Effectiveness of the photochemical reflectance index to track photosynthetic activity over a range of forest tree species and plant water statuses

F. Ripullone\textsuperscript{A,F}, A. R. Rivelli\textsuperscript{A}, R. Baraldi\textsuperscript{B}, R. Guarini\textsuperscript{A}, R. Guerrieri\textsuperscript{A}, F. Magnani\textsuperscript{C}, J. Peñuelas\textsuperscript{D}, S. Raddi\textsuperscript{E} and M. Borghetti\textsuperscript{A}

\textsuperscript{A}Department of Crop Systems, Forestry and Environmental Sciences, University of Basilicata, viale dell’Ateneo Lucano 10, 85100 Potenza, Italy.
\textsuperscript{B}Institute of Biometeorology, National Research Council (CNR), via Gobetti 101, 40129 Bologna, Italy.
\textsuperscript{C}Department of Fruit and Trees, University of Bologna, via Fanin 46, 40127 Bologna, Italy.
\textsuperscript{D}Global Ecology Unit, Center for Ecological Research and Forestry Applications (CREAF), Center for Ecological Research and Forestry Applications, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
\textsuperscript{E}Department of Agricultural and Forest Economics, Engineering, Sciences and Technologies, University of Florence, Via S. Bonaventura, 13 50145 Firenze, Italy.
\textsuperscript{F}Corresponding author. Email: francesco.ripullone@unibas.it

Abstract. In this study, we investigated the potential of the photochemical resistance index (PRI) to track photosynthetic activity under water stress conditions by measuring PRI, leaf fluorescence, the xanthophyll cycle and photosynthetic activity in different forest tree species subjected to progressive drought. The PRI declined with pre-dawn water potential and a significant relationship between PRI and the xanthophyll de-epoxidation state (DEPS) was observed, although with large interspecific variability in the sensitivity of PRI to changes in DEPS. For single tree species, a strong relationship was observed on either PRI light saturated photosynthesis or PRI maximum photochemical efficiency of PSII ($\Delta F/F_{m}$); a larger variability in both relationships was apparent when data from different species were pooled together. However, an improved correlation was shown only in the former relationship by plotting the ΔPRI (dawn PRI minus the midday PRI values). Thus, we conclude that PRI is able to provide a good estimate of maximum CO$_2$ assimilation at saturating light and $\Delta F/F_{m}$ for single tree species, despite the severe drought conditions applied. PRI should be applied more cautiously when dealing with multispecific forests because of confounding factors such as the strong interspecific differences in the initial value of PRI and in the sensitivity of PRI to changes in DEPS in response to drought.

Additional keywords: broadleaves, drought, evergreen, leaf reflectance, photosynthesis, xanthophylls.

Introduction

In recent years, increasing attention has been paid to refine the methodologies suitable for tracking the photosynthetic activity of forest vegetation; this is a necessary step towards assessing carbon uptake by forest ecosystems using remote sensing (Grace \textit{et al.} 2007).

Among several methods suitable for remote sensing applications, the photochemical reflectance index (PRI) (Gammon \textit{et al.} 1992, 1997; Peñuelas \textit{et al.} 1995) has a sound functional basis and provides promising results. When incident light energy exceeds that needed for photosynthesis, plants protect their photosynthetic machinery by dissipating the excess energy through the xanthophyll cycle (Demmig-Adams and Adams 2006; Busch \textit{et al.} 2009), which leads to a change in the leaf reflectance at 531 nm. Changes in leaf reflectance are combined into the PRI index, and the latter can thus be used to obtain information on photosynthetic activity (Gammon \textit{et al.} 1992, 1997; Peñuelas \textit{et al.} 1995).

Several studies reported a good correlation between the PRI and photosynthetic activity at both the leaf (Gammon \textit{et al.} 1997; Evain \textit{et al.} 2004; Guo and Trotter 2004; Naumann \textit{et al.} 2008) and canopy scales (Filella \textit{et al.} 1996; Nichol \textit{et al.} 2000; Dobrowski \textit{et al.} 2005; Garbulsky \textit{et al.} 2008), but also across a range of environmental conditions (Richardson \textit{et al.} 2003; Whitehead \textit{et al.} 2005; Garbulsky \textit{et al.} 2011).

