Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress

José Javier Peguero-Pina\textsuperscript{A}, Domingo Sancho-Knapik\textsuperscript{A}, Fermín Morales\textsuperscript{B}, Jaume Flexas\textsuperscript{C} and Eustaquio Gil-Pelegrín\textsuperscript{A,D}

\textsuperscript{A}Unidad de Recursos Forestales, Centro de Investigación y Tecnología Agroalimentaria, Gobierno de Aragón, Apdo. 727, 50080 Zaragoza, Spain.
\textsuperscript{B}Department of Plant Nutrition, Experimental Station of Aula Del, CSIC, Apdo. 13034, 50080 Zaragoza, Spain.
\textsuperscript{C}Laboratori de Fisiologia Vegetal, Departament de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa, km 7.5, 07071, Palma de Mallorca, Balears, Spain.
\textsuperscript{D}Corresponding author. Email: egilp@aragon.es

Abstract. The ability of three Mediterranean oaks (\textit{Quercus cocifera} L., \textit{Quercus ilex} ssp. ballota (Desf.) Samp and \textit{Quercus suber} L.) to cope with intense drought was investigated. Water stress reduced stomatal conductance and photosynthesis in these species. Drought-mediated changes in photosynthetic-related parameters allowed the characterisation of the specific photo-protective mechanisms. Specifically, \textit{Q. suber} downregulated photosynthetic electron transport rates (ETR) closing PSII reaction centres (i.e. decreasing photochemical quenching) and through an antheraxanthin (A) + zeaxanthin (Z) mediated diminished intrinsic PSII efficiency (\textit{F}_{\text{r,pi}}). These changes were lower in \textit{Q. cocifera} and \textit{Q. ilex} ssp. ballota, which decreased further ETR photo-inactivating PSII centres (evidenced by their low predawn \textit{F}_{v}/\textit{F}_{m} ratios at high water stress). The predawn \textit{F}_{v}/\textit{F}_{m} ratio decreased in \textit{Q. cocifera} largely due to \textit{F}_{m} decreases, whereas in \textit{Q. ilex} ssp. ballota \textit{F}_{v}/\textit{F}_{m} decreases were due to \textit{F}_{0} increases, below −4 MPa. These \textit{F}_{v}/\textit{F}_{m} decreases were well correlated with increases in the A + Z photo-protective pigments. An analysis of dark respiration and photorespiration as alternative electron sinks under intense drought stress also revealed interspecific differences. The largest imbalance between electrons generated and consumed increased potentially oxidative damage in \textit{Q. suber}. Subsequently, only \textit{Q. suber} showed loss of chlorophyll, which is one of the main targets of oxidative damage. Data suggest that \textit{Q. cocifera} and \textit{Q. ilex} ssp. ballota seem more able than \textit{Q. suber} to withstand highly xeric conditions. Therefore, our results question the consideration of Mediterranean evergreen oaks as a homogeneous physiological group.


Introduction

Species growing in the Mediterranean area must cope with periods of summer drought, and should develop mechanisms and strategies to survive under situations of water deficit (Lo Gullo and Salleo 1988). Stomatal closure, as a way of avoiding severe water losses, allows water consumption regulation (Tenhunen et al. 1981; Jarvis and Davies 1997; Flexas et al. 1998; Mediavilla and Escudero 2003; Peguero-Pina et al. 2008). Water economy, between tissue water contents in the different plant parts at maximum stomatal closure and those where risks of irreversible damage occur (Sperry 2000; Brodribb and Holbrook 2004), has been considered critical for estimating plant survival probability (Burghardt and Riederer 2006), when water supply from the soil is severely restricted.

