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Diurnal changes in photoprotective mechanisms in leaves of cork oak (*Quercus suber*) during summer

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Summary Daily variations in photoprotective mechanisms were studied in sun and shade leaves of 40-year-old cork oak (*Quercus suber* L.) trees during early summer in Portugal. Although trees were not severely water stressed because predawn leaf water potentials remained high, photosynthesis and stomatal conductance decreased at midday. The midday depression in gas exchange was not reversed by short-term exposure to "optimal" conditions of temperature, light and vapor pressure deficit. Chlorophyll a fluorescence, maximum photochemical yield of photosystem II and the quantum yield of noncyclic electron transport showed midday depressions, but recovered by the evening. Both short-term changes in the components of the xanthophyll cycle (reversible de-epoxidation of violaxanthin during the day) as well as long-term changes (higher xanthophyll content in sun compared with shade leaves) were detected and may play a role in the dissipation of excess energy at midday. Because the activities of enzymes of the antioxidant system, superoxide dismutase and ascorbate peroxidase, were high enough to cope with the increase in oxygen reactive species likely to arise under the stressful conditions of midday, we conclude that these enzymes may provide an additional mechanism for energy dissipation.

Keywords: antioxidants, photoinhibition, photosynthesis, stomatal conductance, xanthophylls.

Introduction

Cork oak (*Quercus suber* L.) is a sclerophyllous evergreen Mediterranean tree species that is adapted to summer conditions characterized by a 4-month dry period with little or no precipitation, high temperatures with maxima that often reach 35–40 °C and high irradiance exceeding 2000 μmol m^{-2} s^{-1} (PAR) at midday. Among these environmental constraints, soil and atmospheric water deficits are the most important factors limiting photosynthesis and growth, although high irradiance and high temperature also contribute to the inhibition of net carbon uptake, especially in leaves exposed to the sun.

The decrease in stomatal conductance often observed in sclerophyllous trees during the warmest period of the day, even in the absence of a significant leaf water deficit, results in a midday depression of net photosynthesis (Tenhunen et al. 1984). The causes of this depression are not fully understood but seem to involve regulatory mechanisms at both the stomatal (Downton et al. 1988) and chloroplast levels (Correia et al. 1990, Chaumont et al. 1994). Because CO_2 fixation is the main sink for absorbed solar radiation energy and gas diffusion is severely restricted when stomatal conductance is reduced, chloroplasts may be subjected to an excess of energy at midday resulting in down-regulation of photosynthesis (Demmig-Adams et al. 1989). Excessive photon flux can also directly inhibit carbon uptake (Krause 1994). Protection against excess solar energy may be achieved by an increase in the dissipation of excess excitation energy, which is accompanied by a decrease in the quantum yield of photosystem (PS) II (Genty et al. 1989), or by increasing the catabolism of active oxygen species ($^1O_2$, O_2^- and H_2O_2). Energy dissipation is closely associated with the reversible formation of zeaxanthin by de-epoxidation of violaxanthin via antheraxanthin (Demmig and Björkmann 1987, Björkmann and Demmig-Adams 1994). Detoxification of active oxygen molecular species is undertaken by a system of physiological antioxidants, which can be lipophilic (tocopherol, carotenoids) or hydrophilic (glutathione, ascorbate) (Polle and Rennenberg 1994). The production of singlet oxygen ($^1O_2$), which occurs when the energy absorbed by chlorophyll is not dissipated through photosynthesis, can induce lipid peroxidation and oxidation of proteins and nucleic acids. Singlet oxygen can be quenched by the chloroplastic carotenoids (mainly β-carotene), either directly or indirectly, by preventing the formation of the chlorophyll triplet excited state (Larson 1988). The removal of the chlorophyll singlet excited state (by the combined effects of zeaxanthin and antheraxanthin, and a low pH within the thylakoid membrane) should also prevent the formation of the chlorophyll triplet excited state. Although there is controversy over the exact mechanism of the de-excitation of the chlorophyll singlet excited state (Horton et al. 1994), there is a growing consensus that it is the chlorophyll singlet excited state that is removed. Superoxide (O_2^-) may be produced as a consequence of the reduction of O_2 by PSI and is detoxified by superoxide dismutase (SOD) (Asada and Takashashi 1987). The product of this reaction, H_2O_2, is reduced by a four-step enzymatic pathway

involving ascorbate and glutathione as cosubstrates (Asada and Takashashi 1987, Foyer et al. 1994).