It was shown, however, that severe drought conditions tend to introduce a confounding effect in the physiological interpretation of the PRI from different vegetation types, including the Mediterranean macchia (Gammon \textit{et al.} 1992; Peñuelas \textit{et al.} 1994; Winkel \textit{et al.} 2002; Filella \textit{et al.} 2004). Leaf wilting occurring under severe drought and the interconversion of
xanthophyll pigments has often been interpreted as the likely cause of uncoupling between the reflectance signal and photosynthetic downregulation (Peñuelas et al. 1997; Stylinski et al. 2002; Thenot et al. 2002). Furthermore, interspecific differences in the light-use efficiency or the carotenoids : chlorophyll ratio have been also indicated as likely causes of the reduced suitability of PRI in tracking photosynthetic activity under drought conditions (Filella et al. 2004, 2009). This may limit the use of the PRI in geographic regions like Southern Europe, where more frequent and severe water stress are occurring as a consequence of climate change (IPCC Working Group II 2007; De Dato et al. 2008) and where forests are characterised by large spatial variability in species composition.

In this study, we explored whether PRI techniques can be applicable to track photosynthetic activity in different forest tree species, despite severe drought conditions, and the divergence among species in photosynthetic potential and sensitivity to drought. In particular, an experiment on the potential of PRI to track photosynthesis has been performed on tree species from different altitudinal belts, which are expected to diverge in their sensitivity to drought: Abies alba Mill., and Castanea sativa Mill., which are deciduous tree species typical of mid-altitude forest on more mesic sites; Fagus sylvatica L., which is the main species of the montane forest in Southern Europe; and Populus × euroamericana Guinier, which is widely employed as fast-growing tree species on floodplain soils.

Materials and methods
Plant material and experimental conditions
The experiment was carried out in the summer of 2007 (i.e. July to August) in the experimental nursery of the University of Basilicata (40°38′N, 15°48′S; Potenza, Italy) on potted plants belonging to the following species: A. unedo, Q. ilex, Q. pubescens, Q. cerris, Q. robur, C. sativa, F. sylvatica and P. euroamericana.

In April 2007, about 50 potted seedlings of each species, aged 3 to 5 years old, were transferred to the nursery and transplanted to 15-L pots containing soil taken from a nearby oak forest (22% clay, 28% lime, 36% fine sand and 14% peat); the soil was covered with a layer of perlite to minimise evaporation. For P. euroamericana, the experimental material consisted of stem cuttings planted in 15-L pots at the beginning of June 2007. Before the start of the experiment, 20 plants per species were selected for uniformity: 10 plants were randomly chosen and assigned to the drought treatment, and the remaining 10 plants were used as controls. The control plants were well watered by daily irrigation to field capacity; the plants assigned to the drought treatment underwent a period of progressive water stress induced by suspending irrigation between July 31 and August 12 in 2007. The weather during this period was clear, with low relative humidity and high air temperatures, as assessed by a meteorological station located in the same experimental nursery.

Measurement of leaf water potential and leaf relative water content
Leaf water potential was measured on six to eight plants in each species, using a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA); one leaf of each plant was measured. Measurements were performed both well before sunrise (pre-dawn water potential, \( \Psi_{pd} \)) and in the middle of the day (midday water potential, \( \Psi_{md} \)). Measurements were performed every 2 days in the drought treatment plants; in the control plants, leaf water potential was measured on two occasions only, once at the beginning and once towards the end of the experimental period.

The leaf relative water content (RWC) was determined on one fully expanded leaf excised from the same plants sampled for the measurement of leaf water potential. The excised leaves were immediately wrapped in aluminum foil and brought to the laboratory in a portable refrigerator (Fridge-Freeze, Inc., San Diego, CA, USA); the FW was then measured to the nearest mg; next, leaves were soaked for 24 h in distilled water under normal room light and temperature and the turgid weight (TW) was recorded; all leaves were then oven-dried at 80°C to a constant weight, cooled down and weighed to determine the DW. The leaf relative water content was calculated as follows:

\[
RWC = \frac{(FW-DW)}{(TW-DW)}
\]

Barrs and Weatherley (1962).

