Comparative field study of spring and summer leaf gas exchange and photobiology of the Mediterranean trees Quercus ilex and Phillyrea latifolia

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Abstract

Summer-induced changes in gas exchange, fluorescence and reflectance were measured on leaves of two co-occurring Mediterranean small trees, Quercus ilex and Phillyrea latifolia, in May, June and July 1996 in Central Catalonia (NE Spain). The humid 1996 summer only produced mild water stress conditions. However, photosynthesis (A) and stomatal conductance (gs) decreased in June and July in both species. In June P. latifolia had higher net photosynthetic rates and lower stomatal conductances than Q. ilex, thus exhibiting higher instantaneous plant water use efficiencies. In agreement with these results, the photochemical reflectance index (PRI), calculated as (R570−R531)/(R531+R570) of P. latifolia was lower, suggesting a possible lower xanthophyll de-epoxidation state. However, P. latifolia had lower ΔF/ΔFm and therefore a lower electron transport rate (ETR). The behaviour of PRI confirmed previous studies indicating a strong relationship between PRI, ΔF/ΔFm and photosynthetic radiation-use efficiency (PRUE). PRI offers a simple, portable means of assessing PRUE with the potential for remote sensing applications. Finally, the possible ecological consequences of these results on the behaviour of the two species studied under the predicted warmer and drier conditions of global change are discussed.

Key words: Quercus ilex, Phillyrea latifolia, summer, net photosynthetic rates, stomatal conductance, intrinsic water use efficiency, photosynthetic radiation-use-efficiency, reflectance, fluorescence, photochemical reflectance index, photochemical efficiency, water index.

Introduction

Water availability represents a major environmental constraint in the Mediterranean environment. There is usually a long dry summer with low precipitation coinciding with high irradiance and high temperature (Di Castri, 1973). The global change effects on the Mediterranean climate are likely to provide more frequent and longer droughts (Houghton et al., 1995; Peñuelas, 1996; Piñol et al., 1997) such as the recent ones of 1986 and 1994 in Mediterranean Spain. These droughts had significant demographic effects on some typical species such as Quercus ilex subsp. ilex (15% of the individuals died in certain areas of Central Catalonia) and less important effects on other more thermophilic species such as Phillyrea latifolia (only 3% of the individuals died in those areas; Siscart, Dalmases and Lloret, unpublished data).

Q. ilex is a deep-rooted tree widely distributed in the sub-humid areas of the Mediterranean basin between 0 m and 1400 m altitude, whereas P. latifolia is a deep-rooted small tree distributed in warmer and drier Mediterranean areas between 0 m and 1100 m. Studies on leaf gas exchange and ecophysiology of these and other evergreen Mediterranean species have been conducted in a variety of Mediterranean areas, but few comparisons of both species growing in the same site under the same ecological conditions have been made (Tretiach, 1993).

In addition to gas exchange, chlorophyll fluorescence and reflectance methods have many advantages for quantifying stress effects on photosynthesis (Bilger et al., 1995; Peñuelas et al., 1995). These optical measurements are non-destructive and rapid, thereby only minimally affecting the plants (Bolhar-Nordenkampf and Öquist, 1993; Peñuelas et al., 1995). Genty et al. (1989) have...
shown that PSII photochemical efficiency may be estimated from the expression

\[ \frac{(F'\text{m} - F)}{F'\text{m}} = \Delta F/F'\text{m} \]

derived from measurements of leaf fluorescence under ambient light (F), and under saturating light pulses (F'\text{m}) and \( \Delta F/F'\text{m} \) can be used to calculate relative electron transport rates (Schreiber et al., 1995). Another indirect optical measure of photosynthetic performance can be derived from the reflectance changes near 531 nm (Gamon et al., 1993). These changes are associated with xanthophyll pigment interconversion and chloroplast conformational changes, and are correlated with both the epoxidation state of the xanthophyll cycle pigments (Gamon et al., 1990, 1992; Brugnoli and Björkman, 1992) and photosynthetic radiation-use-efficiency (Gamon et al., 1992; Peñuelas et al., 1994, 1995; Filella et al., 1996). For the estimation of plant water content, a reflectance water index (WI) based on the water absorption band at 970 nm and calculated as \( R_{970}/R_{900} \) has also been proposed (Peñuelas et al., 1993, 1996).

