Low temperature during winter elicits differential responses among populations of the Mediterranean evergreen cork oak (*Quercus suber*)

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**Summary** Populations of cork oak (*Quercus suber* L.) were assessed for seasonal and inter-population variability in, and temperature responses of, the ratio between light-induced variable and maximum fluorescence of chlorophyll, \(F_v/F_m\), considered a surrogate for the maximum photochemical efficiency of photosystem II (PSII). Seedlings from 10 populations throughout the distribution range of *Q. suber* in the Mediterranean basin were grown in a common garden in central Spain. The \(F_v/F_m\) ratio of dark-adapted leaves was measured at dawn every month for 2 years. Air temperature was recorded at a nearby climatic station.

During the summer, when maximum air temperatures reached 40 °C, there were no significant differences in \(F_v/F_m\) among populations, but significant differences were seen during the winter. In colder months, \(F_v/F_m\) ranged in all populations between 0.5–0.6 and 0.2–0.3 in 2001 and 2002, respectively. The variance explained by the population effect was greatest during winter months, especially in 2002, reaching a peak value of 10% when minimum air temperature was below −10 °C. Populations originating from warmer sites showed the largest decline in \(F_v/F_m\) between the end of 2001 and the beginning of 2002. Thus, a negative linear relationship was established between mean annual temperature at the population source and population mean \(F_v/F_m\) recorded in the coldest month in 2002 and normalized by the \(F_v/F_m\) spring measurement.

**Keywords:** chlorophyll fluorescence, cold, Mediterranean evergreen oak, photoinhibition.

**Introduction**

Summer drought has often been identified as the main environmental stress to which forest vegetation is exposed in Mediterranean ecosystems (Aussenac and Vallet 1982, Tenhunen et al. 1987, Oliveira et al. 1992, Sala and Tenhunen 1994, 1996). Low rainfall and a high vapor pressure deficit and temperature constrain seedling photosynthetic activity and growth. However, low winter temperatures may also impose a significant stress on seedlings in continental areas of the Mediterranean region (Tenhunen et al. 1987, Terradas and Savé 1992, Tretiach et al. 1997, Boorse et al. 1998, García-Plazaola et al. 1999a, 1999b, Larcher 2000, Nardini et al. 2000, Oliveira and Peñuelas 2001).

Light-energy harvesting is impaired by low temperatures as a consequence of reduced photochemical efficiency of PSII (Ball et al. 1991, Adams and Demmig-Adams 1995). There is some evidence that this phenomenon, which occurs frequently in species at high latitudes (Ottander and Öquist 1991, Vogg et al. 1998), also occurs in forest vegetation in continental areas of the Mediterranean area during winter months (Méthy and Trabaud 1990, Oliveira and Peñuelas 2000, 2001). The reduction in photochemical efficiency of PSII may be greater when overnight frosts are followed by sunny days (Greer 1990, Egerton et al. 2000), as frequently occurs during Mediterranean winters. Under such conditions, loss of photochemical efficiency may be exacerbated by sudden exposure to high light in the absence of mechanisms to dissipate excess light energy (Sonoike 1998).

Cork oak (*Quercus suber* L.) is a widely distributed forest tree species in the Mediterranean basin. It is present in areas with a mean annual rainfall of 400–1500 mm and a mean annual temperature of 13–20 °C (Díaz-Fernández et al. 1995, Montero and Cañellas 1999). The species is not well adapted to low temperatures but is able to maintain photochemical efficiency during periods of drought (Faria et al. 1996, García-Plazaola et al. 1997). It is also tolerant to high temperatures (Ghouil et al. 2003).

Thermal stress is frequently assessed in vivo by chlorophyll fluorescence measurements (Strand and Öquist 1988, Ball et al. 1994, Williams et al. 2003). In this study, we used chlorophyll fluorescence to assess the integrity of maximum photochemical efficiency of photosystem II (PSII) in populations of cork oak grown in a common garden for two years. Specifically, we examined if: (1) maximum photochemical efficiency of PSII in leaves of cork oak varies seasonally; (2) low temperatures during winter stress have a more negative impact on
photochemistry of cork oak leaves than low water availability and high temperatures in summer; and (3) there are inter-population differences in the photochemical response of cork oak to environmental stress. Increased understanding of these three topics will contribute to better management and conservation of genetic resources and allow better selection of provenances for afforestation.

Materials and methods

Open-pollinated seeds from 10 populations, covering the geographic and climatic range of cork oak, were collected in the autumn of 1996 (Table 1). Plants were cultivated in a nursery under standard conditions after seeding in the spring of 1997. Seedlings were well watered every two days.

