cold winters and dry summers has been highlighted by several studies both at a morphological (Corcuera et al., 2004; Crescente et al., 2002; Gratani, 1996; Montserrat-Martí et al., 2009; Morales et al., 2002) and physiological (García-Plazaola et al., 1999; Gratani et al., 2003) level. This adaptation to local conditions is mainly explained by phenotypic plasticity, i.e. how a genotype may express different phenotypes under contrasting environmental conditions (Valladares et al., 2007; Gimeno et al., 2009). However, it is uncertain to what extent photoprotection mechanisms are plastic in response to changing climatic conditions. For instance, if photoprotection mechanisms are triggered by stressful climatic conditions, such responses may have a cost in terms of reduced photosynthetic efficiency (Murchie and Niyogi, 2011).

Our main objective was to evaluate if photoprotection mechanisms are constitutive or plastic in *Q. ilex* by comparing trees living on three sites subjected to contrasting climatic conditions. To fulfil this objective we assessed morphological and physiological photoprotective features in leaves belonging to the three youngest cohorts, which are the ones retained most frequently by *Q. ilex* trees (Montserrat-Martí et al., 2009), in different seasons and during two different growing seasons. We studied leaves of different ages because photoprotective characteristics change as leaves age (Munné-Bosch, 2007). Finally, to assess whether ecotypic differences exist between sites, we evaluated photoprotective mechanisms in greenhouse-grown seedlings subjected to the same environmental conditions.

Materials and methods

Study sites

This study was performed in three sites located in northern Spain and showing contrasting climatic conditions (Table 1 and Fig. 1): Alcubierre (41°49′N, 0°30′W, 580 m a.s.l.) with semiarid Mediterranean climate characterized by hot and dry summers; Soria (41°47′N, 2°27′W, 1050 m) with continental Mediterranean conditions and cold winters; and Tertanga (42°58′N, 3°01′W, 500 m) with oceanic Mediterranean climate, mild temperatures and high precipitation leading to only slight summer drought. The coldest and warmest months in the three study sites are January and July, respectively. The mean maximum air temperatures in July are: 25.3 °C in Tertanga, 27.4 °C in Soria and 32.6 °C in Alcubierre. The mean minimum air temperatures in January are: −2.2 °C in Soria, +1.1 °C in Tertanga and +2.1 °C in Alcubierre. The total rainfall during the driest period of the year (July–August) is 46 mm in Alcubierre, 63 mm in Soria and 76 mm in Tertanga. Soils are acid in Tertanga and Soria and basic in Alcubierre. Texture is sandy in Soria, and sandy-loam type in Tertanga and Alcubierre (see Appendix A).
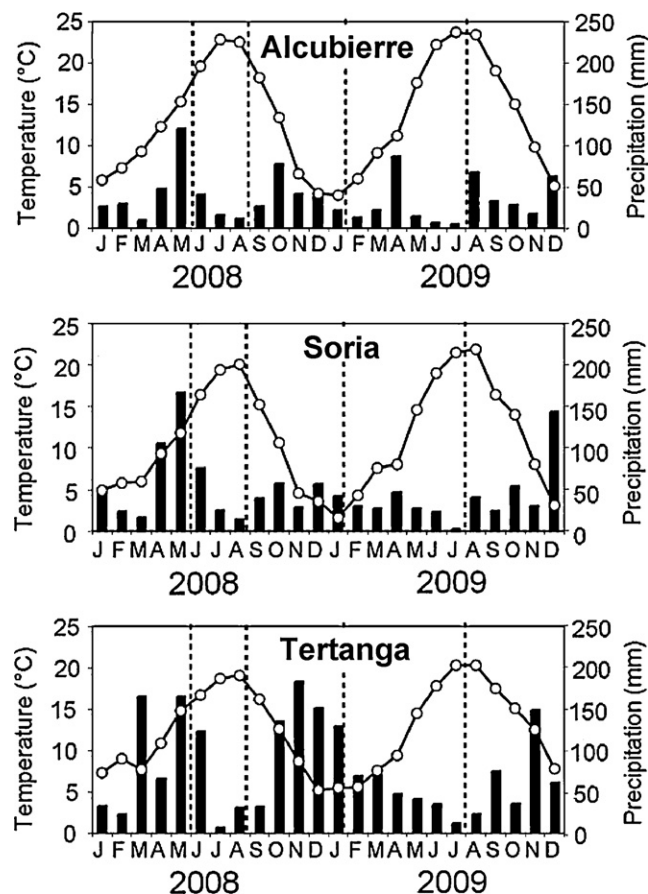

Fig. 1. Monthly climatic conditions (mean temperature – lines with symbols – and rainfall – bars) in the three studied sites during the period 2008–2009. Vertical dashed lines represent the sampling periods.

