Leaf gas exchange and fluorescence of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings in severe drought and high temperature conditions

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Abstract

Saplings of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* were withheld watering for 7 days, followed by reirrigation. Incident photosynthetic photon flux density (PPFD), leaf temperature, net photosynthetic rates, stomatal conductance, and photochemical efficiency of the photosystem II (AF/Fm) were measured three times during the day. The watered plants had higher photosynthetic rates, stomatal conductances, AF/Fm and ETR than non-watered plants. However, watered plants were mildly water stressed as shown by low ratio of variable to maximal fluorescence (Fv/Fm) and high non-photochemical fluorescence quenching (qN). Their AF/Fm was low in the morning and increased in the evening, following the variations in PPFD. Watered plants of *Q. ilex* had lower photosynthetic activity, stomatal conductance and photosynthetic radiation use efficiency than *Ph. latifolia* and *P. lentiscus*, and, conversely, reached the highest AF/Fm and ETR. This seems to indicate a different relationship between photosynthetic activity and electron transport rate in *Q. ilex* compared to the other two species. *Ph. latifolia* and *P. lentiscus* appeared to be better adapted to severe drought than *Q. ilex*. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Drought; AF/Fm; *Phillyrea latifolia*; *Pistacia lentiscus*; Photosynthetic rate; *Quercus ilex*; Stomatal conductance

1. Introduction

In Mediterranean ecosystems, drought, high irradiance and high temperature for short or long periods may dramatically influence the ecophysiology of plants and limit their production. Different species tend to adapt to environmental conditions by different ecophysiological and structural adjustments at different time scales, e.g. the modulation of growth activity (De Lillis and Fontanella, 1992), the maximal carbon fixation early and late in the day, or the sensitive stomatal control of water loss (Tenhunen et al., 1990; Sala and Tenhunen, 1994). When prolonged or more severe droughts occur, the capacity of the different species to avoid damaging effects determines their survival.

Severe droughts in 1986 and 1994 in eastern Spain caused different degrees of injury depending
on the species, e.g., higher mortality in Quercus ilex (13%) than in Phillyrea latifolia (3%) in some N.E. Spain forests (D. Siscart and F. Lloret, pers. comm.). Nearly no mortality was observed in P. lentiscus. Q. ilex is an evergreen sclerophyllous deep-rooted tree dominant in the landscape all around the Mediterranean basin between sea level and 1400 m altitude. However, Q. ilex is sensitive to drought (Tetriach, 1993) and is absent in the most arid areas of the Mediterranean zone. Ph. latifolia is a deep-rooted small tree occurring together with the shrub P. lentiscus in warmer and drier Mediterranean areas than Q. ilex, between sea level and 1100 m. P. lentiscus exhibits less sensitivity to high temperature and low humidity than most other Mediterranean species (Tenhunen et al., 1987) and is considered to be one of the species most tolerant to drought.

As global change effects on Mediterranean climate are likely to provide more and stronger droughts (Houghton et al., 1996; Peñuelas, 1996) like the recent ones of 1986 and 1994, it is important to understand the physiological responses of these species to severe drought in order to predict possible changes in species dominance and in landscape structure. Short-term drought permits observation of changes in some physiological processes (like stomatal conductance or photosynthetic rate) that are typical for progressive stages of drought.

Thus, as previous demographic and distribution data indicate that Ph. latifolia and P. lentiscus are better adapted to drought than Q. ilex, our hypothesis was that Ph. latifolia and P. lentiscus would present a more drought-adapted gas exchange pattern. We studied their leaf gas exchange and chlorophyll fluorescence under progressive drought conditions by subjecting potted saplings to increasing water deficits, in order to understand whether these ecophysiological processes were differently regulated by the studied species.

2. Materials and methods

Q. ilex L., Ph. latifolia L. and P. lentiscus L. saplings, 2 years old were bought in a plant nursery in Girona (4° 75’ E, 4° 46’ N). They were offsprings germinated from seeds of different parent plants from the surrounding natural vegetation. They were grown in 1.8 L pots in a plastic tunnel, 28 m long and 6 m wide, to avoid undesired watering by natural precipitation. From 10 June, 1996 onwards, 20 plants of each species were irrigated once daily to maximum pot capacity to assure good water supply. Water was withheld from 20 other plants until the 17 June to generate severe drought conditions, and then reirrigated again. Leaf gas exchange and fluorescence measurements were conducted the 10, 17 and 25 June in the morning (8–9 h solar time), in the middleday (12–13 h solar time) and in the evening (16–17 h solar time).

