Leaf reflectance and photo- and antioxidant protection in field-grown summer-stressed Phillyrea angustifolia. Optical signals of oxidative stress?

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Summary

• Here we aimed to assess the photo and oxidative stress linked to the Mediterranean summer conditions characterized by drought, heat and high irradiance, and to find possible optical signals of such changes.
• Foliar reflectance was measured in parallel to leaf water content, gas exchange, chlorophyll fluorescence, extent of oxidative stress, and mechanisms of photo- and antioxidative protection in leaves of the evergreen Phillyrea angustifolia (Oleaceae) plants exposed to Mediterranean field spring-summer conditions.
• Relative leaf water content (RWC) of nonirrigated plants (WS) decreased from 82 to 50% after 48 d without rain, and net photosynthetic rates and stomatal conductances decreased c. 90%, but there was no photo- and oxidative damage. The amounts of the photoprotector zeaxanthin and of the antioxidant α-tocopherol increased up to threefold. The β-carotene concentration decreased to 50%. The reflectance indices WI (water index), PRI (photochemical reflectance index), and BPI (brown pigment index) followed the changes in water content, photosynthetic rates, and oxidant status, respectively.
• These results show that severe drought leads to oxidative stress in field-grown P. angustifolia plants, highlights the significance of photo- and antioxidative protection for the survival of drought-stressed plants, and indicates that the changes in oxidant status may be assessed by leaf reflectance (brown pigment index, BPI).

Key words: reflectance, reflectance indices, antioxidants, relative water content (RWC), net photosynthetic rates, summer drought, Phillyrea angustifolia.

Abbreviations

AOS, activated oxygen species; α-T, α-tocopherol; β-C, β-carotene; BHT, butylated hydroxytoluene; BPI, brown pigment index; Chl, chlorophyll a + b; DPS, de-epoxidation state of the xanthophyll cycle; $F_v$ : $F_m$, maximum efficiency of photosystem II photochemistry; H, hydration of leaves; HPLC, high-performance liquid chromatography; IR plants, irrigated plants; L, lutein; LMA, leaf mass area; MDA, malondialdehyde; PPFD, photosynthetically active photon flux density; PRI, photochemical reflectance index; PSI, photosystem II; RWC, relative leaf water content; TW, turgid weight; VPD, vapour pressure deficit; WI, water index; WS plants, nonirrigated plants; Z, zeaxanthin.

Introduction

Soil water availability represents a major environmental constraint under Mediterranean summer conditions (di Castri, 1981). Together with air temperature and solar radiation, it determines the cyclic pattern of vegetation activity. Survival in a Mediterranean climate depends, among other factors, on the ability of plants to make good use of the usually scarce and unpredictable rainfall water during the summer, when water deficit is superimposed on high radiation and high temperatures (Pereira & Chaves, 1993). Many Mediterranean species are evergreen and therefore are ready to use small rain events and short favourable meteorological conditions, but at the same time, they need to further invest in protection to avoid photo- and oxidant damage. Currently, climatic studies of the Mediterranean region have shown a trend of increasing temperature and drought and unpredictability of rainfall in recent decades (Pifol et al., 1998; Houghton et al., 2001; Peñuelas et al., 2002; Peñuelas & Boada, 2003). General circulation models for the next decades forecast even more and stronger droughts for the Mediterranean regions (Houghton et al., 2001).

A number of environmental stresses, including drought, high temperatures and high irradiance, may lead to an imbalance between antioxidant defences and the amount of activated oxygen species (AOS) resulting in oxidative stress (Smirnoff, 1993; Pastori & Foyer, 2002; Xiong et al., 2002). Although AOS are necessary for intracellular signalling (Doke, 1997; Foyer & Noctor, 1999), accumulation of AOS can cause damage at various levels of organization, including chloroplasts (Halliwell & Gutteridge, 1989; Asada, 1999). Apart from the xanthophyll cycle, photosynthesis and other changes in metabolic activity, which may protect the chloroplasts from oxidative damage (Demmig-Adams & Adams, 1996; Kozaki & Takeba, 1996; Eskling et al., 1997; Osmond et al., 1997; Peñuelas & Llusia, 2002), a number of enzymatic and nonenzymatic antioxidants are present in chloroplasts that serve to control oxygen toxicity (Smirnoff, 1993; Foyer et al., 1994; Asada, 1999). Among the latter, tocopherols (vitamin E) and carotenoids play an important role maintaining the integrity of the photosynthetic membranes under oxidative stress (Havaux, 1998; Munné-Bosch & Alegre, 2002a).