Several species of the Mediterranean flora of southern Europe show a high water economy. Among them, \textit{Quercus cocifera} L., \textit{Quercus ilex} ssp. ballota (Desf.) Samp and \textit{Quercus suber} L. are three evergreen oak species with small leaves (Traiser et al. 2005), which have been historically included within the genuine Mediterranean vegetation (Breckle 2002). Furthermore, these species are able to survive even in the most xeric areas of this territory (Martin-Albertos et al. 1998). Stomatal closure occurs in these species at water potential close to −3 MPa (Mediavilla and Escudero 2003; Vilagrosa et al. 2003), and leaf size and cuticular transpiration are lower than in other congener species from mesic habitats (Kerstiens 1996; Esteso-Martinez et al. 2006b). However, these three species show a high resistance to drought-induced cavitation (Tyree and Cochard 1996; Vilagrosa et al. 2003; Corcuera et al. 2005) when compared with the resistance found in some Mediterranean deciduous oaks (Corcuera et al. 2006; Esteso-Martinez et al. 2006a) or temperate deciduous oaks (Tyree and Cochard 1996). So, these three species are able to withstand severe drought periods with leaf water potentials lower than those inducing stomatal closure, but much
higher than cavitation limit. Therefore, *Q. coccaifera, Q. ilex ssp. ballota* and *Q. suber* can be considered as species with a wide ‘safety margin’ (sensu Brodribb and Holbrook 2004).

With the stomata closed, plants minimise water losses at the expense of reducing net CO₂ assimilation. Under these conditions, where light incident on the leaf surface exceeds largely the amount that can be used for photosynthesis (Demmg-Adams and Adams 1996), both A Ph and the de-epoxidation state of the xanthophyll cycle pigments increase, protecting the photosynthetic apparatus through a mechanism that dissipates excess of light as heat (Niyogi 1999; Li et al. 2000; Demmg-Adams and Adams 2006). This photoprotective mechanism varies along the diurnal time course, as well as in response to temperature, water and nutrient stresses (Morales et al. 2006). Moreover, the increase of the photosynthetic pathway in drought-stressed plants can also be important for energy dissipation to prevent photodamage (Guan et al. 2004).

The existence of several photoprotection mechanisms makes the photosynthetic electron transport chain relatively insensitive to drought stress (Cormic and Briantais 1991; Havaux 1992; Tournus and Pelletier 1995). There is no evidence for major sustained photodamage in water-stressed plants, as judged by the lack of effects of drought on the maximum potential PSII efficiency, estimated from the dark-adapted Fv/Fm chlorophyll fluorescence ratio (Epron and Dreyer 1992, 1993; Havaux 1992; Faria et al. 1998; Flexas and Medrano 2002; Morales et al. 2006). It should be noted that although Epron et al. (1993) showed strong Fv/Fm decreases due to water stress in three mesic oak species, these decreases were found at water potentials inducing the whole hydraulic conductivity loss due to cavitation in these species (Tyree and Cochard 1996). Balagué et al. (2002) found Fv/Fm decreases in photoinhibitory processes caused by extreme aridity and associated with strong decreases in chl concentration. Effects of water stress on chl concentrations are highly species-dependent (reviewed by Morales et al. 2006). In response to water stress, some crop species, such as barley, coffee, grapevine, and others, maintain high leaf chl concentrations, while having a decreased capacity of utilisation of solar energy for photosynthesis (Morales et al. 2006). Conversely, other Mediterranean plant species could prevent the absorption of an excess of light in the presence of water stress by decreasing leaf chl concentrations (Kyparissis et al. 2000; Munne-Bosch and Alegre 2000), thereby potentially diminishing the capacity for light harvesting. Evidence, however, suggests that changes in light harvesting capacity play only a small role in photoprotection (Baroli et al. 2003). In any case, chl concentration changes significantly affect light absorption only when chl loss is very important (Morales et al. 1991; Abadia et al. 1999). In cases where chl loss is important, the photoinhibition processes, as a damage-type occurrence, should be considered (Morales et al. 2006). Peguero-Pina et al. (2008) reported a first evidence of drought-mediated increases in PSII efficiency without changes in chl concentration in *Q. coccaifera*, a feature also described recently for some other Mediterranean species (Galmés et al. 2007). This latter case seems to be related to an additional photoprotective mechanism that may play an important role for survival of species living in sites with long and intense summer drought periods.

At this point, the next question is, how are the photosynthesis-related parameters and PSII efficiency affected during the water stress period between the water potential that induces stomatal closure and that inducing cavitation in *Q. coccaifera*, *Q. ilex ssp. ballota* and *Q. suber*? Our main objective here was to investigate whether these species show water stress-mediated predawn *Fv/Fm* decrease, and whether this photoinhibitory process results photodamage or photoprotective mechanisms. In this sense, the wide safety margin of these species (sensu Brodribb and Holbrook 2004) makes them useful for investigations into this process.