Incident PPFD (photosynthetic photon flux density), leaf net photosynthetic rate, and stomatal conductance were determined with a portable gas exchange system (ADC4, configured with chamber model PLC4B, ADC, Hodderdon, Hertfordshire). Branch ends with several leaves were inserted into the chamber of the system. The partial shading of some of the leaves of the branches was unavoidable due to their distribution (this shading may have led to a slight underestimation of photosynthetic rate). Three plants per species, the same one for every sampling time during the day, were measured outside the tunnel. Ambient air temperature was also measured outside the tunnel.

Chlorophyll fluorescence was determined using a portable modulated fluorometer PAM-2000, and a leaf clip holder 2030-B (Heinz Walz GmbH, Effeltrich, Germany). The following parameters were measured: photochemical efficiency of PSII in light adapted state (F’m-Fs)/F’m or ΔF/F’m as defined by Genty et al. (1989), Fv/Fm (maximal PSII photochemical efficiency) (Bolhá-Nordenkampf and Öquist, 1993) and qN (non-photochemical fluorescence quenching) (Ruban and Horton, 1994), as well as leaf temperature and incident PPFD. Fv/Fm was measured as well by applying a brief saturating light pulse (< 5000 μmol m−2 s−1) in dark adapted leaves by covering them in the morning (06.00 h) with an opaque bag for 20 min. ΔF/F’m and qN were measured during the day and previous to gas exchange
measurements, by applying an additional saturating light pulse (> 5000 μmol m⁻² s⁻¹). Apparent photosynthetic electron transport rate (ETR) was calculated by multiplying ΔF/F’m by PPFD, by 0.85 (considering that about 85% of incident quanta are absorbed by the leaf), and by 0.5 (transport of one electron requires absorption of two quanta). These are apparent values (to know proper ones, the exact amount of quanta absorbed by PSII would have to be known).

Midday relative water content (RWC) was measured on 17 June. Twenty leaves (4 leaves × 5 saplings) of each species were detached and weighted (fresh weight, FW). They were then 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 = (FW − DW)/(TW − DW).

Statistical analyses were performed with the Statview 4.5 program package (Abacus Concepts, Berkeley, CA).

3. Results

Daily time courses of PPFD ranged from 1200–1500 μmol m⁻² s⁻¹ in the morning, 1700–2000 μmol m⁻² s⁻¹ in the midday and 1000–400 μmol m⁻² s⁻¹ in the evening. The low irradiation values in the evenings were due to high altitude clouds. Leaf temperature followed a similar pattern with values ranging from 25–27°C in the morning and the evening, and reaching maximum values of 34°C at midday for the three species (Fig. 1). Vapour pressure deficits ranged between 3 and 3.5 kPa at midday and between 1.5 and 2.0 kPa in the morning and the evening.

Net photosynthetic rates in the three species were quite low, even for well watered plants (the maximum daily photosynthetic activity was around 3 μmol m⁻² s⁻¹ at PPFD of 2000 μmol m⁻² s⁻¹ in the midday, for Ph. latifolia and P. lentiscus and around 3 μmol m⁻² s⁻¹ for Q. ilex at PPFD of 1300 μmol m⁻² s⁻¹ in the morning) (Fig. 2). The first day, irrigated Ph. latifolia and P. lentiscus reached maximum photosynthetic ac-
Fig. 2. Daily pattern of photosynthetic rate and stomatal conductance of sun-lit leaves of *Phillyrea latifolia*, *Pistacia lentiscus* and *Quercus ilex* saplings submitted to irrigation and to water withhold for 7 days. Hours are expressed in solar time. Error bars are ± S.E. Day 1 and 8 correspond to the 10 and 17 June 1996, respectively.

Activity at midday. The period between the first and the second measurements were very hot (a maximum of 33–34°C at midday and a minimum of 25°C at night) and with high radiations and vapor pressure deficits (all days had maximum values of 2000 μmol m⁻² s⁻¹ and 3.5 kPa respectively at midday). As a consequence, plants, although watered, presented lower stomatal conductances and assimilation rates during the second measurement day than the first day. With the exception of *Ph. latifolia* in the morning, plants from the drought treatment had negative net photosynthetic rates (respiration dominated).