Ground level remote sensing allows rapid evaluation of plant and canopy physiological status in a noninvasive way (Peñuelas & Filella, 1998; Gamon & Qiu, 1999). Moreover, remote sensing technology from airborne platforms allows rapid evaluation of vegetation properties in large areas. Many high spectral resolution reflectance vegetation indices have been proposed with the aim of monitoring biomass, phenology and physiological conditions of plants and canopies (Peñuelas & Filella, 1998). Among them, several can be proposed to assess the changes in water status, photosynthetic performance, and oxidative status of the Mediterranean plants submitted to the stressing drought, high irradiance and high temperatures of the summer. The reflectance water index (WI) has been defined as the ratio R900 : R970, where R means reflectance and the number stands for the wavelength, and has been shown to be highly correlated with plant water content in several species of trees, shrubs, crops and grasses (Peñuelas et al., 1993, 1994, 1997a). The Photochemical Reflectance Index (PRI, calculated as \( R_{531} - R_{570} / R_{570} + R_{531} \)), provides a new approach of estimating photosynthetic performance using spectral reflectance. Absorbance at 531 nm has been found to be related to several processes of dissipation of excess radiation by plants (xanthophyll pigment interconversion, thylakoid ΔpH formation and chloroplast conformational changes) (Bilger & Bjorkman, 1990; Demmig-Adams, 1990; Demmig & Adams, 1996). The measure of reflectance at this wavelength can thus provide an indirect estimation of photosynthetic performance (Gamon et al., 1990, 1992, 1997; Peñuelas et al., 1994, 1995, 1997b). Several studies have shown a good relationship between PRI and photosynthetic performance when measuring at leaf (Peñuelas et al., 1995; Gamon et al., 1997; Peñuelas et al., 1997b; Richardson & Berlyn, 2002), proximal canopy (Gamon et al., 1992; Filella et al., 1996; Trotter et al., 2002) or at crop or ecosystem scale (Nichol et al., 2000; Peñuelas & Inoue, 2000). Finally, we hypothesized that the oxidative stress linked to the severe summer drought and high temperatures and radiation of the Mediterranean conditions would oxidise compounds such as phenolics to quinones, and would increase the pool of oxidised compounds and pigments often denominated ‘brown pigments’ (Vaughn & Duke, 1984). The quinonoid products are strongly electrophilic and undergo secondary chemical reactions, which lead to the formation of polymeric brown or black pigments (Nicolas et al., 1994). We define here a new reflectance index based on the reflectance in the far red to assess such brown pigments.

While drought responses in the Mediterranean vegetation have received considerable attention (Chaves et al., 2002), pigment and antioxidant responses, and the optical (reflectance) detection of all these changes have received less attention and are still less understood. Moreover, as far as we are aware, they have not been studied simultaneously. We simultaneously measured water relations, photosynthetic activity, photo- and antioxidative protection, and reflectance of field grown plants of the evergreen species Phillyrea angustifolia L. under increasing severe summer drought conditions produced by withholding irrigation. The study was aimed at first characterizing water, photosynthetic, pigment and antioxidant changes in Phillyrea angustifolia plants under the progressively drier conditions of the Mediterranean summer, second evaluating the putative relationships among these changes in drought-stressed plants, and third characterizing these physiological responses by plant reflectance changes, especially in the water band at 970 nm, in the green 531 nm band indicative of xanthophyll pigments and photosynthetic performance,
and in the far-red part of the spectrum associated with brown oxidized compounds, which could provide a more specific assessment of oxidative stress. Our general aim was thus to assess the oxidative stress linked to summer drought and to find its possible optical signals.