At the end of the experiment, two fully expanded leaves were excised from the upper part of each plant. The projected area \( A_p \) of the leaves was determined with an LI-3000 area meter (LI-Cor Biosciences, Lincoln, NE, USA) and their DW was determined after 48 h in oven at 70°C. The leaf mass per area was computed as DW \( A_p^{-1} \).

Measurement of leaf gas exchange
Leaf gas exchanges (the maximum CO₂ assimilation at saturating light \( A_{max} \), transpiration rate \( E \) and stomatal conductance \( g_s \)) were measured with a portable infrared gas analyser (LI 6400, Li-Cor). Measurements were carried out every 2 days over the experimental period on one fully illuminated leaf in the upper part of the crown of six to eight plants per species. Measurements were conducted at around midday (1130–1430 hours), when the PPFD was above 1600 \( \mu \text{mol} \text{ photons m}^{-2} \text{ s}^{-1} \), which corresponds to saturating light conditions for all the studied species, as assessed in a preliminary trial using photosynthesis light curves. During the measurements, the CO₂ concentration inside the cuvette was \(~370 \mu \text{mol} \text{ mol}^{-1} \), and the environmental parameters were similar to the external conditions. Data were recorded after a steady state was reached, generally after 2–3 min of measurement. Furthermore, special attention was paid to minimise modifications in natural leaf orientation during all measurements.

Measurement of chlorophyll fluorescence and leaf reflectance
Leaf chlorophyll fluorescence and leaf reflectance measurements were conducted concurrently with the leaf gas exchange measurements, on the same plants and on the same or adjacent leaves.

Chlorophyll fluorescence was measured on one leaf of each plant with a portable pulse fluorometer (PAM-2000, Walz, Effeltrich, Germany), while maintaining the leaves in their
natural position. The actual photochemical efficiency of PSII (ΦPSII) at a PPFD higher than 1600 μmol m⁻² s⁻¹ was assessed by measuring the steady-state fluorescence (F) and maximum fluorescence (Fm′) during a light-saturating pulse and expressed as ΔF/Fm′ = (Fm′−F)/Fm′⁻¹ according to Genty et al. (1989). The maximum photochemical efficiency of PSII was assessed by measuring the minimum (Fo) and maximum (Fc) fluorescence before dawn and expressed by the equation Fo/Fc = (Fc−Fo)/Fc⁻¹ (Genty et al. 1989). Leaf reflectance was measured using a portable fibre optic spectrometer (USB-2000, Ocean Optics Inc., FL, USA) with an optical resolution of 0.4 nm; the instrument is provided with a bifurcated low-OH optical fibre, connected to a stabilised light source (LS-1, Ocean Optics). During the measurements, the optical fibre was maintained at an angle of 45° on the leaf surface. Four readings were recorded at different positions on the leaf surface and the average value of these four readings was used for subsequent calculations. The photochemical reflectance index was calculated (Gamon et al. 1992) as PRI = (R531 − R570)/(R531 + R570)⁻¹, where R531 and R570 represent leaf reflectance at the wavelengths of 531 and 570 nm, respectively. The normalised value of PRI (ΔPRI) was calculated by the difference between the value of PRI measured at pre-dawn at the beginning of the experiment and the value of PRI measured at midday along the experimental period (Gamon and Surfus 1999; Weng et al. 2006).

Analysis of leaf pigments
Before dawn and at midday of each day of measurement, two leaves were excised from the upper part of the crown of six plants per species; the leaves were immediately plunged in liquid nitrogen and stored at −80°C until subsequent analyses. Two leaf discs (0.64 cm²) were extracted from each leaf, ground in a mortar in liquid nitrogen and the powder was extracted with cold HPLC grade 100% acetone. The extracts were centrifuged at 5000 g at 0°C for 4 min and water was added to give a final concentration of 80% acetone (v/v). Finally, the pigment solutions were filtered through a 0.45 μm syringe filter and analysed with a HPLC system (Model LC-10AS with a SPD-10AV detector, Shimadzu, Kyoto, Japan), according to the procedure described by Baraldi et al. (2008). Peaks were detected and integrated at 445 nm for determining the carotenoid and chlorophyll b contents, and at 410 nm for chlorophyll a. The concentrations of the pigments were calculated from the corresponding peak areas (Munné-Bosch and Alegre 2000). The calibration was performed using commercially available pigment standards (neoxanthin, violaxanthin (V), zeaxanthin (Z), and lutein; DHI Water and Environment, Horsholm, Denmark). The calibration factor for V was also used for A. The xanthophyll de-epoxidation state (DEPS), resulting from the conversion of V to Z via antheraxanthin (A), was calculated as: (Z + 0.5A) (V + A + Z)⁻¹, according to Müller et al. (2006). This index is able to estimate the actual number of partially or totally de-epoxidised molecules within the xanthophyll cycle compared to the maximum possible.

