Chlorophyll fluorescence responses to temperature and water availability in two co-dominant Mediterranean shrub and tree species in a long-term field experiment simulating climate change*

Romà Ogaya *, Josep Peñuelas, Dolores Asensio, Joan Llusia

Global Ecology Unit CREAF-CEAB-CSIC, CREAF (Center for Ecological Research and Forestry Applications), Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain

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ABSTRACT

A rain exclusion experiment simulating drought conditions expected in Mediterranean areas for the following decades (15% decrease in soil moisture) is being conducted since 1999 in a Mediterranean holm oak forest to study its response to the forecasted climatic changes for the coming decades. The maximum PSII quantum yield of primary photochemistry (Fv/Fm) was measured in Quercus ilex, and Phillyrea latifolia, the co-dominant species of the studied forest, from 1999 to 2009 in four plots: two of them were control plots and the other two plots received the rain exclusion treatment. In both species, the Fv/Fm values were highly dependent on air temperatures, and in a second term, in water availability. P. latifolia was the species with the larger decrease in Fv/Fm values induced by low air temperatures, while in hot seasons, the Fv/Fm values in P. latifolia were even higher than in Q. ilex. Rainfall exclusion decrease Fv/Fm values significantly only in few monitoring dates. The most drought resistant species P. latifolia was more affected by the experimental rainfall exclusion than Q. ilex that instead lost number of leaves per tree. There was a synergic effect of drought stress and winter cold in P. latifolia not observed in Q. ilex, but a more conservative strategy in P. latifolia maintaining leaves with a down-regulation of the linear photosynthetic electron transport. These results indicate that, although other physiological and reproductive strategies at whole plant level must be also taken into account, the warmer and drier environment expected for the following decades could favour the species more sensitive to cold and more resistant to drought, the shrub P. latifolia, in detriment of the tree Q. ilex as already observed in the field after severe heat-drought episodes.

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1. Introduction

An increase in air temperatures and a slight decrease in precipitations are forecasted for the Mediterranean Basin by most General Circulation Models (GCMs) (IPCC, 2007). Lower water availability is also projected for this region by ecophysiological models such as GOTILWA (Sabaté et al., 2002; Peñuelas et al., 2005). In fact, increased temperatures and lower water availability have been already experienced in the last decades (Le Houérou, 1996; Piñol et al., 2000; De Luis et al., 2001; Peñuelas et al., 2002, 2005; Peñuelas and Boada, 2003).

Several studies have described restricted plant activity in the Mediterranean area in summer months due to heat and drought stress (Tenhunen et al., 1990; Filella et al., 1998; Peñuelas et al., 1998, 2007; Larcher, 2000; Llusia and Peñuelas, 2000; Yordanov et al., 2000; Ogaya and Peñuelas, 2003a,b). But warming may also alleviate the plant stress produced by minimum temperatures in colder seasons to which some Mediterranean species are sensitive (Mitrakos, 1980; Oliveira and Peñuelas, 2000, 2001, 2004; Ogaya and Peñuelas, 2003a,b, 2007).

The maximal photochemical efficiency, Fv/Fm ratio, is the most common measurement of chlorophyll a fluorescence, and it is inversely proportional to damage in the PSII reaction centres (Farquhar et al., 1989). Reductions in photochemical efficiency in Mediterranean species have been detected in summer in response to drought and also in winter in response to cold stress (Karavats and Manetas, 1999; Gratani et al., 2000; Larcher, 2000; Oliveira and Peñuelas, 2000, 2001, 2004; Llorens et al., 2003a,b; Ogaya and Peñuelas, 2003a,b; Bellot et al., 2004; Prieto et al., 2009).

Quercus ilex L. (holm oak) and Phillyrea latifolia L. are plant species frequently co-occurring in the Mediterranean forests. In particular, Q. ilex is a tree widely distributed in the sub-humid areas of the Mediterranean Basin, whereas P. latifolia is a tall shrub.
distributed in warmer and drier areas (Tretiach, 1993; Lloret and Siscart, 1995; Peñuelas et al., 1998, 2000).

Our aim was to study the effect of a long-term experimental drought on maximal of PSII of two dominant woody species of Mediterranean forests in order to elucidate how these two dominant species respond to increasing drought and to the interacting effects with heat in summer and cold in winter. We also aim to study the possible advantage of photochemical efficiency in P. latifolia compared to the actual dominant species Q. ilex under these drier conditions, as observed in other parameters such as gas exchange (Ogaya and Peñuelas, 2003b) and leaf demography (Ogaya and Peñuelas, 2006). The experimental period covered 10 years (1999–2009) and the four seasons of the year, so as to include inter-annual and inter-seasonal variability in the study of the plants’ responses to drought treatment.