Materials and Methods

Plant material and treatments

Thirty-six plants of *Phillyrea angustifolia* L. (*Oleaceae*), which were purchased in Garden Bordas (Gavà, Barcelona, Spain), were obtained from seeds and grown in pots of 1–5 l capacity (depending on root biomass as plants grew) containing a mixture of soil:peat:perlite (1 : 1 : 1, by vol.). The pots were maintained in a glasshouse with controlled temperature (24/18°C, day/night) and adequate watering conditions. After growing for 3 yr, plants were transplanted to the experimental fields at the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, NE Spain) on 23 February 2002. Plants were distributed homogenously in six flat plots of 3 × 3 m² each, so that all plants had the same orientation to the sun. The plots and their surroundings were always maintained clear of vegetation that could have interfered in the growth of *P. angustifolia*. Before the experiment started (18 May 2002), all plants were watered twice a week, so that they received c. 100 mm each month (watering + rainfall). At the beginning of the experiment, plants were 90 ± 5 cm tall (*n* = 3 plants), and their root and leaf d. wt (*n* = 3 plants), respectively.

Two watering conditions were imposed on plants during the experiment (18 May–5 July), and three plots (six plants each) per treatment were used: first irrigated (IR) plants were watered twice a week, so that they received c. 100 mm per month (watering + rainfall), and second plants exposed to water deficit (no irrigation and no rains) (WS plants). Water deficit was imposed by withholding watering to WS plants, and by covering them with a polyvinyl chloride sheet when it rained.

The environmental conditions were monitored with a Davis weather station (Darrera S.A., Esplugues Ll., Barcelona, Spain) that was situated at c. 50 m of the experimental plot. Vapour pressure deficit (VPD) was calculated from air temperature (*T*) and relative humidity data according to Nobel (1991).

Plant water status, leaf mass area, the extent of lipid peroxidation (malondialdehyde [MDA] contents), chlorophyll fluorescence, photosynthetic pigments, xanthophyll pigments, α-tocopherol (α-T), and high spectral resolution reflectance in the visible and near-infrared were measured in fully developed young leaves exposed to sun on clear sunny days at midday (at maximum diurnal photosynthetically active photon flux density [PPFD]). For measurements of MDA, photosynthetic pigments, and α-T, leaves were collected, frozen in liquid nitrogen and stored at −20°C until analysis.

Water status and leaf mass area

Leaves were weighted and leaf area was immediately measured using a flatbed scanner (model GT-5000; Epson, Nagano, Japan) and an image-processing program. Then, leaves were re-hydrated for 24 h at 4°C in darkness and subsequently oven-dried for 24 h at 80°C. The relative leaf water content (RWC) was determined as 100 × (f. wt − d. wt)/(t. wt − d. wt), where f. wt is the fresh weight, t. wt is the turgid weight after re-hydrating the leaves, and d. wt is the dry weight after oven-drying the leaves. The leaf mass area (LMA) was determined as d. wt/Leaf area. Leaf hydration, H, was calculated as (f. wt − d. wt)/d. wt.

Estimation of lipid peroxidation

The extent of lipid peroxidation in leaves was estimated by measuring the amount of MDA by the method described by Hodges et al. (1999), which takes into account the possible influence of interfering compounds in the assay for thiobarbituric acid (TBA)-reactive substances. In short, samples were repeatedly extracted with 80 : 20 (v/v) ethanol/water containing 1 ppm butylated hydroxytoluene (BHT) using sonication. After centrifugation, supernatants were pooled and an aliquot of appropriately diluted sample was added to a test tube with an equal volume of either first –TBA solution containing 20% (w/v) trichloroacetic acid and 0.01% (w/v) BHT, or second –TBA solution containing the above plus 0.65% (w/v) TBA. Samples were heated at 95°C for 25 min and, after cooling, absorbance was read at 440 nm, 532 nm, and 600 nm. MDA equivalents (nmol ml⁻¹) were calculated as 10⁶ × ((A – B)/157 000), where A = ((Abs 532 + TBA) − (Abs 600 + TBA)) − (Abs 532 – Abs 600 + TBA), and B = ((Abs 440 + TBA) − Abs 600 + TBA) × 0.0571.

Chlorophyll fluorescence

Measurements of the maximum efficiency of PSII photochemistry (*F*ₚ : *F*ₚ) were conducted *in situ* on attached leaves with a portable pulse-modulated fluorimeter PAM-2000 (Walz, Effeltrich, Germany). Leaves were dark-adapted with leaf clips for 1 h, which was determined to be sufficient to allow complete relaxation of energy-dependent quenching. The *F*ₚ : *F*ₚ ratio was calculated as (*F*ₚ − *F*ₖ)/*F*ₚ, where *F*ₚ and *F*ₚ are the maximum and basal fluorescence yields, respectively, of dark-adapted leaves (Genty et al., 1989).