Statistical analyses
The average values recorded for each plant on each experimental day were considered as elementary data in statistical computations. Pairwise Student’s t-test, ANOVA, linear and non-linear regressions and Pearson’s correlation coefficients were computed using the SPSS 17 statistical package (SPSS Inc., Chicago, IL, USA).

Results
Throughout the experiment, the weather was clear, hot and dry; the average values of day: night temperature and vapour pressure deficit were 28.4:18.9°C and 2.3:1.0 kPa, respectively. The maximum air temperature recorded at midday was 35°C and, at this time, PPFD was always above 1600 μmol photons m⁻² s⁻¹ (Fig. 1).

In the drought treatment, the pre-dawn water potential (Ψpd) decreased dramatically over the experimental period (the initial mean value was about −0.3 MPa and the final value differed between species, ranging from −1.5 MPa in F. sylvatica and P. euroamericana to −4.7 MPa in Q. ilex). Concurrently, a progressive decrease of leaf RWC was observed, also with notable differences between species (Fig. 1).
Fig. 2. Relationships between stomatal conductance ($g_s$, left panels), photosynthesis at saturating light ($A_{\text{max}}$, right panels) and pre-dawn water potential ($\Psi_{pd}$). Each symbol represents the mean of six to eight measurements for each species; bars indicate ±1 s.e. Note: Quercus ilex and Arbutus unedo (Mediterranean evergreen tree species), and Quercus cerris, Q. robur, Q. pubescens and Castanea sativa (deciduous tree species from the same altitudinal belt) are represented in the same panel and have been pooled in the same dataset for the regression analysis.
In parallel with decreasing water potential, stomatal conductance \((g_s)\) and light-saturated photosynthesis \((A_{\text{max}})\) decreased over the experimental period, showing rather clear differences between species (Fig. 2). At the beginning, a large variability among species was apparent, with the highest values \((g_s = 320 \text{mmol } H_2O \text{m}^{-2} \text{s}^{-1}, \quad A_{\text{max}} = 20 \text{mmol } CO_2 \text{m}^{-2} \text{s}^{-1})\) recorded in \(P. \text{ euroamericana}\) and the lowest \((g_s = 80 \text{mmol } H_2O \text{m}^{-2} \text{s}^{-1}, \quad A_{\text{max}} = 7 \text{mmol } CO_2 \text{m}^{-2} \text{s}^{-1})\) in \(Q. \text{ ilex}\). As water stress progressed, conditions of almost complete stomatal closure and photosynthesis inhibition were reached, and the differences between the species levelled off: \(g_s\) fell below 70 mmol \(H_2O \text{m}^{-2} \text{s}^{-1}\) in all species tested except for \(F. \text{ sylvatica}\) (Fig. 2). Clear differences in drought sensitivity between species emerged in terms of the drop in stomatal conductance for a given drop in pre-dawn water potential (Table 1).