**Materials and methods**

**Plant materials and experimental conditions**

Five-year-old seedlings of *Quercus coccaifera* L., *Quercus ilex* ssp. *ballota* (Desf.) Samp and *Quercus suber* L. used in this study were from the same provenance for each species [Zaragoza 41°49’N, 0°30’W, 620 m above sea level, Alcarria-Serrania de Cuenca 40°19’N, 2°15’W, 950 m above sea level, and Montes de Toledo 39°33’N, 4°44’W, 600 m above sea level (Spain), respectively]. Seedlings of *Quercus* species showed morphological and photosynthetic characteristics similar to those found in leaves of fully developed trees at the levels of water stress *Quercus* species experience in a typical Mediterranean summer (Morales et al. 2002; Corcuera et al. 2005). Three weeks before the beginning of the experiments, pots (6.31-L) were placed in a transparent greenhouse of alveolar polycarbonate (Unit of Forest Resources, CITTA de Aragón, Zaragoza, Spain) that allowed passing 90% of PPFD (~1300 μmol photons m⁻² s⁻¹) at midday, during the experiment. The use of greenhouses in water-stress experiments had the advantage of performing measurements in more controlled environmental conditions, avoiding re-watering by storms or unwanted rainfall events.

Measurements in well irrigated plants were performed on 10 September 2003. Irrigation was stopped on 11 September, and the drought stress was imposed during 12 days. In the following days, measurements were performed with increasing levels of drought stress: 12, 16, 18, 20 and 22 September for *Q. coccaifera*; 11, 14, 17, 19 and 22 September for *Q. ilex ssp. ballota* and 11, 15, 17, 18 and 19 September for *Q. suber*. Five leaves of each plants of each species were systematically used for all measurements. Measurements were conducted strictly before dawn (water potential and chl fluorescence), at 8 h (gas exchange) and at midday (water potential, chl fluorescence, and gas exchange), in the two latter cases, solar time. Measurements were conducted during consecutive days or even at the same day (see above) and the measured water potential was very similar for the three species studied (see below). Therefore, the development of water stress with time was very similar for all the species investigated.

**Water potential, gas-exchange and chlorophyll fluorescence measurements**

Predawn and midday leaf water potentials (MPa) were measured in shoots of *Q. coccaifera, Q. ilex ssp. ballota* and *Q. suber* (with leaves still attached to the shoots) with an Scholander pressure chamber (Scholander et al. 1965), following the methodological procedures described by Turner (1988).

Gas-exchange measurements were performed at 8 h (solar time) and at midday (solar time) in fully developed current-year
attached leaves of *Q. coccifera*, *Q. ilex* ssp. *ballota* and *Q. suber* with a portable gas exchange system (CIRAS-1, PP-Systems, Herts, UK). Net CO₂ uptake (A) and stomatal conductance (gₛ) were registered. Measurements were performed at controlled CO₂ external concentration (Cₐ = 350 μmol mol⁻¹), PPFD incident on the leaf surface [800 and 1300 μmol photons m⁻² s⁻¹ at 8 h and midday (solar time) respectively], and ambient relative humidity. Dark respiration (DR) was studied by measuring CO₂ exchange in darkness (i.e. at 0 μmol photons m⁻² s⁻¹). Photorespiration (PR) was studied by measuring CO₂ exchange in an atmosphere containing less than 1% O₂ (connecting the air inlet of the CIRAS-1 to a N₂ oxygen-free gas cylinder), and subtracting the net CO₂ uptake measured at atmospheric O₂ concentration. DR and PR were measured at midday (solar time) at three representative water potentials during the drought period: well irrigated (control plants; CO), loss turgor point (about −3 MPa for the three species investigated, according to Corcuera et al. 2002; S1) and at the moment when drought stress was more intense (near −7 MPa; S2).