Photosynthetic pigments and α-tocopherol

The extraction and HPLC analyses of photosynthetic pigments and α-T were carried out essentially as described by Munné-Bosch & Alegre (2000). In short, leaves were repeatedly extracted with ice-cold 85 (v/v) and 100% acetone using sonication. Pigments were separated on a Dupont
nonendcapped Zorbax ODS-5 µm column (250 × 4.6 mm, 20% C, Scharlau, Barcelona, Spain) at 30°C at a flow rate of 1 ml min⁻¹. The solvents consisted of (A) acetonitrile/methanol (85: 15, v/v) and (B) methanol/ethyl acetate (68: 32, v/v). The gradient used was: 0–14 min 100% A, 14–16 min decreasing to 0% A, 16–28 min 0% A, 28–30 min increasing to 100% A, and 30–38 min 100% A. Detection was carried out at 445 nm (Diode array detector 1000S, Applied Biosystems, Foster City, CA). α-T was separated on a Partisil 10 ODS-3 column (250 × 4.6 mm, Scharlau, Barcelona, Spain) at a flow rate of 1 ml min⁻¹. The solvents consisted of (A) methanol/water (95: 5, v/v) and (B) methanol. The gradient used was: 0–10 min 100% A, 10–15 min decreasing to 0% A, 15–20 min 0% A, 20–23 min increasing to 100% A, and 23–28 min 100% A. α-T was quantified through its absorbance at 283 nm (Diode array detector 1000S, Applied Biosystems). Compounds were identified by their characteristic spectra and by coelution with authentic standards, which were obtained from Fluka (Buchs, Switzerland).

Photosynthetic rates and stomatal conductance

Calibrated LCA-4 IRGA porometers (ADC, Hoddeson, Hertfordshire, UK) were used for determination of CO₂ and H₂O exchange. Intact leaves were clamped in Parkinson Chambers (PLC-2) adapted to ADC-LCA-4. Net carbon dioxide assimilation rates and stomatal conductances were calculated from changes in CO₂ and H₂O exchange according to Von Caemmerer & Farquhar (1981).

Reflectance measurements and indices

Leaf-based reflectance was measured with a UniSpec Spectral Analysis System/Reflectometer (PP Systems, Haverhill, MA, USA), operated with a palmtop PC. Individual leaves from six plants per treatment and date were individually sampled by placing them into a leaf clip (adaxial side upwards) attached to a fibre-optic halogen light source and detector. Four scans per sample were integrated (integration time 10 ms). Reflectance measurements were preceded by a dark scan, and were compared with reflectance from a Spectralon (Labsphere Inc., North Sutton, NH, USA) white standard to obtain percent reflectance. From the reflectance data, various spectral indices and features were derived. The following reflectance indices were computed (Rx means the reflectance at x nm):

- WI (Water Index) = R900/R970 (Peñuelas et al., 1993, 1997a; Peñuelas & Filella, 1998)
- PRI (Photochemical reflectance index) = (R531 − R570)/(R531 + R570) (Gamon et al., 1992; Peñuelas et al., 1995)
- BPI (Brown Pigment Index) We defined this index as the difference between the wavelengths where the second derivative of the reflectance in the far-red region intercepts 0, that is the difference between the wavelength with maximum reflectance slope (λ red edge) and the wavelength with the minimum reflectance slope in the near infrared (Fig. 1). This way, the higher brown pigment concentrations will be noticed by the higher BPI values, that is longer wavelength distances from the red edge to the near infrared asymptotic reflectances.

![Fig. 1 Reflectance, first derivative reflectance, and second derivative reflectance spectra of an irrigated (IR) and a nonirrigated (WS) plant on 5 July after 48 d without rain. Only the red edge region between 670 and 770 nm is depicted. The brown pigment index (BPI) is calculated as the wavelength difference between the wavelengths where the second derivative of the reflectance in the far-red region intercepts 0, that is the distance between the red edge and the wavelength with the minimum reflectance slope in the near infrared.](https://www.newphytologist.org)
Statistical analyses

Statistical differences between measurements on different treatments or on different times were analysed following the Student’s \( t \)-test. Only the average value for each plot was used in the analyses. Differences were considered significant at a probability level of \( P < 0.05 \). These analyses and additional correlational and regression analyses were conducted using SPSS (Chicago, IL., USA). For regression analyses of mean (average), and therefore of values having known associated variance, a model II regression was used (Coleman et al., 1994). To compute this model II regression, we used the reduced major axis method (Sokal & Rohlf, 1995).