Stomatal conductance values were low (Fig. 2) and their daily variation was parallel to that of photosynthesis. The highest values were reached the first day, and *Ph. latifolia* and *P. lentiscus* showed higher values than *Q. ilex* (Fig. 2). There were hardly positive values for plants under drought. At drought lower gs of *Ph. latifolia* was accompanied by still positive photosynthetic rates while *Q. ilex* or *P. lentiscus* had negative photosynthetic rates (Fig. 2). After 1 week without irrigation, RWC of *Ph. latifolia* ranged between 62 and 84%, while in *Q. ilex* plants it dropped below 40%. The third sampling date is not shown in Fig. 1 and Fig. 2 for sake of clarity and because it rained during the evening. At that sampling date, plants had been reirrigated for 1 week and the values of all studied variables were very similar to these of irrigated plants at the first sampling date (photosynthetic rate between 1 and 3.5 μmol CO₂ m⁻² s⁻¹, and stomatal conductance between 0.02 and 0.06 mol H₂O m⁻² s⁻¹) except for *Q. ilex* plants being so severely affected by drought treatment that they did not recover.

The fluorescence variable ΔF/F’m, which indicates the quantum efficiency of PSII electron flow, was very low (0.1–0.2) in the morning and increased only in the evening (0.3–0.4) in the watered plants (Fig. 3) following the variations in PPFD (Fig. 4). However, values were low, even in watered plants. *Q. ilex* presented the highest ΔF/F’m in the watered treatment and the lowest in drought treatment. *Ph. latifolia* showed the highest values during drought treatment. Their ΔF/F’m decreased at midday and increased again in the evening similar to that of watered plants. In
the three species there was a steady decline of $\Delta F/F'm$ with increasing PPFD. In *Q. ilex* $\Delta F/F'm$ reached the change saturation at 800 µmol m$^{-2}$ s$^{-1}$, while in *P. lentiscus* and *Ph. latifolia* $\Delta F/F'm$ was already saturated around 600 µmol m$^{-2}$ s$^{-1}$ (Fig. 4). We considered the change in $\Delta F/F'm$ versus PPFD saturated, when the slope of the curve was lower than 0.00005 $\Delta F/F'm$ µmol$^{-1}$ m$^{-2}$ s$^{-1}$.

In the three species ETR was higher in the watered treatment than in the drought treatment. Watered plants of *Q. ilex* had the highest ETR values (Fig. 3). The relationships between photosynthetic rate and ETR for the three species are presented in Fig. 5. *Q. ilex* reached higher ETR values than the other two species for lower photosynthetic rates as indicated by the lower slope ($P < 0.05$).

Fv/Fm morning values were around 0.6–0.7 for watered plants of *Ph. latifolia* and *P. lentiscus* and 0.7 for watered plants of *Q. ilex*. These Fv/Fm values decreased to zero in *Q. ilex* and to around 0.4 in *Ph. latifolia* and *P. lentiscus* after 8 days withholding watering. qN presented high values (mostly between 0.6–0.8) in all three species.

4. Discussion

Different studies of leaf gas exchange in Mediterranean plants found similar leaf responses to those described here. Net photosynthetic rates and stomatal conductances decreased with increasing drought (Oechel et al., 1981; Tenhunen et al., 1987; Damesin and Rambal, 1995; Peñuelas et al., 1998), but the sensitivity and the response rate depended on the species (Acherar and Rambal, 1992). The closure of stomata protects plants from exposure to severe water stress. Stomata generally remained fully open until a critical leaf water potential was reached (Ludlow, 1980), then, as water availability decreases, they begin to narrow until full closure, with the cessation of photosynthetic CO$_2$ uptake and stomatal transpiration. This critical leaf water potential for complete closure is generally much lower for field grown...
plants than for plants grown under controlled conditions (Fitter and Hay, 1987). This fact together with the low capacity of the pots could have accelerated the attainment of extremely low levels of assimilation rates or stomatal conductances in the drought treatment of this study. The intense radiation (around 2000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) at midday) and the high temperatures (up to 35°C) and VPD (up to 3.5 kPa) may have also contributed to the low photosynthesis.