Chl fluorescence parameters were measured on attached leaves at predawn and midday (solar time) in fully developed current-year leaves of *Q. coccifera*, *Q. ilex* ssp. *ballota* and *Q. suber* with a PAM 2000 portable pulse amplitude modulation fluorimeter (Heinze Walz, Effeltrich, Germany). Plants were covered with a black bag and kept in darkness for 30 min to estimate the minimum (F₀) and maximum (Fₘ) chl fluorescence. F₀ and Fₘ were measured at predawn and midday. F₀ was measured by switching on the modulated light at 0.6 kHz in presence of far-red light (7 μmol m⁻² s⁻¹); PPFD was below 0.1 μmol m⁻² s⁻¹ at the leaf surface. Fₘ was measured at 20 kHz with a 1-s pulse of 6000 μmol m⁻² s⁻¹ of white light. The chl fluorescence at steady-state photosynthesis (Fₛ) was measured at midday, when PPFD was ~1300 μmol photons m⁻² s⁻¹, and a second pulse of high-intensity white light was used to determine the maximum chl fluorescence in the light-adapted state (Fₘ). Leaves were then covered and the minimum chl fluorescence after illumination in presence of far-red light (7 μmol m⁻² s⁻¹) was determined (F₀). The experimental protocol for the analysis of the chl fluorescence quenching was essentially as described by Genty et al. (1989) with some modifications. These involved the measurements of F₀ and F₀ᵣ, which were measured in presence of far-red light (7 μmol m⁻² s⁻¹) in order to fully oxidise the PSII acceptor side (Belkhodja et al. 1998; Morales et al. 1998). The dark-adapted, maximum potential PSII efficiency was calculated as Fₛ/Fₘ (Kitajima and Butler 1975; Morales et al. 1991; Abadia et al. 1999). The actual (Φₚₛₛ) and intrinsic (Φₑₛₛ) PSII efficiency was calculated as (Fₘ − F₀ᵣ)/Fₘ and Fₛ/Fₘ, respectively (Genty et al. 1989; Harbinson et al. 1989). Photochemical quenching (qP) was calculated as (Fₘ − F₀ᵣ)/Fₘ according to van Kooten and Snel (1990). The fraction of light absorbed that is dissipated in the PSII antenna (1 − Φₑₛₛ) was also estimated (Demming-Adams et al. 1996; Morales et al. 1998). Electron transport rate (ETR) was estimated according to Kraul and Edwards (1992), multiplying Φₚₛₛ by PPFD by 0.5 (because we assumed an equal distribution of excitation between PSII and PSI) and by 0.84, which is considered the foliar absorbance coefficient more common for C₃ plants (Björkman and Demmig 1987) including *Q. coccifera* and *Q. ilex* ssp. *ballota* (Morales et al. 2002).

**Photosynthetic pigment measurements**

Immediately after measuring chl fluorescence (at predawn and midday), leaf disks were cut with a calibrated cork borer from the same leaves in which chl fluorescence was measured, wrapped in aluminum foil, frozen in liquid nitrogen, and stored (still wrapped in foil) at −20°C. Leaf pigments were later extracted with acetone in the presence of Na-ascorbate and stored as described previously (Abadia and Abadia 1993). Pigments extracts were thawed on ice, filtered through a 0.45 μm filter and analysed by an isocratic HPLC method (Labre et al. 2004). All chemicals used were of HPLC quality.

Despite inter-conversions within the xanthophyll cycle that will be reported in detail during the whole drought period, other photosynthetic pigments will be presented more coarsely. Simplifying, only the concentration of photosynthetic pigments at three representative water potentials during the drought period is reported: well irrigated (control plants; CO), loss turgor point (about −3 MPa for the three species investigated, according to Corcuera et al. 2002; S1) and at the moment where drought stress was more intense (near −7 MPa; S2).

**Statistical analysis**

The non-parametric test of Kruskal-Wallis was used. ANOVA was not used, because the experimental data did not show a normal distribution (data not shown).

**Results**

Both net photosynthesis (A) and stomatal conductance (gₛ) decreased in *Q. coccifera*, *Q. ilex* ssp. *ballota* and *Q. suber* at 8 h (solar time) and midday (solar time) when water potential diminished (Fig. 1). At the end of the drought period (when water potential was approximately −7 MPa), both *A* and *gₛ* reached negligible values.

At predawn, values of the de-epoxidation state of the xanthophylls cycle pigments \([A + Z]/(A + Z)\) and maximum potential PSII efficiency \((Fₛ/Fₘ)\) showed non-significant changes during the drought period in *Q. suber* (Fig. 2). At midday, there was conversion of violaxanthin (V) into anteraxanthin (A) and zeaxanthin (Z) and, when water potential decreased below −5 MPa, a slight decrease of \(Fₛ/Fₘ\) (after 30 min of darkness). However, *Q. coccifera* and *Q. ilex* ssp. *ballota* did not follow this pattern. At predawn, when predawn water potential decreased below −3 MPa, A + Z were progressively retained overnight, which was accompanied by gradual decreases in the predawn \(Fₛ/Fₘ\) ratios. In the final stages of the drought period (about −7 MPa) practically all the pool of the xanthophylls cycle pigments was in de-epoxidated forms (A + Z) and \(Fₛ/Fₘ\) ratios were −0.3–0.4 for *Q. coccifera* and *Q. ilex* ssp. *ballota* (Fig. 2).