Results and Discussion

Environmental conditions

Environmental conditions during the experimental period (18 May–5 July) were typical of the Mediterranean climate. Maximum diurnal PPFD ranged between 1898 and 2003 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) throughout the experiment (Table 1). Maximum diurnal air temperature \((T_a)\) ranged between 26.8 and 33.6°C throughout the study (Table 1). Maximum diurnal VPD ranged from 2.15 to 3.85 KPa (Table 1).

Water content and the reflectance index WI

RWC in IR plants was kept nearly constant around 80% throughout the experiment. Plants exposed to drought in the field experienced progressive leaf water losses. After 48 d of water deficit, RWC in WS plants decreased from 80% to c. 50% and the leaf hydration, \( H \), from 1.25 to 0.6 g [g d. wt] \(^{-1} \) (Fig. 2).

Reflectance WI distinguished between IR and WS plants, being lower at reduced leaf water content, that is it decreased in WS plants and in day 48 when water content had decreased after several days with no rain and increasing evaporative demand (Fig. 2). WI therefore followed \( H \) (Figs 2 and 8).

Photosynthetic rates, stomatal conductances and the reflectance index PRI

Net photosynthetic rates decreased 50% in WS plants relative to IR plants the day 15 and c. 90% the day 48 when the atmospheric VPD conditions allowed the IR plants to increase their net photosynthetic rates 2.5 fold those of the day 15 (Fig. 3). Parallel changes were found in stomatal conductances although it must be highlighted that WS plants had almost closed stomata (Fig. 3). Similar trends were found for foliar PRI which this way was therefore a good estimator of net photosynthetic rates (Figs 3 and 8).

Table 1 Photosynthetically active photon flux density (PPFD), air temperature (\( T_{air} \)), and vapour pressure deficit (VPD) at midday during the measurement days between May and July 2002

<table>
<thead>
<tr>
<th>Date</th>
<th>PPFD (( \mu \text{mol} \text{ m}^{-2} \text{ s}^{-1} ))</th>
<th>( T_{air} ) (°C)</th>
<th>VPD (KPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 May</td>
<td>1898</td>
<td>26.8</td>
<td>2.20</td>
</tr>
<tr>
<td>3 June</td>
<td>1922</td>
<td>33.6</td>
<td>3.85</td>
</tr>
<tr>
<td>5 July</td>
<td>2003</td>
<td>30.6</td>
<td>2.15</td>
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Fig. 2 Relative water content (RWC), foliar hydration (\( H \)), and reflectance ‘Water Index’ (WI, R900/R970) of irrigated (IR) and nonirrigated (WS) field-grown \( \text{Phillyrea angustifolia} \) plants during the experiment (from 18 May to 5 July). IR plants were watered twice a week, so that they received c. 100 mm of water per month (rainfall + watering). Water deficit was imposed by withholding water to WS plants, and by covering them with a polyvinyl chloride sheet when it rained. Presented data are the means ± SE (\( n = 3 \) plot averages of three independent leaves per plot). Leaves were collected at midday (at maximum diurnal photosynthetically active photon flux density (PPFD)).
Photo- and antioxidant protection and the reflectance index BPI

Although after the 48-d period of study, Chl levels decreased by c. 20% in IR plants and 25% in WS plants (Fig. 4), they did not present significant differences between IR and WS plants. No significant differences were found either for their leaf mass per area (data not shown), their maximum efficiency of photosystem II photochemistry (\( F_v : F_m \) ratio) or their malondialdehyde (MDA) accumulation in leaves of irrigated (IR) and nonirrigated (WS) Phillyrea angustifolia plants. IR plants were watered twice a week, so that they received c. 100 mm of water per month (rainfall + watering). Water deficit was imposed by withholding water to WS plants, and by covering them with a polyvinyl chloride sheet when it rained. Data correspond to the means ± SE (\( n = 3 \) plot averages of two independent leaves per plot). Measurements were made on leaves collected at midday.