Because drought, high irradiance and high temperature may enhance the production of reactive oxygen species, an increase in the antioxidant systems of leaves might act as a protective system during the hot dry Mediterranean summer. Although the antioxidant system of coniferous trees has been studied in relation to some environmental stresses, such as high altitude (Polle and Rennenberg 1992), pollutants (Wingsle and Hällgren 1993) and low temperatures (Nakagara and Sagisaka 1984), few studies have focused on the activity of such systems in Mediterranean sclerophyllous species. However, it has been shown that leaves of herbaceous plants acclimated to sunny habitats have a larger pool of the xanthophyll cycle components than shade leaves (Demmig-Adams and Adams 1992) indicating a higher potential for thermal energy dissipation in sun leaves than in shade leaves. In addition, large increases in zeaxanthin content during the period of peak irradiance were reported in leaves of the Mediterranean shrub *Arbutus unedo* L. (Demmig-Adams et al. 1989). Also, recent data for conifers (Adams and Demmig-Adams 1994) and two broad-leaved evergreen species (Adams and Demmig-Adams 1995) indicate that much more zeaxanthin is present in leaves at dawn following chilling nights than following warm nights.

We have characterized the responses of the various systems associated with the regulation of carbon assimilation to high light and temperature in leaves of *Q. suber* trees growing in the field in early summer. Besides measuring CO_2 uptake and stomatal conductance in early morning, midday and evening, we also related the components of the zeaxanthin–violaxanthin (VAZ) cycle and various enzymes of the antioxidant system with parameters indicative of photochemical efficiency and dissipation of excess excitation energy.

Materials and methods

Field site and plant material

The study was carried out on 40-year-old *Q. suber* trees at Herdade do Paço de Camões, Azaruja, near Évora, in central Portugal (38°34′ N, 07°54′ W). The site has a Mediterranean climate with hot dry summers, during which maximum temperatures exceed 30 °C on 88% of the days, and the average precipitation per month is less than 5 mm.

The experiments were conducted on clear days in June 1994 before the occurrence of severe water deficits. During the measurements, daily maximum temperatures ranged between 33 and 35.5 °C. All measurements were performed on sun and shade leaves of six trees at (solar time): predawn (about 0430 h), morning (about 0800 h) (3 h after sunrise), midday (1200 h) and evening (1700 h) (3 h before sunset). For the biochemical analyses, individual leaf samples were collected, immediately frozen in liquid nitrogen and stored at −80 °C until analyzed.

Methods

Predawn and midday leaf water potentials (Ψ) were determined with a Scholander pressure chamber (PMS Instrument Co., Corvallis, OR). Specific leaf area (SLA) was calculated as the ratio between area and dry weight (after 48 h at 80 °C) of leaf discs. The nitrogen concentration of leaf tissue was measured by the standard Kjeldahl method (AOAC 1980) with a Kjeltec 1030 analyzer (Tecator, Sweden). The carbohydrate concentrations of leaves were measured as described by Stitt et al. (1989).

Carbon assimilation rate (A) and transpiration rate were measured under natural light conditions with a portable CO_2/H_2O analyzer (LCA 4, Analytic Development Corp., Hoddesdon, Herts, U.K.). Photosynthesis and stomatal conductance at 22.5 and 32.5 °C were measured at ambient CO_2 concentrations, constant light (1300 μmol m^{-2} s^{-1}) and relative humidity (40%) with a compact minicuvette system (Heinz Walz, Effeltrich, Germany) with the bypass humidity control unit. Photosynthetic capacity, defined as the rate of carbon assimilation measured with saturating CO_2 and light, and oxygen quantum yield were determined in a leaf-disc oxygen electrode (Hansatech Ltd., Kings Lynn, U.K.). The measurements were made in the morning (about 0800 h) at 25 °C with saturating CO_2 (10% CO_2 provided by mixing air and CO_2), photosynthetic photon flux density (PFD) between 40 and 150 μmol m^{-2} s^{-1} (for O_2 quantum yield) and 1500 μmol m^{-2} s^{-1} (for photosynthetic capacity) immediately after detaching the leaves. Chlorophyll a fluorescence was measured with a portable pulse amplitude modulation fluorometer (PAM 2000, Heinz Walz). The efficiency of excitation energy capture by open PSII reaction centers was estimated by the fluorescence ratio (F_v/F_m) in dark-adapted leaves (30 min). The quantum yield of noncyclic electron transport (ϕ_e) of light-adapted leaves was calculated by the $(\phi F_m' - \phi F_s)/\phi F_m'$ ratio (Genty et al. 1989).

Pigments were extracted from frozen leaf discs, ground in a mortar with acetone (about 0.2 cm^2 of leaf tissue per ml of solvent) in the presence of sodium ascorbate and analyzed by HPLC as described by Rivas et al. (1989). The amounts of zeaxanthin (Z), antheraxanthin (A) and violaxanthin (V) are presented on a chlorophyll a basis as well as the ratio (A + Z)/(V + A + Z) (Adams et al. 1994), which shows the concentration of the de-epoxidized forms in relation to the total xanthophyll concentration.