Sampling design

We sampled along three climatically contrasting periods during 2008: June 2008 (hereafter abbreviated as "spring 08"), September 2008 (hereafter abbreviated as "summer 08") and January 2009 (hereafter abbreviated as "winter 08"). Additional sampling was performed during August 2009 (hereafter abbreviated as "summer 09"). Summer 2009 was preceded by a much drier spring than summer 2008 (Fig. 1). The sampling dates allowed us characterizing physiological responses under a wide range of contrasting climatic conditions. Leaves were already fully expanded in all sampling dates. In each sampling we collected three years-old branches from sun and shade positions from ten adult trees in each case ($n = 10$), except for Tertanga sun leaves, where we sampled five trees because the rest of trees were too high for performing an adequate sampling. Trees showed similar mean diameters (±SE) at 1.3 m between sites (Alcubierre, 14.6 ± 0.3 cm; Soria, 13.6 ± 0.2 cm; Tertanga, 18.5 ± 0.4 cm). Sun leaves were considered those facing south in the outer part of the crown. Each branch included leaves belonging to several cohorts (usually three). Analyses were performed using current (termed as "new") and previous or one-year old leaves (termed as "old") of the 2007, 2008 and 2009 cohorts. Branches were immediately transported to the laboratory, stored in darkness at saturating humidity and 20 °C to allow comparable conditions between different sites and seasons. One sample of each leaf type was analyzed in each tree, and the same leaf was used for reflectance, fluorescence and pigment content determinations.

In autumn 2008, acorns were collected from the same trees and germinated in wet sand. After germination, seedlings were

Table 1
Means of annual, winter and summer precipitation and temperature, and estimated annual water deficit from the three areas of study for the last 20 years[a].

Site	Precipitation (mm)			Temperature (°C)			Annual water deficit (mm)
	Annual	Winter	Summer	Annual	Winter	Summer	
Alcubierre	458	97	94	14.7	5.9	23.6	350
Soria	572	146	118	10.5	3.0	18.5	154
Tertanga	1059	341	140	12.1	6.2	17.9	74

[a] Data obtained from the nearest meteorological station to the three experimental sites (Pallaruelo de Monegros, located at 27.5 km from Alcubierre site; Soria, located at 2.5 km from Soria site; and Amurrio, located at 8.5 km from Tertanga site). Climatic data were obtained from www.ucm.es/info/cif and annual water deficit was estimated using a Thornthwaite water-budget procedure based on the monthly mean temperature and total precipitation data (see more details in Montserrat-Martí et al., 2009).

grown in a greenhouse under controlled conditions of temperature and humidity (temperature 18–25 °C night-day, 60–80% RH, day-night). The natural light was supplemented with white cold lamps (Phillips SON-T AGRO 400, Belgium) to reach an illumination of 400 μmol photon m^{-2} s^{-1} photosynthetic flux density under a photoperiod of 12 h d^{-1}. Seedlings were cultivated in a substrate blend as 60:20:20 of peat, perlite and vermiculite. Plants were watered every two days with Evans nutrient solution (Evans and Nason, 1953) and water. Ten seedlings coming from acorns collected at each site were analyzed for each parameter.

Leaf indumentum morphology and leaf mass per area

To estimate the trichome density in adult trees from the field and greenhouse-grown seedlings, we counted the number of trichomes located within 7.1-mm^2 circles of the interveinal region on the adaxial surface of leaves ($n = 20$ per site, except Tertanga adult trees where $n = 10$). Trichomes were counted with the aid of a binocular microscope at 10–20 magnification. We identified three types of trichomes according to the classification of Hardin (1976): (*i*) fused-stellate sessile trichomes with fusion of the 7–8 rays beyond the base; (*ii*) fasciculate trichomes with cells clustered and fused at least at the base; and (*iii*) solitary trichomes showing a single, long and erect unicellular hair (Fig. 2).

The leaf mass per area (LMA) was calculated by taking two disks of 6 mm in diameter from each plant ($n = 20$ leaves per site, except Tertanga adult trees where $n = 10$). Disks were ovendried at 70 °C during 48 h and weighted.
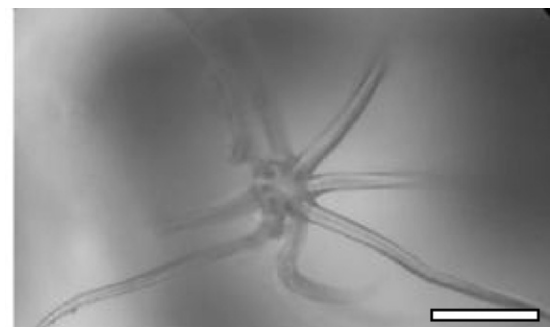
Leaf reflectance