Fig. 3 Net photosynthetic rates, stomatal conductance and photochemical reflectance index (PRI, \( R531 - R570/R531 + R570 \)) in leaves of irrigated (IR) and nonirrigated (WS) Phillyrea angustifolia plants. IR plants were watered twice a week, so that they received c. 100 mm of water per month (rainfall + watering). Water deficit was imposed by withholding water to WS plants, and by covering them with a polyvinyl chloride sheet when it rained. Data correspond to the means ± SE (\( n = 3 \) plot averages of two independent leaves per plot). Measurements were made on leaves collected at midday.

Fig. 4 Chlorophyll a + b (Chl) levels, maximum efficiency of photosystem II photochemistry (\( F_v : F_m \)), and malondialdehyde (MDA) accumulation in leaves of irrigated (IR) and nonirrigated (WS) Phillyrea angustifolia plants. IR plants were watered twice a week, so that they received c. 100 mm of water per month (rainfall + watering). Water deficit was imposed by withholding water to WS plants, and by covering them with a polyvinyl chloride sheet when it rained. Data correspond to the means ± SE (\( n = 3 \) plot averages of two independent leaves per plot). Measurements were made on leaves collected at midday.
malondialdehyde concentrations, which did not change significantly either throughout the period of study or between treatment levels (Fig. 4). These results indicate an absence of a significant photoinhibitory or oxidative damage to the photosynthetic apparatus, and therefore that the photo- and antioxidative protection mechanisms were able to protect the plants under these typical summer drought conditions.

Mechanisms of photo- and antioxidant protection in chloroplasts (xanthophyll cycle and levels of lipophilic antioxidants) during drought were evaluated. The amounts of zeaxanthin of WS plants increased significantly up to 2.5 fold at RWCs of c. 50% at day 48, which caused up to c. 75% increase in the total amount of xanthophylls pigments and in the de-epoxidation state of the xanthophyll cycle (DPS) (Fig. 5). The total amount of the xanthophyll cycle components (VZA) remained unaltered in IR plants, and increased by c. 0.06 µmol [g d. wt]⁻¹ in WS plants (Fig. 5). This was mainly due to the large increases in zeaxanthin, which increased in these plants by c. 0.075 µmol [g d. wt]⁻¹. Enhanced zeaxanthin formation was therefore not only due to the de-epoxidation of the violaxanthin present in irrigated (IR) plants, but also to the de novo synthesis of carotenoids under stress. Zeaxanthin attained maximum levels in WS plants, in which zeaxanthin accounted for 65% of the xanthophyll cycle components present in leaves. The de-epoxidation state of the xanthophyll cycle was c. 0.45 in IR plants and reached its maximum levels (c. 0.75) in WS plants on day 48 (Fig. 5).

Chloroplastic lipophilic antioxidants (carotenoids and tocopherols) differed in their response to drought and recovery in *P. angustifolia* (Fig. 6). While lutein levels did not show significant alterations throughout the experiment in IR and WS plants, β-carotene (β-C) decreased and α-tocopherol (α-T) increased, as drought progressed. β-C decreased with time 20% in IR plants and 45% in WS plants (Fig. 6), and α-T increased up to 2–3-fold, at RWCs of c. 50% of the WS plants on day 48, whereas it remained constant in the IR plants (Fig. 6).

The reflectance index BPI, indicative of brown pigments (oxidized compounds), increased c. 8–10% in the WS plants on day 48 (Fig. 6) and therefore was negatively correlated with the β-carotene concentrations (Fig. 7). This index also presented similar patterns to Z, DPS, and α-T, that is to photo- and antioxidant protection.

**Water and oxidative status under increasing summer drought**

General ecophysiological responses to increasing summer drought in Mediterranean plants have been partially elucidated in numerous studies that have shown similar leaf responses to those described here of decreasing leaf water content, chlorophyll concentrations, net photosynthetic rates and stomatal conductances with increasing drought (Tenhunen *et al.*, 1990; Peñuelas *et al.*, 1998). However, we show here the importance of coordinated physiological responses including photo- and antioxidant protective mechanisms.