Plant material was extracted for analysis of the antioxidant enzyme system as described by Polle et al. (1993) except for ascorbate peroxidase (APOD), monodehydroascorbate radical reductase (MDHAR) and dehydroascorbate reductase (DHAR), where 0.2% (w/v) ascorbate was added to avoid deactivation of these enzymes (Polle and Rennenberg 1994). At least three replicates of individual samples were analyzed. The crude extracts were used for the determination of glutathione reductase (GR) activity, measured directly as described in Wingsle and Hällgren (1993). The extracts were partly purified by Sephadex G-25 filtration (Polle et al. 1993) and used for the determination of SOD activity as described by Polle et al. (1989), catalase (CAT) activity as described by Aebi (1983), and guaiacol peroxidase (GP) activity as described by Polle et al. (1990). From the extracts to which 0.2% ascorbate was added, we determined ascorbate peroxidase and dehy-

droascorbate reductase activities as described by Asada (1984), and monodehydroascorbate radical reductase (MDHAR) activity was measured by following the disappearance of NADH at 340 nm in the presence of ascorbate and ascorbate oxidase.

A commercial Bio-Rad protein assay was used to measure soluble protein content by the Bradford method (Bradford 1976).

Results

Under natural conditions, stomatal conductance (g_s) and net carbon assimilation rate (A) of sun leaves were highest during the morning, declined substantially by midday and showed almost no recovery in the afternoon (Figure 1). The midday decline occurred even though the plants were not suffering severe water deficits as indicated by the high predawn leaf water potentials (−0.31 ± 0.12 MPa). The midday leaf water potential was −2.26 ± 0.25 MPa. The high positive linear correlation between photosynthesis and stomatal conductance suggests that stomatal closure was the main factor limiting photosynthesis under the experimental conditions (Figure 2).

Measurements of CO_2 uptake in the morning and at midday at constant saturating light, leaf to air water vapor pressure deficit and ambient CO_2, but at two different temperatures (22.5 and 32.5 °C) indicated that the declines in g_s and A from morning to midday persisted even in a constant environment (Figure 3). Moreover, the afternoon decline in gas exchange could not be reversed by placing the leaves under the conditions naturally prevailing in the morning, i.e., 22.5 °C. These findings suggest that the midday depression in gas exchange rates is a feature of endogenous metabolic regulation rather than a short-term response to light, temperature or the leaf to air water vapor pressure deficit.

The pool of soluble sugars remained approximately constant

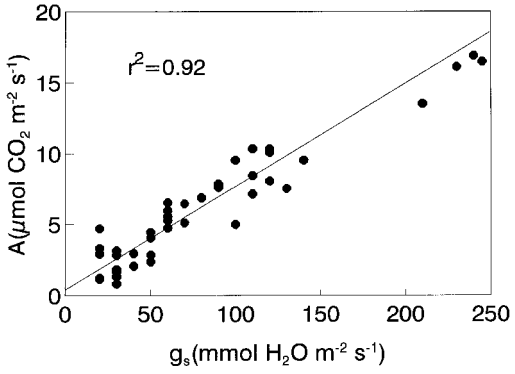

Figure 2. Relationship between net photosynthesis (A) and stomatal conductance (g_s) in sun leaves of *Q. suber.*

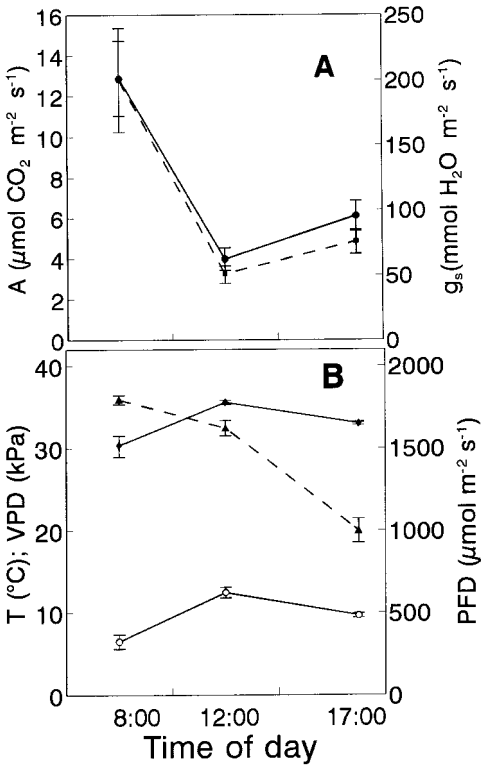

Figure 1. (A) Diurnal changes in net photosynthesis (A) (●) and stomatal conductance (g_s) (■) of sun leaves of *Q. suber*. (B) Diurnal variation in temperature (◆), solar radiation (PFD) (▲) and water vapor pressure deficit (○) during the experiment. Error bars represent the standard errors (SE) of at least five replicates.