Reflectance was measured with a portable spectrophotometer (UNISPEC^{TM}, FieldSpec UV/NIR, PP Systems, Amesbury, USA) on the adaxial surface of leaves of field-grown adult trees and greenhouse-grown seedlings ($n = 20$ leaves per site in both cases, except Tertanga adult trees where $n = 10$). All measurements were made at 2.5 nm intervals from 700 to 400 nm. The spectra obtained showed the characteristic shape of functional leaves. The reflectance data of a particular leaf lot were subtracted from the reflectance values that were measured at the site showing the highest reflectance values at each wavelength (Alcubierre), in order to highlight relative differences between sites.
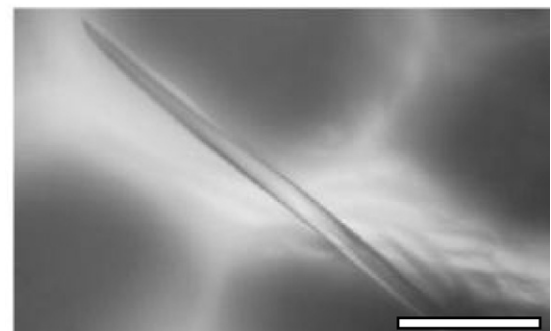
Leaf fluorescence

Fluorescence parameters measurements were made with a portable fluorometer with modular light (OS 5-FL, Optisciences, Tyngsboro, USA), in darkness at room temperature (20 °C). The samples ($n = 10$ leaves per site, except Tertanga adult trees where $n = 5$) were dark-adapted for 12 h to determine the minimum Chl fluorescence yield (F_0). The maximum Chl fluorescence (F_m) was determined under a saturating light pulse. The ratio F_v/F_m represents the maximum quantum yield of PSII and was calculated as $(F_m - F_0)/F_m$.
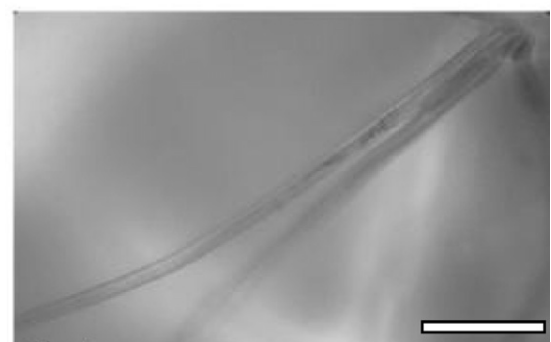
Pigment contents

To quantify pigment contents samples were taken with a cork borer from sun ($n = 5$ leaves per cohort and site) and shade ($n = 5$ leaves per cohort and site) leaves of different cohorts (2007–2009) in the field and for greenhouse-grown seedlings ($n = 10$ leaves

Fig. 2. Contrasting morphologies of the leaf trichomes of greenhouse-grown seedlings from each study site ($n = 20$ leaves per site). Trichome morphological types followed the Hardin (1976) classification including fused-stellate, solitary and fasciculate trichomes. The scale bars are equivalent to 50 μm.

per site). Four leaf disks 6-mm wide were taken from each leaf, immediately frozen in liquid nitrogen, and stored at $-80\,°C$ until use. Frozen leaf disks were grinded and then homogenized with a tissue tearing homogenizer (Model 395, Dremel, Mexico) by mixing the leaf tissue in a pure acetone solution buffered with $CaCO_3$. The extracts were centrifuged at $16,100 \times g$ for 20 min, and supernatants were filtered with 0.2-μm PTFE filters (Teknokroma, Spain). Pigment and α-tocopherol separation were performed in the same extracts by HPLC with a reverse phase C18 column (Waters Spherisorb ODS1, 4.6–250 mm, Massachusetts, USA), following the method of García-Plazaola and Becerril (1999). During processing in the HPLC, samples were maintained at $4\,°C$ in a refrigerated compartment. Identification and quantification was carried out with a photodiodearray (PDA) detector for pigments and a fluorescence detector ($\lambda_{excitation}$ 295 nm, $\lambda_{emission}$ 340 nm) for α-tocopherol. This method allows the separation of all carotenoids and chlorophylls. Specifically, the performance of the xanthophyll cycle was characterized by the ratio of the total pool of the cycle (xanthophyll cycle pigments, i.e. violaxanthin plus antheraxanthin plus zeaxanthin, VAZ) to chlorophyll (Chl) – VAZ/Chl – and the degree of deepoxidation or ratio between antheraxanthin plus zeaxanthin $(A+Z)$ and the xanthophyll cycle pigments – A+Z/VAZ.