The low predawn \(Fₛ/Fₘ\) ratios were mostly due to a water-stress mediated quenching of \(Fₘ\) in the case of *Q. coccifera* and to large increases of \(F₀\) in the case of *Q. ilex* ssp. *ballota* (Fig. 2).

At midday, large decreases in \(Fₛ/Fₘ\) occurred below −4 MPa with the xanthophylls cycle pool almost fully de-epoxidated \([A + Z]/(A + Z) = 0.8\)–0.91 (Fig. 2). \(F₀\) and \(Fₘ\) behave similarly to predawn in *Q. coccifera* and *Q. ilex* ssp. *ballota*, whereas *Q. suber* showed a quenched \(Fₘ\) with respect to predawn (Fig. 2).
There was a strong correlation between $F_v/F_m$ and $(A+Z)/(V+A+Z)$ at predawn ($R^2 = 0.77, F = 16.56, P = 0.007$, Fig. 3). This was mostly because values obtained for *Q. coccifera* and *Q. ilex* ssp. *baltica*, since both $F_v/F_m$ and $(A+Z)/(V+A+Z)$ remained fairly constant for *Q. suber* (see Fig. 2). This correlation was not as good at midday, because there was large water stress-mediated decreases of $F_v/F_m$ with the xanthophyll cycle pigments in de-epoxidized forms $(A+Z)/(V+A+Z) = 0.8-0.9~(F ~3$).

The actual PSII efficiency ($\Phi_{PSII}$) decreased when water potential diminished in the three species investigated (Fig. 4), which was due to both closure of PSII reaction centres (decreased photochemical quenching, qP) and the efficiency of those PSII centres that remained open at steady-state photosynthesis (decreased intrinsic PSII efficiency, $\Phi_{exc}$) (Fig. 4). At the beginning of the experiment, $\Phi_{PSII}, qP$ and $\Phi_{exc}$ values were higher in *Q. suber* than in the other two species. At the end of the drought period, the fraction of light absorbed that is dissipated in the PSII antenna $(1 - \Phi_{exc})$ was higher for *Q. coccifera* and *Q. ilex* ssp. *baltica* than for *Q. suber* (Fig. 4).

Dark respiration (DR) and photorespiration (PR) decreased in all species with severe drought, with the exception of photorespiration in *Q. ilex* ssp. *baltica*, which did not show statistically significant differences between S2 and CO, although it was decreased in S1 (Table 1). The ETR/A and ETR/(A + DR) ratios increased for all species with severe drought (Table 1). These increases were higher for *Q. coccifera* and *Q. ilex* ssp. *baltica*, highlighting the differences between these species and *Q. suber* when midday water potential was below −6 MPa. However, the ETR/(A + DR + PR) ratios were higher for *Q. suber* in S2 due to the lower value of PR found for *Q. suber* in S2 in relation to *Q. coccifera* and *Q. ilex* ssp. *baltica*.

Severe drought did not modify the photosynthetic pigment composition of the three oak species (Table 2), excepting changes within the xanthophyll cycle (see Fig. 2). Chl a and the chl a/b ratio decreased only in *Q. suber* when drought stress
was maximal (referred as S2 in Table 2), and V+A+Z increased only in *Q. cocifera* in S2 (Table 2).