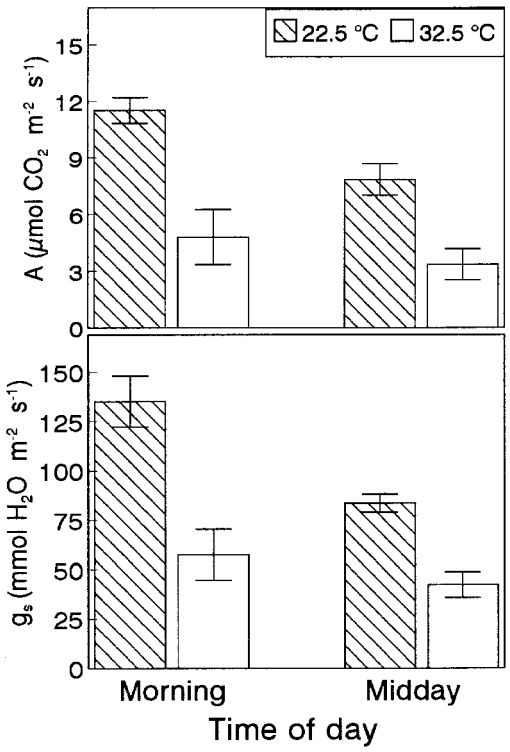

Figure 3. Photosynthesis (A) and stomatal conductance (g_s) in sun leaves of *Q. suber* measured at 22.5 °C (cross-hatched bars) and 32.5 °C (open bars) at ambient CO_2 concentration, constant PFD (1300 μmol m^{-2} s^{-1}) and a relative humidity of 40%. Error bars represent the SE of at least three replicates.

up to midday and only increased in the late afternoon (Figure 4). There were no significant differences in soluble sugar content between sun and shade leaves. However, sun and shade leaves differed in several other characteristics (Table 1). Sun leaves had significantly lower (27%) SLA than shade leaves, which corresponds to thicker leaves. The chlorophyll content per unit of area was the same in both leaf types, but chlorophyll concentration per unit of dry weight was slightly higher in shade leaves than in sun leaves. The ratio of chlorophyll a to chlorophyll b was significantly lower in shade leaves than in sun leaves. Both leaf types had similar carotenoid concentrations per unit of dry weight, but shade leaves had significantly lower concentrations than sun leaves when carotenoid content was expressed per unit of leaf area, implying that sun leaves contained more carotenoid molecules per chlorophyll molecule than shade leaves. Compared with shade leaves, sun leaves also showed a significantly higher photosynthetic capacity at 25 °C but a lower quantum yield of oxygen evolution.

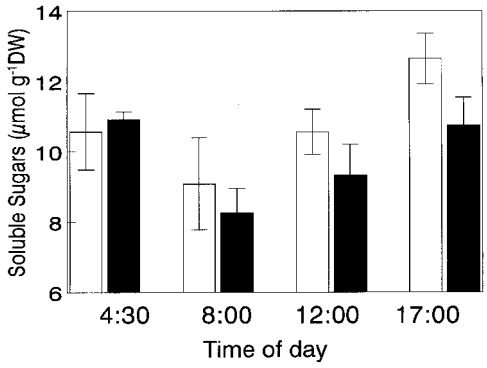

Figure 4. Diurnal changes in soluble sugar concentrations in sun (open bars) and shade (solid bars) leaves of *Q. suber*. Error bars represent the SE of at least four replicates.

Table 1. Morphological and physiological characterization of sun and shade leaves of cork oak (*Quercus suber*).

Parameter	Sun leaves	Shade leaves
Specific leaf area ($cm^2\ kg^{-1}$)	5.85 ± 0.35	8.60 ± 0.03
Fresh weight/dry weight ratio	5.20 ± 0.51	5.48 ± 0.39
Nitrogen concentration (% dry weight)	1.58 ± 0.02	1.57 ± 0.04
Protein content (mg g^{-1} fresh weight)	54.50 ± 8.68	42.89 ± 7.02
Chlorophyll concentration (μmol m^{-2})	615.5 ± 28.9	610.4 ± 38.7
Carotenoid concentration (μmol m^{-2})	173.8 ± 19.9	126.9 ± 6.4
Chlorophyll a/b ratio	3.38 ± 0.08	3.20 ± 0.10
Chlorophyll/carotenoids mole ratio	3.75 ± 0.42	4.93 ± 0.69
Photosynthetic capacity at 25 °C (μmol $O_2\ m^{-2}\ s^{-1}$)	13.73 ± 2.76	8.9 ± 0.71
Quantum yield of oxygen evolution (μmol $O_2\ m^{-2}\ s^{-1}$)	0.070	0.088

The maximum photochemical efficiency of PSII (given by F_v/F_m in dark-adapted leaves) of shade leaves remained constant during the day, whereas in sun leaves, it decreased significantly by 12% during the midday depression as a result of the increase in maximal fluorescence (F_m) (Figure 5). The midday decrease in F_v/F_m was reversed by the late afternoon (1700 h), which contrasts with the absence of recovery of the photosynthetic rate during the late afternoon. The actual quantum yield of noncyclic electron transport (given by $\Delta F/F_m'$) decreased slightly but significantly at midday (20% in sun leaves and 8% in shade leaves), and this decline was also reversed by 1700 h (Figure 6).