Statistical analyses

We used one-way ANOVA to compare photochemical parameters (F_v/F_m), pigment ratios (VAZ/Chl, A+Z/VAZ, α-tocopherol/Chl) and leaf characteristics (trichome density, LMA and reflectance) between provenances. Since mean values of most measured variables, excepting F_v/F_m in summer and A+Z/VAZ in summer 2009, differed significantly ($P<0.05$) between sun and shade leaves, we used the factor sun-shade as covariate in the statistical analyses. Therefore, results are presented for means, irrespective of sun and shade leaf positions, but comparing leaves of different ages and cohorts. Pairwise means were compared using Tukey honestly significant difference (HSD). Data were analyzed with R package (R Development Core Team, 2011).

Results

Reduction in the efficiency of light energy absorption

Adaxial density of trichomes and LMA differed between sites (Table 2). The pattern matched climatic patterns with a decreasing trend in trichome density and LMA values between the xeric site (Alcubierre), the continental site (Soria) and the mesic site (Tertanga). Trichome type (Fig. 2) was fused-stellate in the three populations. Despite the above-mentioned differences in adaxial trichome density we did not find significant differences in total reflectance in the 400–700 nm spectrum range between leaves from adult trees of the three sites, although there was a nonsignificant, decreasing trend from Alcubierre to Tertanga (Table 2).
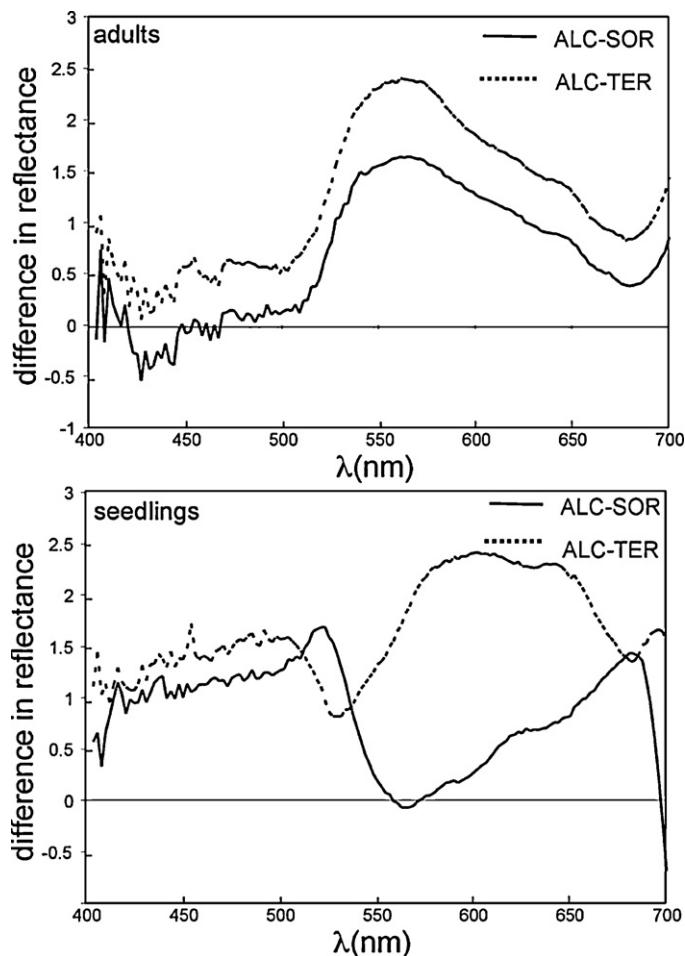

Fig. 3. Differences in reflectance of leaves from adult trees growing in the field (n = 10 per site, except Tertanga where n = 5) and greenhouse-grown seedlings (n = 20 per site). The solid line represents the difference between, respectively, the spectra of leaves from the xeric (ALC, Alcubierre) and those of leaves from the continental (SOR, Soria) site (ALC–SOR), and the dotted line represents the difference between, respectively, the spectra of leaves from the xeric and those from the mesic (TER, Tertanga) site (ALC–TER).

Greenhouse-grown seedlings showed a much higher density of adaxial trichomes in seedlings from the xeric site than elsewhere (Table 2). Interestingly, the nature of trichomes also differed between populations with Alcubierre seedlings having leaves with fused-stellate trichomes, Soria seedlings having solitary trichomes, and Tertanga seedlings showing both of the aforementioned types plus fasciculate trichomes (Fig. 2). In the case of leaves from adult trees, reflectance along the 530–600 nm range (corresponding to green light) was significantly higher ($P<0.05$) in the xeric site (Alcubierre) than elsewhere (Fig. 3). The differences in reflectance for wavelengths above 530 nm between the xeric and the mesic sites

Table 2
Mean values of leaf mass area (LMA), percentage of leaf reflectance, density of trichomes and type of trichomes in greenhouse-grown seedlings and field-grown adult plants of *Quercus ilex* from the three study sites. Data are means ($\pm$SD) of n = 10 adult trees, except Tertanga (n = 5) and n = 20 greenhouse-grown seedlings per site. Statistical analyses compared pairs of data between study sites for seedlings and adult plants. Data followed by different letters are significantly different between sites at the $P<0.05$ probability level.