In control plants, the water potential, stomatal conductance, light-saturated photosynthesis and PRI did not change significantly throughout the experiment (Table 2). As the drought progressed, the maximal photochemical efficiency of PSII in dark-adapted leaves \((F_v/F_m)\) did not decrease significantly \((R^2 = 0.11, P > 0.05)\) (a decline would indicate photoinhibitory damage); in fact, in 84% of the cases, \(F_v/F_m\) remained above 0.75. Only in \(F. \text{ sylvatica}\) (from the beginning of the experiment) and in \(Q. \text{ ilex}\) (towards the end of the drought treatment) were lower \(F_v/F_m\) values observed (Fig. 3). The xanthophyll DEPS was measured twice a day, before dawn (DEPS\(_{\text{pd}}\)) and at midday (DEPS\(_{\text{md}}\)), both values increased with increasing drought conditions in all species. A significant positive linear relationship was estimated for both DEPS\(_{\text{pd}}\) \((R^2 = 0.30, P = 0.005)\) and DEPS\(_{\text{md}}\) \((R^2 = 0.54, P = 0.0004)\) (Fig. 4b). The DEPS\(_{\text{md}}\), however, showed a steep initial rise that tended to level off at high water stress. The leaf chlorophyll content did not change significantly with increasing drought (Fig. 4b).

The leaf PRI was affected by drought; it decreased significantly with reduced \(\Psi_{\text{pd}}\) in \(A. \text{ unedo}\) \((R^2 = 0.86, P < 0.01)\), \(Q. \text{ ilex}\) \((R^2 = 0.66, P < 0.05)\), \(Q. \text{ pubescens}\) \((R^2 = 0.91, P < 0.01)\), \(C. \text{ sativa}\) \((R^2 = 0.95, P < 0.001)\) and \(F. \text{ sylvatica}\) \((R^2 = 0.68, P < 0.05)\), but a decreasing trend with increasing drought was also apparent in the remaining species (i.e. \(Q. \text{ cerris}\), \(Q. \text{ robur}\) and \(P. \text{ euroamericana}\)) (Fig. 5). In addition, a significant correlation was calculated even when data from \(Q. \text{ ilex}\) and \(A. \text{ unedo}\) (both Mediterranean evergreen tree species) and from \(Q. \text{ cerris}\), \(Q. \text{ robur}\), \(Q. \text{ pubescens}\) and \(C. \text{ sativa}\) (deciduous tree species from the same altitudinal belt) were pooled together (Fig. 5).

A significant overall relationship could also be established between PRI and DEPS\(_{\text{pd}}\) (Fig. 6), although with strong interspecific differences in the sensitivity of PRI to changes in DEPS. For example, in \(P. \text{ euroamericana}\), DEPS varies from 0.2 to 0.6 with very little change in PRI, whereas in \(F. \text{ sylvatica}\), DEPS varies from 0.4 to 0.7 with higher changes in PRI.

The relationship between PRI and light-saturated photosynthesis \((A_{\text{max}})\) showed significance for single tree species over the physiological conditions experienced in the drought experiment \((A. \text{ unedo}\) \((R^2 = 0.77, P < 0.05)\), \(Q. \text{ ilex}\) \((R^2 = 0.93, P < 0.01)\), \(Q. \text{ pubescens}\) \((R^2 = 0.79, P < 0.05)\), \(C. \text{ sativa}\) \((R^2 = 0.75, P < 0.05)\), \(Q. \text{ robur}\) \((R^2 = 0.80, P < 0.01)\), \(Q. \text{ cerris}\) \((R^2 = 0.78, P < 0.05)\) and \(P. \text{ euroamericana}\) \((R^2 = 0.95, P < 0.01)\), \(F. \text{ sylvatica}\) was the exception \((R^2 = 0.62, P > 0.05)\). A significant correlation was calculated even when data from \(Q. \text{ ilex}\) and \(A. \text{ unedo}\), and from \(Q. \text{ cerris}\), \(Q. \text{ robur}\), \(Q. \text{ pubescens}\) and \(C. \text{ sativa}\) were pooled together (Fig. 7a). However, when the data from all species were pooled together, no clear relationship was apparent (Fig. 7b), although the correlation trend to improve by using \(\Delta PRI\) (Fig. 7b). Similar significant relationships have been also observed between the PRI and the maximum photochemical efficiency of PSII in \(A. \text{ unedo}\) \((R^2 = 0.72, P < 0.05)\), \(Q. \text{ ilex}\) \((R^2 = 0.79, P < 0.05)\),

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**Table 1.** Percent reduction of stomatal conductance \((g_{\text{max}})\) as a drop in values of pre-dawn water potential \((\Psi_{\text{pd}})\) between 0.2 and 0.8 MPa