The concentration of xanthophylls (V + A + Z) was twofold higher in sun leaves than in shade leaves (Figure 7). On the other hand, the ratio A + Z/(V + A + Z) increased at midday in both types of leaves (Figure 7), corresponding to increases in antheraxanthin and zeaxanthin at the expense of violaxanthin in both leaf types (Figure 8). The ratio (A + Z)/(V + A + Z) declined to predawn values during the late afternoon. The other carotenoids (neoxanthin, lutein, β-carotene) showed a nonsig-

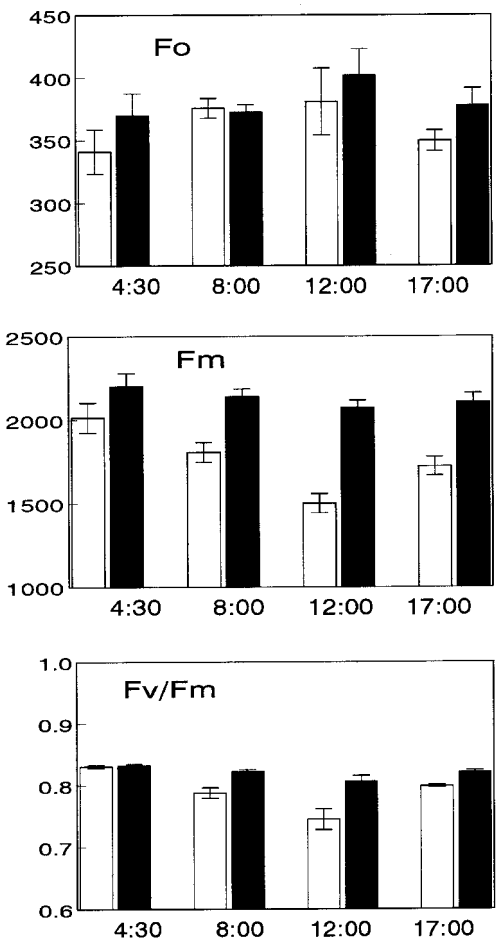

Figure 5. Diurnal changes in basal (F_o) and maximal (F_m) chlorophyll fluorescence and maximal photochemical efficiency of PSII (F_v/F_m) in sun (open bars) and shade (solid bars) leaves of *Q. suber*. Error bars represent the SE of at least six replicates.

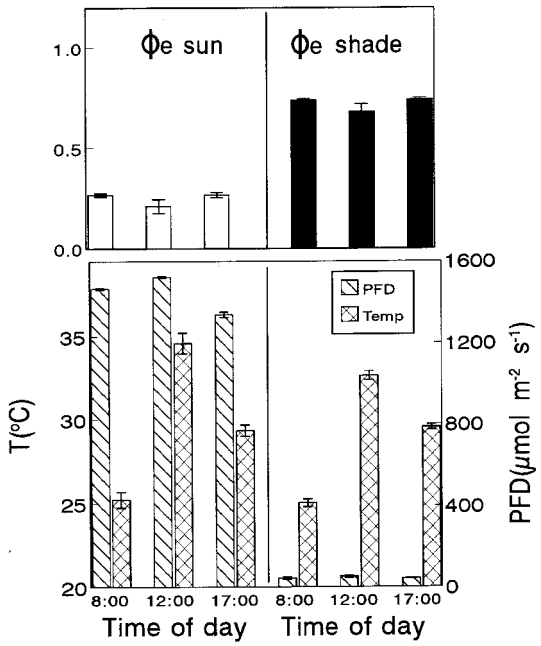

Figure 6. Diurnal changes in the actual quantum yield of noncyclic electron transport (ϕ_e) in sun (open bars) and shade (solid bars) leaves of *Q. suber*, measured under the temperature and PFD conditions indicated in the figure. Error bars represent the SE of at least 10 replicates.

nificant decrease in both shade and sun leaves at midday (Figure 9). The chlorophyll a + b concentrations did not change throughout the day (Figure 9).