Site	LMA (mg cm^{-2})		% Leaf reflectance (λ = 400–700 nm)		Density of trichomes (mm^{-2})		Type of trichomes[a]	
	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults	Seedlings	Adults
Alcubierre	8.91 ± 0.35 a	22.65 ± 058 a	7.26 ± 0.16	5.59 ± 0.15	2.82 ± 0.24a	8.68 ± 1.43 a	FS	FS
Soria	5.48 ± 0.18 b	21.66 ± 0.88 a	6.44 ± 0.19	4.97 ± 0.11	0.03 ± 0.02b	6.14 ± 1.17 a	S	FS
Tertanga	6.82 ± 0.61 b	16.10 ± 1.84 b	5.63 ± 0.16	4.40 ± 0.11	0.18 ± 0.07b	4.46 ± 2.38 b	FS, S, F	FS

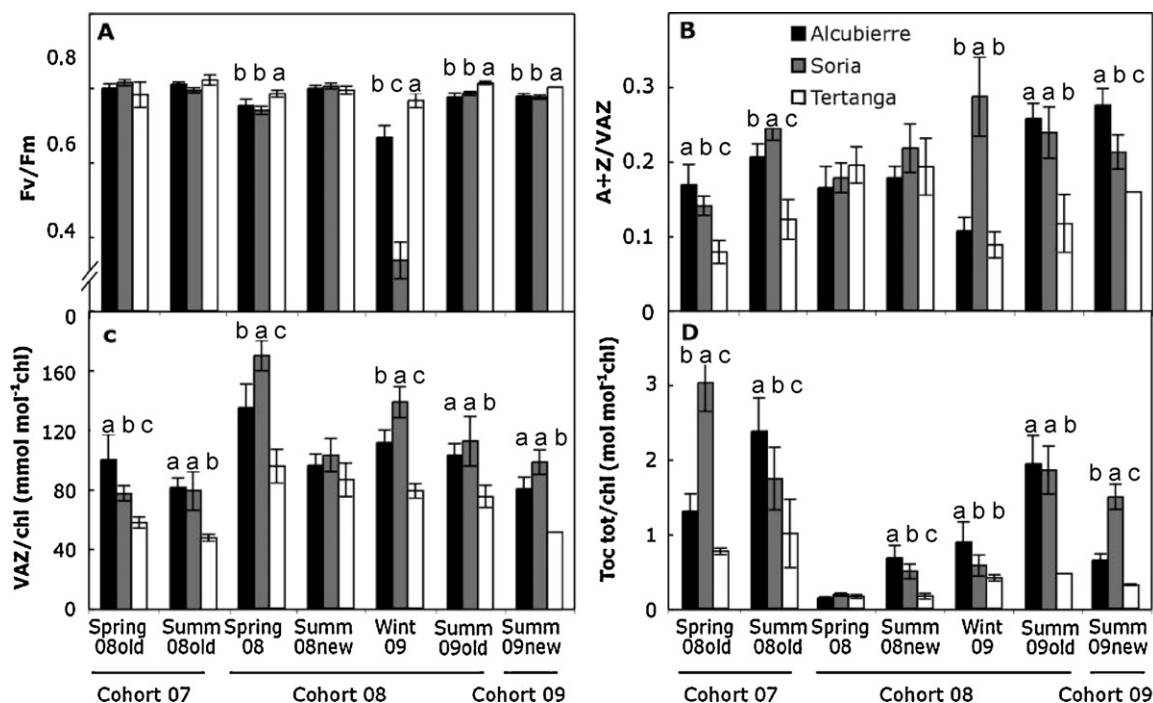[a] Trichome morphological types: FS, fused-stellate; S, solitary; F, fasciculate.

Fig. 4. Physiological and biochemical parameters in field-grown adult trees ($n = 10$ per site and season, excepting Tertanga where $n = 5$) along the three sampling seasons (spring, summer, winter), measured in sun leaves belonging to three different cohorts (cohort 07, 2007; cohort 08, 2008; cohort 09, 2009). Data are presented for current- (new) and one-year old (old) leaves. Mean ($\pm$SE) values are shown for: maximal photochemical efficiency (A), deepoxidation state of xanthophyll cycle (B), xanthophylls cycle pigments content (C), total tocopherol content (D). Different letters above the bars indicate significant differences between sites within each season ($P < 0.05$).