2. Materials and methods

2.1. Study site

The present study was carried out in Prades holm oak forest in Southern Catalonia (NE Spain) (41°21′N, 1°2′E), at 950 m asl and on a south-facing slope (25% slope). The soil is a Dys- tric Cambisol over Paleozoic schist, and its depth ranges from 35 to 90 cm. This holm oak forest has a very dense multi- stem crown (16,616 stems ha−1) and it is dominated by Q. ilex (8633 stems ha−1), P. latifolia (3600 stems ha−1) and Arbutus unedo L. (2200 stems ha−1), with abundant presence of other evergreen species well adapted to dry conditions (Erica arborea L., Juniperus oxycedrus L., Cistus albidus L.) and occasional individuals of deciduous species (Sorbus torminalis (L.) Crantz, and Acer monspessulanum L.). This forest is non-perturbed since 60 years, and the maximum height of the dominant species is about 6–10 m tall.

2.2. Experimental design

In the study site, four 15 m × 10 m plots were delimited at the same altitude along the mountain face. Two of them (randomly selected) received the treatment consisting of partial rain exclusion by suspending PVC strips at a height of 0.5–0.8 m above the soil (covering 30% of the soil surface), and the excavation of a 0.8 m deep ditch at the upper part of the plots to intercept runoff water supply. The rain exclusion treatment started in January 1999. Water interception by strips and ditches was conducted outside the plots, below their bottom edge. The other two plots did not receive any treatment and were considered control plots.

An automatic meteorological station installed between the plots monitored temperature, photosynthetic active radiation, air humidity, and precipitation. Soil moisture was measured each month throughout the experiment by time domain reflectometry (Tektronix 1502C, Beaverton, OR, USA) connecting the time domain reflectometer to the ends of three stainless steel cylindrical rods, 25 cm long, fully driven into the soil (Zegelin et al., 1989). Four sites per plot were randomly selected to install the steel cylindrical rods for soil moisture measurements.

2.3. Chlorophyll fluorescence

The maximum PSII quantum yield of primary photochemistry (Fv/Fm) was measured in each of the 4 annual seasons under clear-sky conditions during the overall studied period (11 years). Fv/Fm was measured with a PAM-210 fluorometer (Walz, Effeltrich, Germany) in sunlit leaves at the top of the canopy of the two most abundant species of this forest, Q. ilex and P. latifolia. Before each measurement, the leaves were dark-adapted for 20 min with leaf clips. In each plot, two trees per species were randomly selected and five current-year leaves in each tree were measured during midday solar time.

2.4. Statistical analyses

Simple linear regressions were conducted to examine the relationships between Fv/Fm values and air temperature (during the measurement, mean temperature of the day of measurement, and minimum temperature of 1, 3, or 5 days before measurement), soil moisture, and rainfall (of the 1, 2, 3, or 4 months before measurement). Later, multiple linear regressions were conducted to test the meteorological influence on the maximal photochemical efficiency of PSII. Fv/Fm was the dependent variable and air temperature, and soil moisture or rainfall the predictor variables. In these multiple regressions the forward stepwise regression technique was used. Repeated measurements analysis of variance (ANOVA) was conducted with soil moisture values in each plot as dependent variable and treatment as independent factor. Another ANOVA was conducted with Fv/Fm values as dependent variable and season of measurement, species, and treatment as independent factors. Fv/Fm values were arcsin transformed to reach the normality assumptions of the ANOVAs and regression analysis. All analyses were performed with the Statistica software package (Statsoft Inc., Tulsa, OK, USA).

3. Results

The climate of the area studied is of mountain mesic Mediterranean type. During the study, the mean annual temperature was 12.3 °C, and the mean annual rainfall 668 mm (Fig. 1). Rainfall was concentrated in spring and autumn seasons, whereas the summers were the driest periods coinciding with higher air temperatures (Fig. 1). The drought treatment reduced about 13% soil moisture of drought plots compared to control plots (Fig. 1), but this reduction was larger during rainfall seasons and lower during dry seasons (P < 0.01).