The activities of SOD and APOD of sun leaves did not vary during the day (Table 2). Glutathione reductase and DHAR activities increased three- to fourfold between predawn and midday; however, their activities were 15 to 40 times lower than APOD activity. Monodehydroascorbate radical reductase (MDHAR) activity also increased significantly, but to a lesser extent than the activities of GR and DHAR. Catalase and GP activities both exhibited a significant decline between the predawn and midday measurements.

Discussion

Despite numerous reports of a midday depression in gas exchange of leaves of Mediterranean sclerophyllous trees and shrubs, including *Q. suber*, occurring before the onset of severe water deficits in summer (Stocker 1956, Lange et al. 1982, Tenhunen et al. 1984, Pereira and Chaves 1993), the causes underlying this response have not been elucidated. Stomatal closure in response to an increased leaf to air water vapor pressure deficit is likely to occur during the warmest part of the day. However, the inhibition was still observed when we measured A and g_s at the same light, temperature and leaf to air water vapor pressure deficit as would naturally occur in the morning. It is possible that the stomata closed at midday as a result of either an increase in ABA concentration in the transpiration stream or the interaction of this signal with low leaf water potential (Gowing et al. 1993, Tardieu et al. 1993). However, in grapevine plants growing in the field under conditions comparable to those of the present study, we could not explain the midday decline in g_s by an increase in xylem ABA concentration or the rate of delivery of this compound by the transpiration stream (Correia et al. 1995). We cannot rule out the

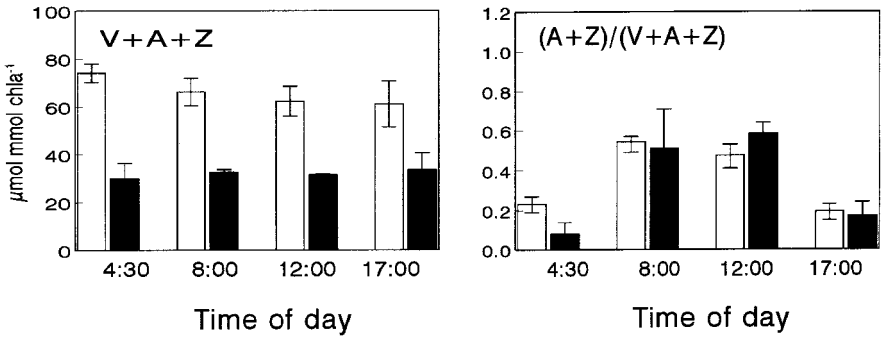

Figure 7. Total xanthophyll concentration (V + A + Z) and the (A + Z)/(V + A + Z) ratio in sun (open bars) and shade (solid bars) leaves of *Q. suber*. Error bars represent the SE of at least four replicates.

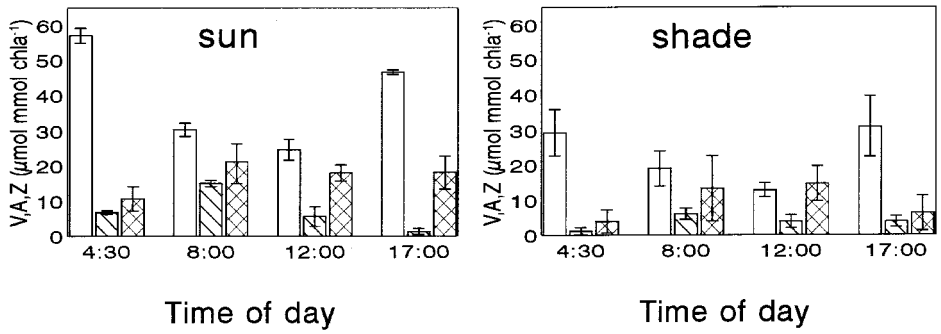

Figure 8. Diurnal changes in violaxanthin (open bars), antheraxanthin (diagonally dashed bars) and zeaxanthin (crossed bars) in sun and shade leaves of *Q. suber*. Error bars represent the SE of at least four replicates.