**Discussion**

This work reports marked differential performance of three Mediterranean evergreen oaks, *Quercus cocifera*, *Quercus ilex* ssp. *bailota* and *Quercus suber*, in response to an intense water stress. Differences appeared once CO$_2$ fixation was almost negligible (at $\sim$4 MPa), and increased when drought continued progressing (down to $-7$ or $-8$ MPa). Drought can reduce the net CO$_2$ assimilation in three ways: limiting the entrance of CO$_2$ into the leaf (stomatal limitation); decreasing the CO$_2$ diffusion within the mesophyll (mesophyll limitation); or inhibiting the photochemical and metabolic processes associated with photosynthesis (photochemical and enzymatic limitations) (Flexas *et al.* 1998, 2002; Flexas and Medrano 2002). Under
such circumstances, since the light harvesting complexes of both PSI and PSII continue collecting light, an excess of excitation energy can occur that can or cannot be directed to the photosynthetic electron transport chain. Electrons not consumed in CO₂ fixation, may react with O₂ generating reactive oxygen species and increasing the possibility of oxidative damage. *Q. cocifera*, *Q. ilex* ssp. *ballota* and *Q. suber* responded to intense water stress through photoprotective mechanisms, different for each species, which allowed them to avoid damage to the photosynthetic apparatus or to evidence some signs of photo-damage (see below).

Thermal dissipation of the energy excess at midday was common to all species, although with some differences. The amount of absorbed energy that was dissipated in the PSII antenna (1−Φexc) reached 55% in *Q. suber* at the end of the drought period, whereas in the other two species it was 65%. Differences were also observed in the functioning of the xanthophylls cycle. In *Q. suber*, most of the midday A+Z were converted into V during the night, irrespective of the degree of water stress. However, intense water-stressed *Q. cocifera* and *Q. ilex* ssp. *ballota* plants retained overnight the de-epoxidated forms A+Z accumulated during the day. This behaviour was much more evident at the final stages of the drought period, and coincided with the decrease of predawn Fv/Fm ratios (see the correlation between predawn Fv/Fm and (A+Z)/(V+A+Z) ratios in Fig. 3).

The persistence of A+Z (not only at midday, but also) at predawn allow us to suggest that the photo-protection strategy of *Q. cocifera* and *Q. ilex* ssp. *ballota* could be related in some way to the existence of a permanent low luminal pH <5.8 at the end of the drought period. It is known that V de-epoxidase enzyme is inactive above pH 6.5 and operates at an optimum pH below 5.8 (Pfündel and Dilley 1993). The persistence of a low luminal pH would inhibit the inverse reaction of epoxidation to V along the evening and during the night. The absence of a rapid PRI (physiological reflectance index) change in response to sudden increase in light intensity also suggests the presence of a low luminal pH in severe water stressed *Q. cocifera* plants (Peguero-Pina et al. 2008). Other authors (Dennmig-Adams and Adams 2006; Demmig-Adams et al. 2006; Zarter et al. 2006) have pointed out that the dissipation of the energy excess could be linked with PsbS-related proteins in long-lived, slow-growing evergreen species with limited intrinsic capacity for photosynthesis, and ability to survive prolonged periods of severe environmental conditions precluding growth.

Data indicate that the mechanisms of photo-protection against intense summer drought stress could be different in the three species investigated. All oak species downregulated photosynthetic electron transport in response to intense drought, with ETR values all decreasing from 273 (*Q. suber*) or 164 (*Q. cocifera* and *Q. ilex* ssp. *ballota*) to 109 µmol electrons m⁻² s⁻¹ at midday, although doing so in different ways. After an intense drought, *Q. suber* downregulated ETR values closing PSI reaction centres (reflected in a decreased qP) and through an A+Z-mediated diminished intrinsic PSII efficiency (reflected in a decreased Φexc). Although this was also observed in the other two oak species, changes were much less marked. *Q. cocifera* and *Q. ilex* ssp. *ballota*, in addition to downregulate ETR through qP.

**Fig. 4.** Time course of actual PSII efficiency (Φpsii), photochemical quenching (qP), and the fraction of energy dissipated in the PSII antenna (1−Φexc) measured at midday (solar time) with midday water potential (MWP) for *Quercus cocifera* (black dots, solid line), *Q. ilex* ssp. *ballota* (grey dots, dashed line) and *Q. suber* (white dots, dotted line). Error bars indicate the s.e. of the mean value of five measurements.
Severe drought in evergreen oaks

and $\Phi_{\text{esc}}$ changes, photo-inactivated PSII centres, decreasing further ETR. This was evidenced by their low predawn $F_{v}/F_{m}$ ratios after the intense drought stress. The bases for this PSII inactivation are not known (see above for discussion), but seem to be different in *Q. coccifera* and *Q. ilex* ssp. *baltica*. In the former, the decreased $F_{v}/F_{m}$ ratios were due to $F_{0}$ quenching, whereas in the latter a large $F_{0}$ increase was observed. The basis for this PSII inactivation is a matter that deserves further investigation.