(Tertanga) were larger (1.0–2.5) than those between the xeric and cold (Soria) sites (0.5–1.5). Reflectance was also significantly higher for the same spectral range in leaves from seedlings of the xeric site (Alcubierre) when compared with those from the other two sites, but these differences were only high (1.0–2.5) when comparing the xeric and mesic sites (Fig. 3).

Increase in the rate of energy dissipation

No significant differences in maximal photochemical efficiency (F_v/F_m) were found (Fig. 4A) between leaves of adult plants from the three study sites during half of the sampled seasons. However, leaves of trees from the cold site (Soria) sampled in winter showed a significantly ($P < 0.05$) lower F_v/F_m as compared to the Alcubierre and Tertanga sites (Fig. 4A). Winter photoinhibition (low F_v/F_m) was associated with the retention of high Z levels in the dark. The A + Z/VAZ ratio was significantly lower in the mesic site (Tertanga) than in the other sites during four sampling periods, particularly in summer (Fig. 4B). The VAZ/Chl content was also lower in the mesic site than in the other two sites in six out of seven sampled seasons (Fig. 4C). Remarkably, there was also a higher content of VAZ/Chl in the case of old leaves in the three sites when comparing the dry summer 2009 (overall mean $\pm$ SE = 97.67 $\pm$ 8.88 mmol mol^{-1}) with the 2008 one (67.67 $\pm$ 11.35 mmol mol^{-1}). Finally, the total tocopherol content was significantly lower in the mesic site than in the other two sites for all sampled summers, either for one-year old or for current leaves, and also when considering old leaves sampled in spring 2008 (Fig. 4D).

In the case of greenhouse-grown seedlings low F_v/F_m values suggested an excess of light. Apart from this effect, seedlings from the three sites showed the same pattern of differences in the physiological parameters analyzed as adult trees, except for A + Z/VAZ (Fig. 5). Thus, seedlings from the xeric (Alcubierre) and cold (Soria)

sites had higher VAZ/Chl but lower F_v/F_m values than seedlings from the Tertanga mesic site.

Enhancement of the detoxification of oxidative species

Comparisons of the α-tocopherol to chlorophyll (Chl) molar ratio between sites showed that both in summer and winter, their concentrations on leaves were lower in the Tertanga site than elsewhere. However, leaf ageing was the main factor implied in tocopherol content variation and was more important than seasonal variations. This was clearly evidenced in the leaf cohort of 2008, in which the increasing trend of tocopherol as leaves aged was observed in all three populations (Fig. 4). Interestingly, seedlings showed lower α-tocopherol to chlorophyll molar ratios in the mesic site (Tertanga) as compared with the climatically stressful sites Alcubierre and Soria (Fig. 5).

Discussion

Photoprotective mechanisms were characterized at the leaf level during the course of two consecutive growing seasons in three climatically contrasting sites, and leaves of different ages were considered. This provides a detailed perspective on the range of responses to environmental conditions that *Q. ilex* leaves may encounter across climatic gradients during their lifespan. Morphological characters such as trichome density and type and LMA, which contribute to photoprotection through an enhancement of leaf reflectance, differed between sites. This observation supports the potential role of leaf structural mechanisms to deal with climatic stress that can be even more relevant that biochemical and physiological variability in the case of evergreen *Quercus* species (Vaz et al., 2011). The photoprotective role of adaxial pubescence in this species has been demonstrated by experimental treatments in which the loss of trichomes induces short-term decreases in
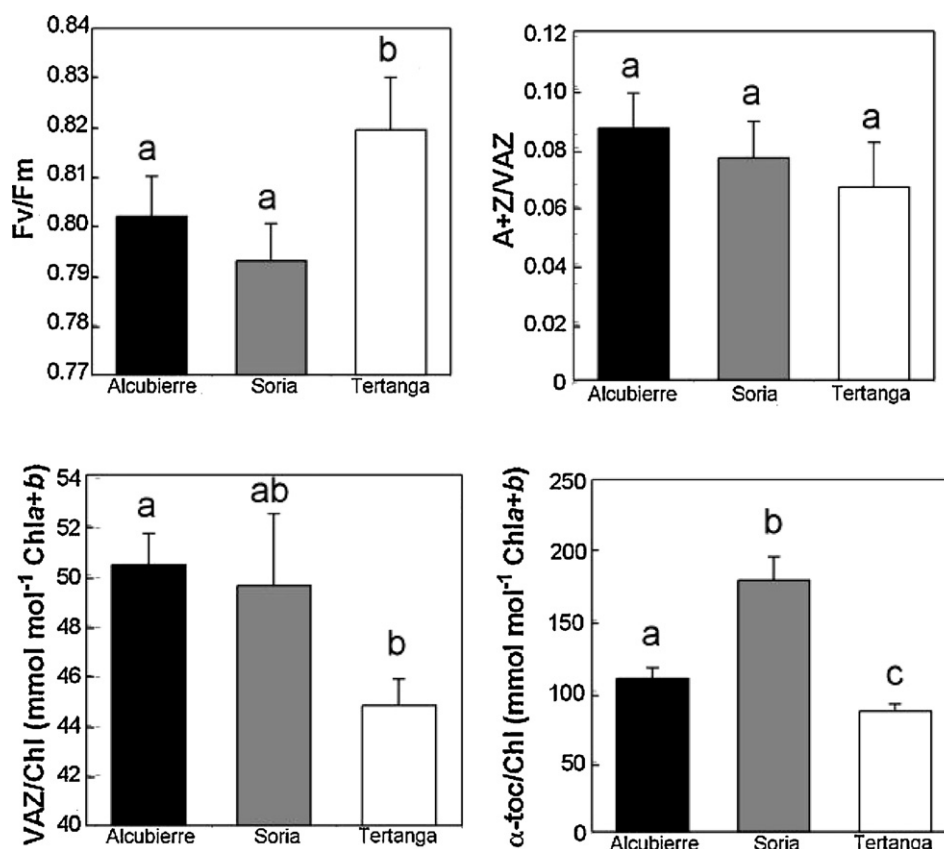
Fig. 5. Physiological and biochemical parameters of the leaves of greenhouse-grown seedlings from the three study sites ($n = 20$ per site). Means ($\pm$SE) are shown. Different letters above the bars indicate significant differences between sites ($P < 0.05$).