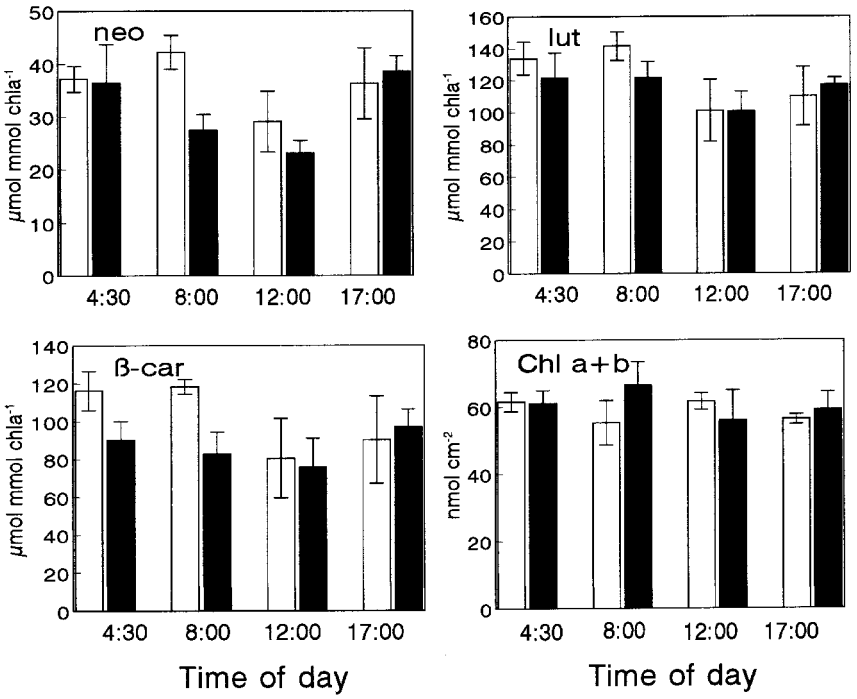

Figure 9. Diurnal changes in neoxanthin (neo), lutein (lut), β-carotene (β-car), and chlorophyll a + b in sun (open bars) and shade (solid bars) leaves of *Q. suber*. Error bars represent the SE of at least four replicates.

Table 2. Predawn and midday activities of SOD, APOD, MDHAR, DHAR, GR, CAT and GP in sun leaves of *Q. suber*. Units are expressed as follows: SOD in units g^{-1} fresh weight; APOD, MDHAR, DHAR, GR, CAT and GP in µmol substrate g^{-1} fresh weight min^{-1}. See text for abbreviations.

Enzyme	Predawn	Midday
SOD	2140.70 ± 505.7	2154.9 ± 284.9
APOD	21.65 ± 3.36	20.72 ± 4.50
MDHAR	3.34 ± 0.62	6.69 ± 2.12
DHAR	0.08 ± 0.05	0.32 ± 0.07
GR	0.50 ± 0.05	1.53 ± 0.08
CAT	21.51 ± 2.98	12.47 ± 1.10
GP	12.53 ± 1.17	5.88 ± 0.41

effects of localized epidermal water deficits on guard cells or the occurrence of endogenous diurnal rhythms on stomatal closure (Snaith and Mansfield 1986, Hagemeyer and Waisel 1987).

The midday depression in mesophyll photosynthesis has been attributed to feedback inhibition resulting from a high carbohydrate content in the source leaves during the day (Krapp et al. 1993). However, we did not find any accumulation of soluble sugars during the morning that could explain the inhibition of *A* by a feedback mechanism.

Down-regulation of PSII is likely to occur at midday as a result of an imbalance between energy input and utilization. Maximal photochemical efficiency (F_v/F_m) of sun leaves exhibited a slight reversible midday depression that occurred concomitantly with the increases in light and temperature. This depression of photochemical efficiency was caused by a small increase in basal fluorescence (F_o) and a more pronounced increase in maximum fluorescence (F_m). The quantum yield of noncyclic electron transport of sun leaves, measured at ambient irradiances (Figure 6), also showed a slight reversible depression at midday when leaf temperature reached its maximum. In shade leaves, the pattern of variation was similar, but more attenuated, than in sun leaves.

We conclude that *Q. suber* leaves have the ability to dissipate excessive light energy at midday by a nonphotochemical mechanism. One of the processes involved could be the conversion of violaxanthin to antheraxanthin and zeaxanthin which would lead to the observed increase in the (A + Z)/(V + A + Z) ratio (Figure 7). The xanthophyll cycle plays an important role in the defense system of leaves of plants subject to high light stress by increasing the content of zeaxanthin, which is involved in the nonradiative dissipation of excess energy (Demmig and Björkmann 1987). It is possible that these carotenoid changes also play a role in the thermoprotection of PSII at high temperatures (Havaux 1995). The increase in zeaxanthin content was associated with a decrease in the photochemical efficiency of PSII, and the increase was reversed in parallel with the de-epoxidation of zeaxanthin in the late afternoon and evening so that the leaves did not retain large amounts of zeaxanthin overnight, i.e., de-epoxidation was readily reversible. This is consistent with the findings of Demmig et al. (1988; see also Björkmann and Demmig-Adams 1994, Adams and Demmig-Adams 1994, 1995) who argue that retention of the de-epoxidized components of the xanthophyll cycle is affected by diurnal changes in temperature, being greatest on cold mornings and less on warm mornings, when photosynthesis can proceed at high rates.