The fluorescence values also showed severe stress conditions. Even the watered plants were mildly stressed, and this may be as a consequence of high vapour pressure deficits producing low stomatal conductances. \( \text{Fv}/\text{Fm} \) morning values were suboptimal if compared to values presented in the literature for unstressed leaves of higher plants (0.75–0.85) (Bjorkman and Demmig, 1987). \( \text{Fv}/\text{Fm} \) has been found to decrease below these values in \( \text{Q. ilex} \) (Methy et al., 1996) and other \( \text{Quercus} \) species only beyond a predawn leaf water potential of about –4 MPa (Epron et al., 1993).

It has been described that photosynthetic efficiency in Mediterranean sclerophylls decreased during drought (Oechel et al., 1981; Lawrence, 1987) as it was found here. In this study, stomatal conductance, net photosynthetic rates, photochemical efficiency (\( \Delta \text{F}/\text{Fm} \)) and ETR severely decreased, thus indicating closed (reduced) photosynthetic reaction centers. However, some functional differences were found between \text{Q. ilex}, \text{Ph. latifolia} and \text{P. lentiscus}. ETR may be compared to the rate of CO\(_2\) assimilation because four electrons are needed for every CO\(_2\) assimilated. However, this relationship was not constant because there were other processes that drive electrons besides CO\(_2\) assimilation, e.g. photorespiration, nitrite reduction or electron cycling at PSII. Watered plants of \text{Q. ilex} presented the lowest photosynthetic activities (Fig. 2) and photosynthetic radiation use efficiencies, and, conversely, reached the highest \( \Delta \text{F}/\text{Fm} \) and ETR values (Figs. 3 and 4). This seems to indicate a different relationship between photosynthetic activity and electron transport in \text{Q. ilex} than in the other two species, as it is shown in Fig. 5, and as it was also found on adult trees at natural field conditions (Peñuelas, 1996). Fig. 4 also shows a different response of \( \Delta \text{F}/\text{Fm} \) to PPFD in \text{Q. ilex}. This discrepancy may be explained by the larger use of reductant in other processes besides carboxylation. High photorespiration rates in \text{Q. ilex} (Heber et al., 1990; Scarascia-Mugnozza et al., 1996) was probably the main cause for the maintenance of its higher electron flow. Increased photorespiration has also been suggested as a possible mechanism of dissipating excess photochemical energy in water-stressed plants (Cornic and Briantais, 1991; Kozakici and Takeba, 1996). In this study, \text{Q. ilex} might dissipate more photochemical energy (ETR) in photorespiration than \text{Ph. latifolia} and \text{P. lentiscus}, what would be more detrimental under severe drought conditions, when available photochemical energy (ETR) decreased.
At severe dry conditions, after the week of drought (watering withheld), only *Phillyrea latifolia* showed positive net photosynthetic rates, indicating lower sensitivity to drought or high temperature than *Quercus ilex*. Moreover, *Q. ilex* did not recover after reirrigation, although it could not be elucidated which part of this extreme response was due to physiological traits, and which to the small pot capacity.

In summary, *Phillyrea latifolia* and *Pistacia lentiscus* presented higher photosynthetic rates and lower stomatal conductances at water stress conditions and *Phillyrea latifolia* survived severe short-term drought conditions, whereas *Q. ilex* did not. Higher assimilation rates during short periods of drought do not assure survival during prolonged stress periods. Thus, to compare different adaptive strategies it is very important to conduct long term observations (Tetriach, 1993). However, in this case, not only the short-term results but the evidence of long-term demographic effects of severe droughts such as those of 1986 and 1994 (D. Siscart and F. LLoret, pers. comm.) indicate a better adaptation to drought in *Phillyrea latifolia* and *Pistacia lentiscus* than in *Quercus ilex*.

The ecophysiological characteristics described in this study are in agreement with the different distribution of these three species. They are also in agreement with predictions of a possible change in dominance of *Quercus ilex* by dominance of *Phillyrea latifolia* and other species with similar adaptations to drought, such as *Pistacia lentiscus*, in a warmer drier climate (Peñuelas et al., 1998), such as the one predicted in the Mediterranean region by global change circulation models (Houghton et al., 1996). Changes towards warmer and drier conditions have already occurred during this century in the studied region (Piñol et al., in press). However, these predictions on change in species dominance of *Quercus ilex* by dominance of *Phillyrea latifolia* are based on water availability. Other environmental factors such as winter low temperatures and latitude also strongly influence these species (Sakai and Larcher, 1987; Tetriach, 1993), and have to be considered.

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