The highest values of the maximal photochemical efficiency of PSII (Fv/Fm) were measured in spring, under the optimum combination of air temperature and water availability (Fig. 2); the lowest Fv/Fm values were reached in winter when air temperatures were the lowest ones (Fig. 2); and intermediate values were measured in summer and autumn, either because of low water availability or low air temperature, respectively (Fig. 2). The Fv/Fm values ranged from 0.8 in warm and humid periods to 0.2 in winter 2005, when an exceptional cold and dry period occurred (Fig. 3). These results and those obtained by the regression analyses showed clearly that the Fv/Fm values were dependent on air temperature and water availability, decreasing in parallel with the temperature and water availability. Air temperature was a more important factor determining Fv/Fm values than water availability as shown by the semi-partial correlation values for these two variables in the multiple regressions (Table 1).

Fv/Fm values were positively related with rainfall of the previous 2, 3, and 4 months, but the strongest relationship was obtained with soil moisture. Fv/Fm values were also positively related with the air temperature during the measurements, and the mean temperature of the day of measurement, but the strongest relationship was obtained with the minimum temperature of the preceding 24 h, rather than the minimum temperature of the preceding 3 or 5 days. Stepwise multiple regressions showed a positive combined effect of both minimum air temperature and soil moisture in the Fv/Fm values. The effect of minimum air temperature on Fv/Fm values was larger in P. latifolia than in Q. ilex (Fig. 2 and Table 1), and the effect of soil moisture was larger in Q. ilex than in P. latifolia (Table 1).
Table 1

Multiple linear regression equations for Fv/Fm values in Q. ilex and P. latifolia as a function of different climatic variables: "Tmin′′ is the minimum temperature of the preceding 24h of measurement, and "SM" is the soil moisture (% v/v) measured with TDR method. Semi-partial correlations of these two variables are also depicted.

<table>
<thead>
<tr>
<th>Species</th>
<th>Multiple regression</th>
<th>R² value</th>
<th>P value</th>
<th>Semi-partial correlations Tmin</th>
<th>Semi-partial correlations SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q. ilex</td>
<td>Fv/Fm = 0.449 + 0.0148Tmin'′ + 0.0082SM</td>
<td>0.32</td>
<td>&lt;0.001</td>
<td>0.53</td>
<td>0.43</td>
</tr>
<tr>
<td>P. latifolia</td>
<td>Fv/Fm = 0.445 + 0.0157Tmin'′ + 0.0062SM</td>
<td>0.25</td>
<td>&lt;0.001</td>
<td>0.50</td>
<td>0.29</td>
</tr>
<tr>
<td>Both species</td>
<td>Fv/Fm = 0.447 + 0.0152Tmin'′ + 0.0072SM</td>
<td>0.28</td>
<td>&lt;0.001</td>
<td>0.51</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Fig. 1. Mean values of air temperature, total values of precipitation, and mean values of soil moisture (% v/v) in the four annual seasons during the overall studied period. Error bars indicate the standard error of the mean (n = 10 years).

Fv/Fm values were higher in Q. ilex than in P. latifolia for the overall studied period (P < 0.01), especially during cold seasons (Fig. 3). On the other hand, Fv/Fm values were lower in drought plots than in control plots (P < 0.05) in 4 out of the 31 sampling dates (Fig. 3). In general, drought treatment has decreased Fv/Fm values in P. latifolia (P < 0.05), but in Q. ilex there was even an increment of Fv/Fm values in the drought treatment (Fig. 3).

Fig. 2. Mean values of maximum quantum yield of primary photochemistry (Fv/Fm) of Q. ilex and P. latifolia during the four annual seasons. Error bars indicate the standard error of the mean (n = 10 years).

4. Discussion

The maximum PSII quantum yield of primary photochemistry (Fv/Fm) exhibited lower values in the colder seasons confirming the results of several previous studies of Mediterranean plants (Larcher, 2000; Oliveira and Peñuelas, 2000, 2001; Ogaya and Peñuelas, 2003a). There was also an important effect of low water availability decreasing Fv/Fm values (Ogaya and Peñuelas, 2003b; Galmés et al., 2007; Prieto et al., 2009), but as shown in the semi-partial correlations of the multiple regressions, the effect of minimum air temperatures was stronger than the effect of soil moisture. The minimum Fv/Fm values were reached when cold was combined with drought conditions, for example during autumn 2004 and the exceptional cold and dry winter 2005, as also observed in other study conducted in a nearby area during the same period (Prieto et al., 2009), whereas under optimal conditions of temperature and water availability, the Fv/Fm values were ca. 0.8 (considered