The concentrations of neoxanthin, lutein and β-carotene and their proportions in relation to total carotenoids (Figure 9) did

not differ significantly during the day, and there were no significant differences between sun leaves and shade leaves. Similar results have been reported by Thayer and Björkmann (1990) and Adams and Demmig-Adams (1992).

The largest difference between the two types of leaves was a 37% increase in SLA in shade leaves compared with sun leaves. The chlorophyll a/b ratio was only slightly lower in shade than in sun leaves indicating a modest shade acclimation, presumably as a result of a relatively open canopy. This is consistent with the observation that the maximal fluorescence (F_m) measured before sunrise was only slightly higher in shade leaves than in sun leaves. We conclude that the two leaf types did not differ substantially in their light harvesting complex structure because the light harvesting pigments neoxanthin and lutein were present in similar concentrations when expressed per unit of chlorophyll mass. Sun leaves had higher concentrations of the photoprotective carotenoids, mainly xanthophyll, than shade leaves. However, the xanthophyll pool was relatively small compared with that determined for other species that develop leaves in deep shade (Demmig-Adams 1990, Thayer and Björkmann 1990, Demmig-Adams and Adams 1994). Nevertheless, the higher predawn values of the VAZ pool in sun leaves than in shade leaves may indicate that sun leaves have a higher capacity to respond to the rapid increase in the light environment by forming zeaxanthin in the early hours of the morning.

Superoxide dismutase activity of sun leaves was similar at predawn and midday. However, because SOD activity was relatively high compared with that of other tree species (e.g., *Picea abies* (L.) Karst.; Polle et al. 1989) and it indirectly protects chloroplastic membranes from lipid peroxidation (Larson 1988), we suggest that SOD activity is high enough to cope with the predicted increase in superoxide production at midday. A small response of SOD activity to environmental changes has also been reported under other potentially photoinhibitory conditions including cold acclimation (Badiani et al. 1993*a*) and water stress (Moran et al. 1994)

Dismutation of superoxide generates H_2O_2 which is removed by an APOD with ascorbate as cosubstrate (Asada 1992). The activity of APOD was high in the morning and remained constant at midday. It seems likely that the regeneration of ascorbate was mainly achieved by MDHAR because its activity was higher than the activities of GR and DHAR of the other pathway of regeneration. Badiani et al. (1993*b*) also found that MDHAR activity was 10 to 20 times higher than the activities of GR and DHAR. Nevertheless, the activities of the enzymes of both pathways increased at midday, especially the GR–DHAR pathway, which is responsible for maintaining a high reduced glutathione/oxidized glutathione ratio. The GR–DHAR pathway could also limit photooxidative damage by lowering the $NADPH/NADP^+$ ratio. Because CO_2 assimilation was reduced by 75% at midday, whereas the quantum yield of noncyclic electron transport only decreased by 15%, the excess of reducing power generated was likely diverted to photorespiration or to other NADPH-consuming processes. The threefold increase in GR and DHAR activities that we detected during midday could cope with much of this excess thereby providing an additional mechanism for energy dissipation, as suggested by Schreiber et al. (1994).

Because stomatal closure reduces the internal CO_2 concentration, and high temperature enhances the affinity of Rubisco for O_2, we predicted an increase in photorespiration during the midday depression in gas exchange. An increase in photorespiration may also play a role in protection against photodamage (Cornic et al. 1989). Photorespiration results in the production of H_2O_2 within the peroxisomes, and H_2O_2 is removed by catalase. However, in our study, CAT and GP activities decreased by 40–45% during the midday depression of gas exchange. The nature of these declines is unclear, although reductions in CAT and GP activities have been described in response to stress treatments (Badiani et al. 1993*a*, Moran et al. 1994).

In conclusion, *Q. suber* leaves undergo a midday depression of photosynthesis in the summer even before the occurrence of severe soil water deficits. This depression in carbon assimilation was strongly correlated with stomatal closure, and it was not relieved by short-term exposure to "optimal" conditions of temperature, light and air to leaf water vapor pressure deficit during the afternoon. Simultaneously with the midday depression of gas exchange, a slight reversible depression of photochemical efficiency was observed that was associated with short-term changes in the xanthophyll cycle (reversible de-epoxidation). In the long-term, acclimation to the light environment prevailing during ontogeny led to a higher xanthophyll content in sun than in shade leaves. These adjustments may play a role in the protection against excess light and high temperatures (Björkmann and Demmig-Adams 1994, Havaux 1995). High activities of the enzymes of the antioxidant system, namely SOD and APOD, were maintained throughout the day, suggesting that these enzymes catalyze the catabolism of the oxygen reactive species that arise under the stressful conditions of midday. The midday increase in the activities of the enzymes involved in the regeneration of the reduced state of antioxidative substrates (e.g., ascorbate and reduced glutathione) could provide an additional mechanism for energy dissipation during the midday period.

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