This initial common range of \(\Psi_{\text{pd}}\) has been chosen as to avoid the large variability in \(\Psi_{\text{pd}}\) drop among the species

<table>
<thead>
<tr>
<th>Species</th>
<th>(g_{\text{max}}(%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Populus \times \text{ euroamericana}) Guinier</td>
<td>66</td>
</tr>
<tr>
<td>(Castanea \text{ sativa}) Miller</td>
<td>61</td>
</tr>
<tr>
<td>(Fagus \text{ sylvatica}) L.</td>
<td>41</td>
</tr>
<tr>
<td>(Quercus \text{ robur}) L.</td>
<td>40</td>
</tr>
<tr>
<td>(Quercus \text{ cerris}) L.</td>
<td>28</td>
</tr>
<tr>
<td>(Arbusus \text{ unedo}) L.</td>
<td>23</td>
</tr>
<tr>
<td>(Quercus \text{ pubescens}) Wild.</td>
<td>20</td>
</tr>
<tr>
<td>(Quercus \text{ ilex}) L.</td>
<td>17</td>
</tr>
</tbody>
</table>

**Table 2.** Mean values of pre-dawn water potential \((\Psi_{\text{pd}})\), stomatal conductance \((g_s)\), photosynthesis at saturating light \((A_{\text{max}})\) and PRI at the beginning and at the end of the experimental period in control plants

Significant differences are marked by an asterisk \((P < 0.05)\)

<table>
<thead>
<tr>
<th>Species</th>
<th>(\Psi_{\text{pd}}) (MPa)</th>
<th>(g_s) (mmol m(^{-2}) s(^{-1}))</th>
<th>(A_{\text{max}}) (mmol m(^{-2}) s(^{-1}))</th>
<th>PRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Abusus \text{ unedo}) L.</td>
<td>–0.21</td>
<td>120</td>
<td>7.8</td>
<td>0.010</td>
</tr>
<tr>
<td>(Quercus \text{ ilex}) L.</td>
<td>–0.33</td>
<td>106</td>
<td>7.0</td>
<td>0.007</td>
</tr>
<tr>
<td>(Quercus \text{ pubescens}) Wild.</td>
<td>–0.43</td>
<td>134</td>
<td>10.9</td>
<td>–0.008</td>
</tr>
<tr>
<td>(Castanea \text{ sativa}) Miller</td>
<td>–0.26</td>
<td>165</td>
<td>10.6</td>
<td>–0.021</td>
</tr>
<tr>
<td>(Quercus \text{ robur}) L.</td>
<td>–0.23</td>
<td>147</td>
<td>8.8</td>
<td>–0.035</td>
</tr>
<tr>
<td>(Quercus \text{ cerris}) L.</td>
<td>–0.33</td>
<td>161</td>
<td>10.1</td>
<td>–0.040</td>
</tr>
<tr>
<td>(Fagus \text{ sylvatica}) L.</td>
<td>–0.21</td>
<td>178</td>
<td>9.5</td>
<td>–0.075</td>
</tr>
<tr>
<td>(Populus \times \text{ euroamericana}) Guinier</td>
<td>–0.15</td>
<td>284</td>
<td>18.9</td>
<td>0.021</td>
</tr>
</tbody>
</table>
This experiment allowed us to monitor the variation in photosynthetic activity and leaf reflectance of a wide range of tree species belonging to different forest vegetation belts, which experienced varying water stress conditions (Figs 1, 2).

We found a significant relationship between PRI and the water stress conditions achieved in the drought treatment, as assessed by pre-dawn water potential (Fig. 5). In our experiment, PRI decreased significantly by 30–50% in water-stressed plants throughout the drought treatment, with the exception of *P. euroamericana* (*P* = 0.07). To our knowledge, very little evidence of a close relationship between plant water status and PRI has been shown in literature (Evain *et al.* 2004; Dobrowski *et al.* 2005; both on grapevine plants (*Vitis vinifera* L.)). The inconsistencies found between PRI and plant water stress in some experiments have been attributed to normal ontogenetic changes during seasonal cycles (Winkel *et al.* 2002). PRI changes due to the confounding effects of temporal changes in the pool of total carotenoids or chlorophylls have also been reported by other authors (Sims and Gamon 2002; Stylinski *et al.* 2002; Filella *et al.* 2009). In our short-term experiment, we were able to exclude the likely effects of leaf ontogeny because no significant differences between the PRI values measured at the beginning and at the end of the experiment in control plants were observed (Table 2). Furthermore, no changes have been observed in the pool sizes of carotenoids or chlorophylls in control plants (data not shown).