Alternative electron sinks may help plants to cope with excess electrons generated through the photosynthetic electron transport chain when the intense water stress close stomata and largely decreases photosynthetic rates. Although all oak species downregulate photosynthetic ETR in response to intense drought, the largest unbalance between electrons generated photosynthetically and consumed in photosynthesis, dark respiration and photorespiration processes was observed in *Q. suber* (see Table 1). This would imply a potential risk of oxidative damage in this species, if electrons in excess react with $O_{2}$ generating reactive oxygen species. Further, this seems to be the case. This is clearly seen in the chlorophylls, noticeably in chl a, one of the first targets of oxidative damage, which was significantly lowered at the end of the experiment when water stress was intense (see Table 2). In *Q. coccifera* and *Q. ilex* ssp. *baltica*, the photosynthetic pigment composition remained fairly unchanged during the whole drought period, despite interconversions within the xanthophylls cycle.

In the case of *Q. coccifera* and *Q. ilex* ssp. *baltica*, we would not interpret predawn $F_{v}/F_{m}$ decreases as permanent damage to the photosynthetic apparatus because: (i) there was not evidence of oxidative damage to photosynthetic pigments during the whole drought period, and (ii) plants recovered normal physiological parameters of well irrigated conditions only a few hours after irrigation, whereas *Q. suber* did not and died after the intense drought stress (data not shown). Furthermore, the preservation of an intact photosynthetic pigment machinery in *Q. coccifera* and *Q. ilex* could contribute to the rapid recovery of these species after a long summer stress period. This fact should not be attributed to the duration of the experiment, because this pattern has been also observed with longer periods of drought (Peguero-Pina et al. 2008).

The view that these $F_{v}/F_{m}$ decreases as an additional protective mechanism suggests that *Q. coccifera* and *Q. ilex* ssp. *baltica* seem more able than *Q. suber* to acclimate to more

| Table 1. Temporal evolution of dark respiration (DR), photorespiration (PR) and the ratios between electron transport rate (ETR) and, respectively, net CO₂ assimilation (A), (A + DR), and (A + DR + PR), in *Quercus coccifera*, *Q. ilex* ssp. *baltica* and *Q. suber* at predawn at three stages of the experiment: control (CO), around the loss turgor point (S1) and when drought stress was maximal (S2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Dark respiration (µmol CO₂ m⁻² s⁻¹)</th>
<th>Photorespiration (µmol CO₂ m⁻² s⁻¹)</th>
<th>ETR/A</th>
<th>ETR/(A + DR)</th>
<th>ETR/(A + DR + PR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Q. coccifera</em></td>
<td>2.04 ± 0.72a</td>
<td>2.90 ± 1.24a</td>
<td>12.78</td>
<td>10.96</td>
<td>9.11</td>
</tr>
<tr>
<td>S1</td>
<td>1.78 ± 0.20a</td>
<td>1.20 ± 0.04b</td>
<td>93.91</td>
<td>81.94</td>
<td>74.14</td>
</tr>
<tr>
<td>S2</td>
<td>0.84 ± 0.12b</td>
<td>1.45 ± 0.46a</td>
<td>166.84</td>
<td>143.29</td>
<td>128.53</td>
</tr>
<tr>
<td><em>Q. suber</em></td>
<td>3.40 ± 0.10a</td>
<td>7.22 ± 0.02a</td>
<td>17.62</td>
<td>14.27</td>
<td>12.96</td>
</tr>
<tr>
<td>S1</td>
<td>4.02 ± 0.28a</td>
<td>0.94 ± 0.18b</td>
<td>23.51</td>
<td>19.73</td>
<td>17.90</td>
</tr>
<tr>
<td>S2</td>
<td>0.40 ± 0.15b</td>
<td>1.14 ± 0.04b</td>
<td>112.38</td>
<td>88.58</td>
<td>75.70</td>
</tr>
<tr>
<td><em>Q. ilex</em></td>
<td>1.67 ± 0.38a</td>
<td>5.02 ± 0.74a</td>
<td>13.46</td>
<td>11.18</td>
<td>9.80</td>
</tr>
<tr>
<td>S1</td>
<td>1.54 ± 0.29a</td>
<td>0.66 ± 0.40b</td>
<td>28.81</td>
<td>22.89</td>
<td>21.06</td>
</tr>
<tr>
<td>S2</td>
<td>0.40 ± 0.03b</td>
<td>4.42 ± 0.67a</td>
<td>322.92</td>
<td>264.20</td>
<td>237.74</td>
</tr>
</tbody>
</table>