The aim of this work was to investigate dysfunctions of photosynthesis induced by the gradual natural summer water deficits in the two co-occurring species, Q. ilex and P. latifolia, and to discuss the possible ecological consequences. Leaf gas exchange, fluorescence and reflectance of adult trees growing in culminal forest isles on rocky hills, on the same soil type and with the same water availability, were compared. A parallel aim of this study was to compare the reflectance changes at 531 nm with fluorescence and gas exchange to evaluate its use in the optical estimation of photosynthetic efficiency.

**Materials and methods**

**Experimental conditions**

The experiment was performed in forest isles located at 950–1050 m altitude on rocky hills in Montcau, Sant Llorenç de Munt natural park (central Catalonia, NE Spain). The vegetation isles are about 30–50 m long and 5–10 m wide on the SE facing slopes of rocky hills. The dominant trees are 3–5 m tall Q. ilex and P. latifolia. These trees root in a poorly developed soil above conglomerates with high water losses through drainage and high evaporation due to high radiation, and frequent and strong wind.

The climate diagram of the area under study is shown in Fig. 1. The climate is sub-humid mediterranean-temperate, with cool wet winters and warm dry summers, with a mean annual temperature of 10.2 °C and a mean annual precipitation of 850 mm. Temperature trends are relatively regular, but precipitation is very variable, which can cause drought periods sometimes in winter or spring which can strongly influence the productivity of the vegetation. Precipitation data (Fig. 1) were provided by a weather station (Mura) located 3 km from the test site.

The measurements were conducted on 13 May, 27 June and 29 July 1996. Measurements were taken on five sun-exposed healthy trees per species (only three for gas exchange measurements on 13 May), on apparently healthy leaves, among the third to the seventh fully developed leaves from the growing branch end of each tree. They were located facing southward in the lower part of the crown, at 1.5–2 m above-ground.

**Methods**

The leaves were studied with successive measurements of leaf gas exchange, reflectance and fluorescence: in the morning (08.30–10.00 h solar time), in the middle of the day (11.30–13.00 h solar time) and in the evening (15.30–17.00 h solar time).

Net CO₂ assimilation and stomatal conductance were measured with a portable gas exchange system (ADC4, configured with chamber model PLC4B, ADC Inc., Hoddesdon, Hertfordshire, England). Photosynthetic radiation-use-efficiency (PRUE) was calculated as follows:

\[ \text{PRUE} = \frac{\text{net CO}_2 \text{ assimilation rate}}{\text{incident PPFD}} \]

Incident PPFD in the leaf was calculated from incident PPFD outside the chamber corrected for chamber light attenuation (previously measured with sensors above and below the chamber lid).

Chlorophyll fluorescence was measured using a portable modulated fluorometer PAM-2000, with the leaf clip holder part 2030-B (Heinz Walz GmbH, Effeltrich, Germany). Photochemical efficiency of PSII \( (F_m' - F)/F_m' \) or \( \Delta F/F_m' \) as defined by Genty et al. (1989), as well as leaf temperature and incident PPFD were measured.

Spectral radiance was measured by directing a fibre optic probe to the leaf surface at the same angle as the leaf clip holder part 2030-B of the fluorometer PAM-2000. The other end of the fibre optic was attached to a spectroradiometer.
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(model SE590 with detector CE390 WB-R, Spectron Engineering, Denver, Colorado). Reflectance was calculated from radiance by normalizing leaf radiance by the radiance of a 99% reflective standard (Spectron, Labsphere, North Sutton, NH). Situations where uneven illumination occurred, e.g. due to partial shading, were avoided. Changes in the angle of the leaf were avoided by careful handling, and as the days were mostly clear, the shading of the leaves by the fibre optics can be considered negligible (Bilger *et al.*, 1995).