photosystem II efficiency because of a higher light interception by mesophyll cells (Morales et al., 2002). In fact, these authors estimated that trichomes reflect ca. 5% of the incident light in *Q. ilex*. In the present study the positive relationship between trichome density and xeric conditions was observed in adult trees in the field and also in greenhouse-grown seedlings, indicating that differences in trichome density may depend to some degree on genotypic control. The difference in trichome types between populations poses the question whether the trichome type has an adaptive function since the type of trichomes is affected by several environmental conditions in *Quercus* species (Hardin, 1979). Nevertheless, the small differences in leaf reflectance between sites (less than 2%) and the loss of trichomes observed during leaf ontogeny (Hardin, 1979) suggest that the type and density of trichomes in leaves may also respond to other environmental factors. Trichomes may regulate transpiration (Bacelar et al., 2004), increase leaf water repellency to enhance CO_2 diffusion (Brewer et al., 1991), or reduce herbivory by accumulating phenolic compounds (Karioti et al., 2011).

The xeric population (Alcubierre) also had the highest LMA both in greenhouse-grown seedlings and in adult trees from the field. *Q. ilex* increases LMA and leaf thickness after the exposition to high-light levels, drought and/or cold conditions (Filella et al., 1998; Filella and Peñuelas, 1999; Oliveira and Peñuelas, 2000; Peña-Rojas et al., 2005; Vaz et al., 2011). The increased LMA usually occurs at expenses of a lower photosynthetic potential (Peña-Rojas et al., 2005) representing a trade-off between photochemical efficiency and photoprotection. In agreement with the higher LMA and trichome density, reflectance was also highest in the adaxial side of seedlings leaves from the xeric site, particularly in the 550–650 nm range which corresponds to green light (Fig. 3).

The driving factor that characterizes physiological responses in evergreen species as *Q. ilex* is that their long-lived leaves must cope with different stress factors throughout their lifespan (Montserrat-Martí et al., 2009). In the case of Mediterranean forests climatic stress is mainly due to a period of summer drought and a cold winter (Mitrakos, 1980). In general it could be expected that these climatic constraints would be more extreme in the continental site (Soria) in winter and in the xeric site (Alcubierre) in summer. The most evident response to low-temperature stress was a dramatic reduction (50%) of photochemical efficiency (F_v/F_m) in leaves from the Soria population in winter (Fig. 4). This decreased winter photochemical efficiency was not reversible and should be considered as "chronic photoinhibition" (Werner et al., 2002) in the sense that this process is due to a combination of photoprotection and photodamage (Müller et al., 2001). Such winter photoinhibition has photoprotective value, as was evidenced by the accumulation of xanthophylls cycle pigments and the higher level of de-epoxidation (Fig. 4). This mechanism is typical for boreal conifers (Adams et al., 1995), but it has been also observed in some Mediterranean evergreen species under continental conditions (García-Plazaola et al., 2003). Hence, this mechanism is probably a general character in evergreens acclimated to cold environments. In the present work, winter photoinhibition was strongly activated in trees from the coldest site as indicated by the drop in F_v/F_m there and the increase in the VAZ/Chl ratio, but not elsewhere. This suggests the idea that local physiological adaptation of different *Q. ilex* provenances in response to contrasting climatic conditions may override plasticity (Gratani et al., 2003). We also found larger differences between sites in morphological traits, such as LMA and trichome density, than in physiological features as in the study of Italian *Q. ilex* populations performed by Gratani et al. (2003). In addition, seedlings from the cold site grown under controlled environmental conditions also displayed low F_v/F_m values which concur with the expected local adaptation patterns. However, the morphological and