Interspecific differences have been observed in the relationship between pre-dawn leaf water potential and the PRI, which tend to be a little closer for evergreens compared with deciduous species (Fig. 5). The higher variability in this latter case may be caused by, for example, the fact that we pooled together more species with different relationships and with higher interspecific differences in the initial reflectance properties (Fig. 5; Table 2) that can affect the PRI signal. However, we cannot exclude the probable differences in midday leaf wilting between Mediterranean species, which are adapted to drought-
prone conditions, compared with deciduous species that are adapted to more mesic conditions. In fact, morphological changes caused by midday leaf wilting have also been indicated by some authors as being responsible for alteration of the PRI signal (Gamon et al. 1992; Thenot et al. 2002).

Measurements of the xanthophyll DEPS confirmed the substantial involvement of the xanthophyll cycle in PRI changes. As the drought progressed and photosynthesis declined in response to stomatal closure, several tree species activated effective photoprotective mechanisms, as shown by the following: (a) the photochemical efficiency of PSII measured before dawn ($F_v/F_m$) remained fairly constant over the experiment, with values that were not far from 0.83, which is the maximum value recorded under optimal environmental conditions for most plant species (Björkman and Demmig 1987); (b) the xanthophyll de-epoxidation cycle was reversible, as assessed by the temporal development of the DEPS values measured before dawn and at midday (Figs 3, 4), except towards the end of the water stress treatment, where a slight increase in pre-dawn DEPS values was recorded.

This scenario is coherent with previous findings on the implication of the xanthophyll cycle in PRI variation and photoprotective mechanisms, across a range of species and environmental conditions (Peñuelas et al. 1994; Sims and Gamon 2002; Peguero-Pina et al. 2008).

One exception in our experiment was represented by $F. sylvatica$ plants, which showed signs of photoinhibition before the onset of substantial water stress, as evidenced by the low $F_v/F_m$ values measured at the beginning of the experiment (Fig. 3). Such result can be related to the shade-tolerant nature of $F. sylvatica$ (Mátyás et al. 2009), the photosynthetic machinery of which may have been damaged when exposed to high PPFD loads, like those measured during the experiment (Fig. 1). Photoinhibition was demonstrated to occur in $F. sylvatica$ plants grown under low light conditions and successively exposed to high light conditions (Tognetti et al. 1998).

The effectiveness of PRI for tracking photosynthetic activity was therefore explored across a wide range of tree species and under varying physiological conditions (Fig. 7). The relationship between PRI and $A_{\text{max}}$ was strong and significant within single tree species, and when data from $Q. ilex$ and $A. unedo$, both Mediterranean evergreen tree species from same altitudinal belt, were pooled together. Other studies carried out at leaf scale show a reduced suitability of PRI for tracking photosynthetic activity under water stress;
however, these studies are few and limited to herbaceous plants (Gamon et al. 1992; Winkel et al. 2002) or shrubland vegetation (Peñuelas et al. 1997; Filella et al. 2004).

On the other hand, a greater scatter and no clear pattern emerged when data from deciduous tree species (Q. cerris, Q. robur, Q. pubescens and C. sativa) or all species were considered together (Fig. 7a). The picture tended to change if the relationship between PRI and the photochemical efficiency of PSII was considered. Here, the scatter among species tends to reduce, as shown by the more robust curvilinear relationship obtained in Fig. 7c. Comparable results have been shown by Peñuelas et al. (1995) for Mediterranean species, by Stylinski et al. (2002) for chaparral species, and by Raddi et al. (2005) across a range of environmental conditions and Mediterranean vegetation types. Likewise, other studies report consistency between PRI and ΔF/Fm′ or light-use efficiency across species and environmental conditions (Gamon et al. 1992; Guo and Trotter 2004), although in these latter cases, no drought treatment was applied.