To follow the reflectance changes at 531 nm associated with the dissipation of excess energy, the ‘photochemical reflectance index’ PRI was used (Gamon *et al.*, 1990, 1992; Peñuelas *et al.*, 1993, 1995), isolated from narrow waveband spectral measurements, and calculated as follows:

$$PRI = \frac{(R_{570} - R_{531})}{(R_{570} + R_{531})}$$

To assess leaf water content the ‘water index’ WI was used (Peñuelas *et al.*, 1993, 1996), calculated as:

$$WI = \frac{R_{970}}{R_{900}}$$

and to characterize the plant water status further leaf water potential in five leaves per plant at midday was determined using a Scholander pressure-bomb (Soilmoisture 3005) in May. In June, this pressure-bomb broke down and in July the midday relative water content (RWC) was measured. Twenty leaves of each species were detached and weighted (fresh weight, FW). Then they were hydrated until saturation (constant weight) for 48 h at 5 °C in darkness (turgid weight TW). Leaves were then dried in an oven at 105 °C for 24 h (dry weight, DW). Relative water content was calculated according to the expression:

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

Statistical analyses (regressions, calculations of curve intervals of confidence, ANOVA of repeated measures, dendograms, and principal component analysis based in correlation matrix) were performed with the Statview 4.5 program package (Abacus Concepts Inc., Berkeley, CA, USA). The principal component analysis was performed with the aim of summarizing the several variables studied in two or three new comprehensive ones that would show the main factors of variation, and in this way separating diurnal and seasonal changes from species-specific changes.

**Results**

**Environmental conditions and water status**

The three months were unusually wet with monthly precipitations close to 70 mm and well distributed.
throughout dates (Fig. 1). Average leaf temperatures during the diurnal range of measurements were 18.9 ± 0.2°C in May, 24.1 ± 0.2°C in June and 30.7 ± 0.2°C in July (Fig. 2). Leaf incident PPFD is depicted in Fig. 2 showing lower values in the evenings due to shading of the leaves studied through the changing sun angle, and lower values at midday of 27 June because of clouds. The values of midday leaf water potential in *Q. ilex* trees (−1.26 ± 0.16 MPa), and in *P. latifolia* (0.98 ± 0.13 MPa) showed the absence of water stress in May. The RWC at 29 July were 78 ± 2% in *Q. ilex* and 78 ± 3% in *P. latifolia*, i.e. far from the values of the 40% drop suggested by some authors (Chaves, 1991) as causing irreversible perturbations in photosynthetic machinery. *WI* was about 0.91 ± 0.02 in both species in all the months tested, i.e. also far from values around 1 corresponding to the low water content (Peñuelas et al., 1993, 1996). Therefore, the available data suggest the absence of severe water stress even in July.

**Gas exchange**

Net photosynthetic rates were higher in May than in June or July (*P* < 0.01, ANOVA of repeated measurements). *Q. ilex* presented similar values of *A* to *P. latifolia* in May, but lower values in June (*P* < 0.01, ANOVA) and slightly lower in July (at low PPFDs). Photosynthetic radiation-use-efficiency (*PRUE*) was thus higher for *P. latifolia* in June and in the evening in July. These differences are shown in Fig. 3 where net photosynthetic rates are plotted against PPFD and *g*ₐ. Leaf stomatal conductance of both species was also lower in June and July (averages of 25–50 mmol m⁻² s⁻¹) than in May (averages of 100–200 mmol m⁻² s⁻¹) (Fig. 4). In June *g*ₐ was significantly lower in *P. latifolia* than in *Q. ilex* (*P* < 0.01, ANOVA). It was also lower (although not significantly) in May and July (Fig. 4). In the June and July evenings, as a consequence of *g*ₐ being lower and *A* being higher, the ratio between *A* and *g*ₐ (intrinsic water use efficiency) was higher for *P. latifolia* than for *Q. ilex* (*P* < 0.01, ANOVA) (Fig. 4).

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**Fig. 3.** The relationship between net photosynthetic rates (*A*) and incident PPFD and leaf conductance (*g*ₐ) of sun-lit leaves of *Q. ilex* and *P. latifolia* in May, June and July 1996. An asterisk is depicted when the difference between intercepts or slopes of the curves for the two species was significant at *P* < 0.05.
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Fig. 4. Daily time-courses of stomatal conductance and intrinsic water use efficiency (A/gs) of studied sun-exposed leaves of Q. ilex and P. latifolia in May, June and July 1996. An asterisk is depicted when the difference between the two species was significant at P<0.05. Error bars are ±SE.

Fluorescence and reflectance

ΔF/Fm decreased with PPFD (Fig. 5) as expected (Bilger et al., 1995). The ΔF/Fm reached the lowest values in June and in P. latifolia. Values followed an almost linear increase with only minor scattering at low PPFD levels, and tended to saturate, and with increased data scattering, at high PPFDs. In June ΔF/Fm of P. latifolia were lower than those of Q. ilex while A was higher (Fig. 3).