Data are mean ± s.e. of five measurements. Different letters within columns indicate significant differences at $P<0.05$

| Table 2. Temporal evolution of photosynthetic pigment composition in *Quercus coccifera*, *Q. ilex* ssp. *baltica* and *Q. suber* at predawn at three stages of the experiment: control (CO), around the loss turgor point (S1) and when drought stress was maximal (S2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Chlorophylls</th>
<th>Carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl a</td>
<td>Chl b</td>
</tr>
<tr>
<td><em>Q. coccifera</em></td>
<td>320.7 ± 22.1a</td>
<td>69.6 ± 10.4a</td>
</tr>
<tr>
<td>S1</td>
<td>270.9 ± 10.3a</td>
<td>82.1 ± 3.8a</td>
</tr>
<tr>
<td>S2</td>
<td>290.7 ± 18.1a</td>
<td>83.8 ± 9.1a</td>
</tr>
<tr>
<td><em>Q. suber</em></td>
<td>370.3 ± 28.4a</td>
<td>103.5 ± 14.8a</td>
</tr>
<tr>
<td>S1</td>
<td>380.2 ± 22.4a</td>
<td>103.4 ± 7.7a</td>
</tr>
<tr>
<td>S2</td>
<td>280.3 ± 23.8b</td>
<td>91.4 ± 8.7a</td>
</tr>
<tr>
<td><em>Q. ilex</em></td>
<td>332.3 ± 23.8a</td>
<td>88.5 ± 6.9a</td>
</tr>
<tr>
<td>S1</td>
<td>391.4 ± 52.7a</td>
<td>111.3 ± 19.3a</td>
</tr>
<tr>
<td>S2</td>
<td>346.1 ± 65.7a</td>
<td>97.3 ± 24.2a</td>
</tr>
</tbody>
</table>
stressful conditions. However, it can be concluded from literature that Q. coccifera is slightly more resistant to summer embolism (water potential at which a 50% loss in hydraulic conductivity occurs, PLC50 = -7 MPa, Vilagrosa et al. 2003) than Q. ilex ssp. ballota (PLC50 = -5.5 MPa, Corcuera et al. 2004) and Q. suber (PLC50 = -5 MPa, Tyree and Cochard 1996). Therefore, our results and those of other research groups, taken together, question the suggestions of several authors that Mediterranean evergreen oaks can be regarded as a homogeneous physiological group (Kummerov 1973; Poole and Miller 1975; Tenhunen et al. 1981; Ackerel and Rambal 1992; Turner 1994). Moreover, other authors showed that there were different strategies in the response to water deficit behind the convergence in leaf morphology of these species (Lo Gullo and Salleo 1988; Salleo and Lo Gullo 1990; Martinez-Vilalta et al. 2003; Baquedano and Castillo 2006), which was probably originated in the tropical vegetation of the Tertiary, before the origin of Mediterranean climate and vegetation in the Quaternary (Verdu et al. 2003; Ackerel 2004).

In summary, although Q. coccifera, Q. ilex ssp. ballota and Q. suber have all a large safety margin, with similar cavitation and loss turgor point water potentials (Corcuera et al. 2002), the performance of their photosynthetic machinery in response to an intense summer stress period varied markedly between species. Differences accentuated when the CO2 photosynthetic fixation rates were almost negligible. These findings indicate a PSII adaptation, which goes beyond the limits of genetic links and the common morphological traits of these species, showing that morphological convergence is not expressed in the physiological performance of these species under drought stress conditions.

Acknowledgements

We thank CITIA-DGA for technical support. This work was supported by the Spanish Ministry of Education and Science projects AGL2004-00194/AGR and BFU2004-05906/BFI, and by an University of Valencia/EEAD-CSIC contract – within the ESA ESRIN contract 19187/05/1-EC – to F. M. Moreover, this study was partially supported by INIA project SUM2008-00004-C03-03 (Ministerio de Ciencia e Innovación). Financial support from Gobierno de Aragón (A03 research group) is also acknowledged.

References


Manuscript received 21 November 2008, accepted 6 March 2009