Interspecific differences in the initial reflectance properties seem to be the cause of the strong variability shown in Fig. 7a (i.e. between 0.022 in P. euroamericana and –0.078 in F. sylvatica). Changes in leaf brightness due to changing surface properties (e.g. cuticle thickness or density of leaf hairs) will necessarily influence the apparent reflectance properties independently of pigment content (Gamon and Surfus 1999). However, the scatter tends to reduce a little when ΔPRI is considered in the relationship with A max (R² = 0.44 comparing to R² = 0.21) (Fig. 7b). As has been shown by other authors (Gamon and Surfus 1999; Weng et al. 2006) ΔPRI appeared to be a more robust measure. Normalisation by the difference between the PRI value measured before dawn at the beginning of the experiment and the PRI value measured at midday during the experiment can reduce possible artefacts due to additional confounding effects as changing illumination, atmospheric conditions etc. (Gamon and Qiu 1999).

Nevertheless, normalisation can explain only part of the variability observed. Thus, we argue that additional confounding affects in the PRI-A max relationship when the responses from several species are considered collectively. The most plausible explanation for the large scatter seems the strong difference in the sensitivity of PRI to changes in DEPS. This is

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**Fig. 7.** (a, c) Leaf midday PRI and (b, d) normalised leaf PRI (ΔPRI) versus (a, b) maximum CO₂ assimilation at saturating light (A max) and (c, d) photochemical efficiency of PSII (ΔF/Fm′). Each symbol represents the mean of six to eight measurements in each species. Regression models for PRI v. A max are: ○ $y = 110x + 10.6$, $R^2 = 0.35$, $P < 0.01$; ● $y = 80.9x + 6.3$, $R^2 = 0.93$, $P = 0.000$; ▲ $y = 86.0x + 12.5$, $R^2 = 0.63$, $P = 0.06$; △ $y = 1220x - 5.9$, $R^2 = 0.95$, $P < 0.01$. Regression models for PRI v. ΔF/Fm′ are: ○ $y = 2.0x + 0.21$, $R^2 = 0.77$, $P < 0.01$; ● $y = 1.4x + 0.14$, $R^2 = 0.74$, $P < 0.01$; ▲ $y = 0.19x + 0.11$, $R^2 = 0.05$, $P = 0.7$; △ $y = 9.3x + 0.1$, $R^2 = 0.78$, $P = 0.06$.  

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clearly shown in Fig. 6, where, for example, in *P. euroamericana*, DEPS varies from 0.2 to 0.6 with very little change in PRI; whereas in *F. sylvatica*, DEPS varies from 0.4 to 0.7 with higher changes in PRI. Thus, this confounding factor can have important implications for the use of PRI as an estimator of light-saturated photosynthesis in multispecific forests.

On the other hand, Fig. 7d showed a weaker ΔPRI–ΔF/Fm’ relationship (R² = 0.47 compared to R² = 0.62). In this case, normalisation by including *F. sylvatica*, a species that showed evident signs of photoinhibition before the onset of experiment (Fv/Fm = 0.7), may have contributed to the weakened correlation. In fact, if we exclude *F. sylvatica* values, the relationship was greatly improved (R² = 0.75) (data not shown).

**Conclusions**

The main conclusions that can be drawn from this study on the relationship between PRI and photosynthetic activity in a range of forest tree species belonging to different vegetation belts subjected to a progressive drought are as follows:

(i) We observed a clear relationship between PRI and pre-dawn leaf water potential in the short term, during which no effect of leaf ontogeny was apparent.

(ii) We found a strong and significant relationship between PRI and light-saturated photosynthesis or between PRI and the photochemical efficiency of PSII within single tree species. Wider scatter emerged, when data from all species were pooled together; in contrast, a clearer relationship seems to emerge by plotting ΔPRI and light-saturated photosynthesis.

(iii) We conclude, therefore, that, other than upscaling difficulties from leaf to canopy, the estimate of photosynthesis from PRI could be difficult when dealing with multispecific forest canopies in drought-prone areas, e.g. patchy Mediterranean vegetation, due to the confounding effects of high interspecific variability in the initial value of PRI and in the sensitivity of PRI to changes in DEPS as water stress progresses.

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