The reflectance index PRI followed an asymptotic response to increasing incident PPFD, with similar curves for both species. However, in June PRI was slightly lower in P. latifolia than in Q. ilex (Fig. 5) possibly indicating lower zeaxanthin formation (lower de-epoxidation state; Peñuelas et al., 1995). This correlated with its higher photosynthetic radiation-use-efficiency (Fig. 3). Moreover, there was an inverse relationship in both species between PRI and photochemical efficiency ΔF/Fm, and between PRI and photosynthetic radiation-use-efficiency (PRUE) (Fig. 6).

The multivariate analyses principal components (Fig. 7) and dendrogram (data not shown) of the different variables under study distinguished three groups. One group was formed by variables linked to hour of the day such as photochemical efficiency ΔF/Fm, PRUE, PRI or PPFD, another was formed by variables linked to month such as A/gs, temperature, or A, and another group was formed by variables linked to species such as A/gs or gs (Fig. 7). The first component (49% of the total variance) may be interpreted as the daytime variation, the second component (14% of the total variance) as the seasonal variation, and the third component (12% of the total variance) as the species variation. This species variation was found mainly in June, when different behaviour was found for the two species studied accompanying mild drier conditions.

Discussion

Gas exchange

Numerous studies of leaf gas exchange in Mediterranean type climates have demonstrated similar leaf responses to
those described here: decreasing net photosynthetic rates and stomatal conductances from spring to summer with increasing drought (Oechel et al., 1981; Tenhunen et al., 1990). However, in the 1996 summer period, the severe water stress usually experienced in Mediterranean ecosystems, which would probably have accentuated these responses, was not so marked. Similar photosynthetic activities to those found here in spring and summer in Q. ilex have also been reported in the same region of north-east Spain (Castell et al., 1994; Sala and Tenhunen, 1994) in Italy and in other Mediterranean regions (Tretiach, 1993; Chaves et al., 1995; Scarascia-Mugnozza et al., 1996).

The decrease in stomatal conductance in June and July produced an increase in tree instantaneous water use efficiency and altered the efficiency of conversion of intercepted light energy to photoproducts. In fact, it is well-known that the photosynthetic efficiency of Mediterranean sclerophylls in converting light energy to photoproducts decreases progressively during summer drought (Oechel et al., 1981; Lawrence, 1987) and it becomes more strongly influenced by atmospheric environmental conditions and time of day (Tenhunen et al., 1989). However, as the 1996 summer drought was minimal, maximal carbon fixation did not occur during periods of relatively low incident light intensity and relatively low temperature early and late in the day as is usual under summer drought (Beyschlag et al., 1986).

Instead, net photosynthetic rates and stomatal conductances followed the PPFD levels (Figs 3, 4) which also indicates the absence of severe water stress. In fact, the multivariate analyses showed that the main factor of variation (49%) in these data was the daytime variation (and the corresponding variation of temperature). The variance associated to species only appeared in second place (14% of variance). The difference between the two species studied was mainly linked to their different A/gs and gs, and only secondarily linked to their different photochemical efficiency ΔF/F∞ and PRI in June and July.

While gas exchange response was nearly identical...
between the two species in May, it was different in June and slightly different in July. The seasonal progression from one phase to the next depends on whole plant function in maintaining the water supply, i.e. on gas exchange, on photobiology, on canopy leaf area or on root system development (Tenhunen et al., 1994). In this study some functional differences on gas exchange and on photobiology between the two species were found mainly on 27 June. The stomatal conductance ($g_s$) was lower in $P$. latifolia than in $Q$. ilex. Moreover, in June lower $g_s$ of $P$. latifolia was accompanied by higher photosynthetic rates (Figs 4, 5). Thus, the relation of net photosynthetic rate to conductance ($A/g_s$), increased in response to the mild water stress of summer 1996, especially in $P$. latifolia.

Fluorescence and reflectance: PRI assessment of PRUE

The decrease of photochemical efficiency of these plants in June was reversible as represented by the July recovery, indicating a down-regulation of photosynthesis rather than a photoinhibitory process.