photoprotective differences were obvious only when comparing the xeric and the mesic sites, and variability between coexisting trees was very high indicating that plasticity, particularly in mesic sites and considering physiological measures, is also a relevant factor to understand the responses of *Q. ilex* to climate (Gimeno et al., 2009). Overall, the photoprotective strategies during winter may differ between local ecotypes and involve different photoprotective mechanisms apart from those associated with xanthophylls (Martínez-Ferri et al., 2004).

Contrasting with the acute effect of winter on photochemical efficiency, summer drought did not generate a comparable photoinhibitory effect even during the stressful summer 2009. This may be explained by the high variability in the expression of their photoprotective capacity between co-occurring trees within the same site, highlighting the importance of the high intraspecific variability in *Q. ilex* (Gimeno et al., 2009; Nicotra et al., 2010). Furthermore, the differences between old and recently formed leaves suggest an additional source of variability of photoprotective strategies within the same tree as leaves age.

The antioxidant metabolism was exemplified through the study of changes in tocopherol content, since this is among the most stress-responsive antioxidants despite other antioxidants measured and other compounds may be more effective in photoprotection (Munné-Bosch, 2005). In sun leaves, the main response observed was the accumulation of a large pool of α-tocopherol during the driest summer 2009 in the xeric site. Increases in the concentrations of this molecule have been reported in response to water deficit (Munné-Bosch et al., 1999). However it should be noticed that the content of α-tocopherol is also affected by its continuous accumulation during leaf ontogeny (Hormaetxe et al., 2005) and the age-dependence of tocopherol levels may confound the observed differences between sites.

Several authors have shown the existence of differences in morphological and physiological traits between seedlings originated from acorns from *Q. ilex* trees living under contrasting climates (Bonito et al., 2011; Gimeno et al., 2009; Gratani et al., 2003; Sánchez-Vilas and Retuerto, 2007). In the present work we report the existence of different intrinsic photoprotective parameters at the morphological and physiological levels when seedlings are cultivated under comparable environmental conditions. In spite of not considering the maternal effects mediated by acorn size on seedlings, our results remain valid since Gimeno et al. (2009) found no significant effect of seed or seedling size on physiologic performance. Basically VAZ and tocopherol contents increased in the xeric site and decreased in the mesic site, with F_v/F_m showing the reverse pattern. These differences, together with the higher LMA and trichome density and the lower photochemical efficiency of the xeric site evidence for the more conservative strategy of *Q. ilex* trees that are locally adapted to xeric conditions.

Conclusions

To sum up, morphological photoprotection traits in *Q. ilex* differed between sites, apparently being affected by the local climatic stressors prevailing on each site. Physiological photoprotective mechanisms showed higher variability between sites than morphological traits. The population from the xeric site was most photoprotected both at physiological and morphological levels. The differences detected between *Q. ilex* provenances in the photoprotection mechanisms may help in the selection of highly stress-tolerant and well photoprotected ecotypes from severely stressed populations. Such trees may hold the stronger protective mechanisms and be the most resistant to face the expected drier, warmer climate predicted for Mediterranean *Q. ilex* forests.

Acknowledgements

Appendix A.

Soil characteristics of the three study sites.

	Alcubierre	Soria	Tertanga
pH	7.75 ± 0.09	6.12 ± 0.24	4.63 ± 0.31
Conductivity (mS cm^{-1})	0.20 ± 0.07	0.09 ± 0.05	0.09 ± 0.02
Organic C (%)	1.98 ± 0.53	1.69 ± 0.57	4.56 ± 0.50
Organic matter (%)	3.41 ± 0.91	2.92 ± 0.98	7.85 ± 0.87
CaCO$_3$ (%)	32.18 ± 7.75	0	0
Nitrogen (%)	0.19 ± 0.06	0.17 ± 0.06	0.39 ± 0.07
C/N	11.65 ± 0.93	10.32 ± 0.39	11.97 ± 0.84
Sand (%)	55.32 ± 5.95	84.76 ± 2.44	50.25 ± 4.04
Silt (%)	27.37 ± 3.15	13.7 ± 2.18	34.21 ± 2.76
Clay (%)	17.31 ± 6.0	1.53 ± 0.27	15.54 ± 1.88

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