Enhanced de-epoxidation of the xanthophyll cycle and antioxidant levels have been associated with increased drought stress resistance in several Mediterranean species (Chaves *et al.*, 2002). The mechanisms that *Phillyrea angustifolia* plants have evolved to withstand stress are of special interest because
Fig. 7 Relationships of the leaf hydration (H), the net photosynthetic rates, and the β-carotene concentrations with the corresponding reflectance indices WI, PRI and BPI, respectively. Data correspond to the means ± SE (n = 3 plot averages of two independent leaves per plot). Measurements were made on leaves collected at midday.

Fig. 6 α-tocopherol (α-T), Lutein (L), β-carotene (β-C) and Brown Pigment Index (BPI) levels in leaves of irrigated (IR) and nonirrigated (WS) Phillyrea angustifolia plants. IR plants were watered twice a week, so that they received c. 100 mm of water per month (rainfall + watering). Water deficit was imposed by withholding water to WS plants, and by covering them with a polyvinyl chloride sheet when it rained. (BPI was defined as the wavelength distance between the red edge and the near infrared reflectance stabilization). Data correspond to the means ± SE (n = 3 plot averages of two independent leaves per plot). Measurements were made on leaves collected at midday.
these shrubs are typical of the Mediterranean climate, and can withstand a severe summer. In the present study, dissipation of excess excitation energy in the xanthophyll cycle and increased α-T levels (which detoxify AOS and lipid peroxyl radicals) seem to afford protection to the photosynthetic apparatus in drought-stressed P. angustifolia plants. Thus, this species did not suffer from photoinhibitory damage to its photosynthetic apparatus (as indicated by the absence of significant changes in \(F_v:F_m\) and MDA levels), at least at the drought stress levels here tested (RWCs above 50%). The slight trend of Chl breakdown with the increasing summer drought (Fig. 4), decreases leaf light absorption, and thus decreases Chl molecules as a source for potentially harmful singlet oxygen production (Halliwell & Gutteridge, 1989) and increases the photo- and antioxidative protection per amounts of photons absorbed (Kyparissis et al., 1995; Havaux et al., 1998; Munné-Bosch & Alegre, 2000). As a result, plants did not suffer from photo or oxidative damage as indicated by the absence of any change or difference in the \(F_v:F_m\) ratio and MDA foliar concentrations (Fig. 4). These results therefore indicate that P. angustifolia leaves can avoid damage to the photosynthetic apparatus by changing pigment and antioxidant levels in response to drought-induced oxidative stress.

Optical signals

These ecophysiological changes were followed by reflectance changes, being WI a good index for water content (Peñuelas et al., 1993, 1997a), and PRI a good index for photosynthetic performance (Gamon et al., 1992; Peñuelas et al., 1994, 1995, 1997b), confirming previous studies (Peñuelas & Filella, 1998). However, what is novel in these results is that they show the possibility that the brown pigment index, BPI, here defined, might become good optical signals for oxidative stress since it was positively correlated with \(Z\) and α-T concentrations, that is with increased photo- and antioxidant protection, and with \(β\)-carotene degradation. This is what should be expected from an index defined to assess brown pigments, that is polymers of oxidized compounds such as quinones. These quinones are produced as a result of the oxidation of phenolic compounds in a reaction catalyzed by the polyphenol oxidase (PPO). The exact physiological role of PPO in plant cells is not well established, but PPO has been implicated to function in the oxygen scavenging in chloroplast (Trebst & Depka, 1995). The \(α\)-quinones are highly reactive in plant cells and are subsequently involved in the formation of polymeric dark-colored pigment deposits (Nicolas et al., 1994). In any case, the concentrations of brown pigments will have to be measured in future studies to test these relationships.

Conclusions

In conclusion, we have demonstrated first that the evergreen P. angustifolia plants activate several mechanisms of photo- and antioxidant protection to withstand drought stress during the Mediterranean summer, second that, when the drought is not extremely severe, these mechanisms are able to protect the tissues from lipid peroxidation, and to allow the maintenance of high photosynthetic rates if meteorological conditions are adequate, and third that all these processes can be reasonably followed by the optical sensing provided by reflectance indices in the visible, the far red and the near infrared. However, further studies on these issues are warranted before these optical signals, especially the BPI here defined, can be used in a broad range of species and conditions.

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