In June, $P$. latifolia had a lower photochemical efficiency than $Q$. ilex in spite of having higher $A$ and PRUE. High photorespiration rates (Heber et al., 1990; Scarascia-Mugnozza et al., 1996) or even higher terpene emission rates (Peñuelas and Llusia, unpublished results) in $Q$. ilex may play an important role in the maintenance of its higher electron flow. But discrepancies may also arise from the profound natural variation in exposure of leaves that introduced large scattering in these relationships. In June, $P$. latifolia PRIs were also lower than in $Q$. ilex, probably indicating a lower de-epoxidation state (Gamon et al., 1990, 1992; Peñuelas et al., 1995; Field et al., 1995) in agreement with its higher PRUE.

PRI and $\Delta F/F_m$ have been shown to exhibit slightly different kinetics upon light transitions (PRI lags behind $\Delta F/F_m$; Peñuelas et al., 1995). These kinetic differences suggest the involvement of multiple processes with separate time constants affecting reflectance and fluorescence to different degrees (Peñuelas et al., 1995). But, despite these slight kinetic differences, a linear relationship between PRI and $\Delta F/F_m$ for each plant species was found here over a wide range of irradiances (Fig. 6). This relationship reinforces previous results on a wide range of species (Peñuelas et al., 1995) and supports the use of PRI as a rapidly responding index of instantaneous photosynthetic radiation-use-efficiency at the level of PSII.

Much of the scatter in the relationships between PRI, $\Delta F/F_m$, and PRUE could be attributed to several factors that can affect the exact matching of PSII photochemical efficiency and whole-leaf CO$_2$ assimilation. In addition, microenvironment changes including the Mehler reaction (Harbison et al., 1990; Neubauer and Yamamoto, 1992), photorespiration (Harbison et al., 1990) and nitrate reduction (Bloom et al., 1989), can all compete with carboxylation for reductant generated by photosynthetic electron transport. The significant correlation within each

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**Fig. 6.** Relationship of photosynthetic radiation use efficiency (PRUE) and $\Delta F/F_m$ with photochemical reflectance index (PRI) in $Q$. ilex and $P$. latifolia leaves.

- **A.** Relationship of PRUE ($\mathrm{mol\ CO}_2\ \mathrm{mol^{-1}\ PFD}$) with PRI.
- **B.** Relationship of $\Delta F/F_m$ with PRI.
species of this study and previous ones (Peñuelas et al., 1995) suggests that the overall photosynthetic system is sufficiently regulated to maintain consistent relationships between PSII processes and carboxylation. This consistency is supported by laboratory studies, which also demonstrate highly reproducible correlations between $\Delta F_i/F_m$ and photosynthetic radiation-use-efficiency over several disparate species (Seaton and Walker, 1990).

Understanding the exact relationship and the multiple factors that can cause these relationships to change will require further experimental work. However, PRI can provide an interesting tool for non-contact assessment of photosynthetic function of leaves and canopies.

**Sensitivity to drought**

The extensive and deep root system of *Q. ilex* appears to support a higher leaf conductance and little conservative use of water in mature plants at times of high water availability (Thomas and Davis, 1989). At drier conditions, however, net photosynthetic rates are lower than in *P. latifolia* indicating a greater sensitivity to drought or/and high temperature as has already been reported (Tretiach, 1993). During the summer drought and high temperatures, the photosynthetic activity of *Q. ilex* is strongly reduced, more than in *P. latifolia*, as also shown in our results even though they were obtained under a mild dry summer.

*P. latifolia* did not have greater leaf reflectance in the visible spectrum than *Q. ilex* (data not shown), nor a decrease in chlorophyll (there was no difference in chlorophyll concentration; both species 52 SPAD units) through which to absorb less photons. Additional adaptations to withstand the adverse conditions of Mediterranean drought should include the transformation of photosynthetic machinery to a more efficient radiation user. PRI of *P. latifolia* was lower than in *Q. ilex* indicating a lower de-epoxidation state that was accompanied by higher PRUE. Although higher assimilation during short periods of drought does not ensure survival during prolonged stress periods, these results together with those of demographic responses to severe droughts (Siscart, Dalmases and LLoret, unpublished data) indicate a better adaptation of *P. latifolia* to drought than *Q. ilex*. They explain the slightly different distribution of the two species and allow the hypothesis that there will be a change of dominance by *P. latifolia* of *Q. ilex* in the warmer drier climate predicted for the Mediterranean region by global change circulation models (Houghton et al., 1995) or under the climatic changes already occurring in the region under study, where drier and warmer years than in the first part of the century are currently being experienced (Piol et al., 1997).

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