Fig. 3. Seasonal course of maximum quantum yield of primary photochemistry (Fv/Fm) of Q. ilex and P. latifolia during the experimental period. Error bars indicate the standard error of the mean (n = 2 plot means of 10 measurements each). An asterisk indicates statistical significance of the difference between control and drought treatment (ANOVA) (P < 0.05).
close to an optimum value). The low Fv/Fm values, below 0.75, in summer, autumn, and winter were a sign of photoinhibition. Some works have reported a decrease in Fv/Fm values under very high air temperatures (Guissé et al., 1995; Chen et al., 2009). However, the reduction in photochemical efficiency at high temperatures was not significant, maybe because in our study site the air temperatures were never so high as to reach the necessary heat stress to decrease the photochemical efficiency of these two Mediterranean species.

P. latifolia showed lower Fv/Fm values than Q. ilex, especially during the colder seasons, which is in accordance with its distribution in warmer and drier sites and therefore with larger heat and drought resistance but lower cold resistance (Tretiach, 1993; Peñuelas et al., 1998, 2000; Ogaya and Peñuelas, 2003a). The results of multiple relationships were also consistent with a different performance of these species in response to cold and drought stresses, because the effect of minimum air temperature on Fv/Fm values was larger in P. latifolia than in Q. ilex (Table 2 and Fig. 1) and the effect of soil moisture was larger in Q. ilex than in P. latifolia (Table 1).

Despite of the reduction of soil moisture induced by drought treatment, and its dependence on air temperature and water availability, Fv/Fm values were lower in drought plots than in control plots only in 4 out of the 31 sampling dates. The slight reduction of soil moisture (13%) combined with the larger effect of air temperature could explain this lack of a strong drought effect. For example in the same experiment drought treatment have decreased net photosynthetic rates of Q. ilex only during few of the sampling dates (Ogaya and Peñuelas, 2003b). On the other hand, the low R2 values of the multiple regression analyses including temperature and soil moisture indicate that there are also some other factors determining Fv/Fm values such as solar radiation in addition to temperature and water availability. It has been observed that in Q. ilex and other Mediterranean species, the leaves subjected to higher solar radiation during winter cold were more photoinhibited and showed lower photochemical efficiency of PSII (Oliveira and Peñuelas, 2002).

Drought treatment decreased Fv/Fm values in the most drought resistant species, P. latifolia, and on the contrary, it did not influence the more drought sensitive Q. ilex. This lack of Fv/Fm decrease in Q. ilex in response to the drought treatment is likely to be the result of the decrease in the number of leaves per tree reported in Q. ilex and not in P. latifolia in the drought plots of this experimental forest site (Ogaya and Peñuelas, 2006). Moreover, P. latifolia leaves may present a more conservative strategy than Q. ilex by down-regulating more the linear photosynthetic electron transport to a reduced demand at the level of the final acceptors Rubisco (Guidi et al., 2001).

These results indicate that, although other physiological and reproductive strategies at whole plant level must be also taken into account (Lloret et al., 2004), the warmer and drier environment expected for the following decades could favour the species more sensitive to cold and more resistant to drought, the shrub P. latifolia, in detriment of the tree Q. ilex as already observed in the field after severe heat-drought episodes (Peñuelas et al., 1998, 2000, 2001; Lloret and Siscart, 1995). The structural, functional and landscape consequences would thus be strong.

5. Conclusions and outlook

In this long-term (10 years) study simulating climate change, minimum air temperatures and, in second term, soil moisture determined the photochemical efficiency of PSII in both Q. ilex and P. latifolia, but the effect of cold temperatures was stronger in P. latifolia. P. latifolia experienced lower Fv/Fm values than Q. ilex, especially during cold seasons, and P. latifolia was also the only one species with Fv/Fm changed by the drought treatment. Q. ilex did not change its Fv/Fm but instead shed a significant number of leaves per tree. It seems that in a warmer and drier environment, as the one projected for the Mediterranean areas for the following decades, the performance of the dominant species, the tree Q. ilex, could be less competitive in front of the other co-dominant species, the more drought and heat resistant shrub species, P. latifolia. Although not only plant growth but also plant reproduction and recruitment must be taken into account to correctly assess plant response to changing environmental conditions, and for example, in the same site it has been observed higher survival rates in resprouts of Q. ilex than in seedlings of P. latifolia (Lloret et al., 2004), this leaf level fluorescence data provides another indication of the possible gain in dominance of the shrub P. latifolia relative to the tree Q. ilex with all the resulting landscape and ecosystemic consequences of land cover change from a forest to a shrubland.

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