A common garden field trial with 15 one-year-old seedlings per population was established in Madrid (40°25′ N, 3°44′ W) at the beginning of 1998. During the following 2 years (1999–2000), plants were well watered each week during the first growing season. After the establishment period, irrigation was terminated and seedlings endured summer drought in each of the following years.

For 2 years after establishment of the field trial (November 2000–November 2002), chlorophyll fluorescence was recorded monthly in 10–12 seedlings per population. Precipitation was scarce from June to September in both years. Minimum pre-dawn water potential evaluated in five of the populations reached –2.1 and –1.4 MPa at the end of the summer in 2001 and 2002, respectively (data not shown).

The ratio between light-induced variable fluorescence and maximum fluorescence, \( F_v / F_m \), was measured as a surrogate for maximum quantum efficiency of PSII (Kitajima and Butler 1975, Demmig-Adams et al. 1989). Maximum fluorescence \( (F_m) \) and ground fluorescence \( (F_o) \) were measured in one dark-adapted leaf per seedling from south-oriented shoots in the uppermost canopy (PSM Chlorophyll Fluorimeter, Biomonitor S.C.I. AB, Umeå, Sweden). Leaves were included in a leaf-clip that allowed dark acclimation of the leaves and the maintenance of the same distance between the tip of the fiber-optic and the sample in all measurements, thereby minimizing the error associated with the sensitivity of \( F_v \) and \( F_m \) to the distance between the point of light pulse emission and the leaf sample. Variable fluorescence was calculated as \( F_v = F_m - F_o \).

The degree of maximum photoinhibition during winter months was estimated by standardization of the winter value of \( F_v / F_m \) for each plant by its spring value, which was assumed to approximate the optimum value of about 0.8.

Chlorophyll fluorescence was recorded at dawn after 30 min of dark acclimation on cloudless days. Whenever possible, measurements were repeated on the same leaves throughout the study. Occasionally, a leaf was replaced by another one from the same shoot. Bud burst took place at the end of May and unfolding of new leaves was completed by mid-June. Following bud burst, measurements were made on the newly expanded leaves as well as on leaves from the previous growing season. Afterward, measurements were made on leaves of the current season only because the old leaves dropped at the end of June. Only a few seedlings supported both types of leaves until mid-July.

Statistical analysis

Chlorophyll fluorescence variables were analyzed mainly by non-parametric statistics. The requirements for a normal distribution and homoscedasticity of variances were not met by the parameters related to chlorophyll fluorescence (Lázár and Naúš 1998). Therefore, the influence of population source on fluorescence variables was evaluated by analysis of molecular variance (AMOVA; Excoffier et al. 1992, Schneider et al. 2000). A hierarchical analysis of variance was applied to partition the total variance into covariance components due to inter-population differences and those due to intra-population differences. The significance of the components was tested using a non-parametric permutation approach (Excoffier et al. 1992). Under this procedure, neither the normality assumption nor the equality of variance among populations is necessary. Statistical analyses were performed using Arlequin (Schneider et al. 2000).

Table 1. Locations and climatic characteristics of the source locations of the cork oak populations studied. Minimum and maximum mean temperatures are referred to as the mean of the lowest and highest annual temperatures. Abbreviations: precip. = precipitation; \( T = \) temperature; min. = minimum; and max. = maximum.

<table>
<thead>
<tr>
<th>Population</th>
<th>Code</th>
<th>Country</th>
<th>Latitude and longitude</th>
<th>Altitude range (m)</th>
<th>Mean annual precip. (mm)</th>
<th>Min. mean ( T ) (°C)</th>
<th>Max. mean ( T ) (°C)</th>
<th>Mean ( T_{annual} ) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alburquerque</td>
<td>Alb</td>
<td>Spain</td>
<td>39° N 7° W</td>
<td>450–500</td>
<td>642</td>
<td>7.0</td>
<td>25.7</td>
<td>15.5</td>
</tr>
<tr>
<td>La Almoraíma</td>
<td>Alm</td>
<td>Spain</td>
<td>36° N 5° W</td>
<td>50–60</td>
<td>993</td>
<td>11.4</td>
<td>23.3</td>
<td>16.6</td>
</tr>
<tr>
<td>Santa Coloma de Farnés</td>
<td>Col</td>
<td>Spain</td>
<td>41° N 2° E</td>
<td>200–500</td>
<td>802</td>
<td>7.5</td>
<td>23.6</td>
<td>15</td>
</tr>
<tr>
<td>Potes</td>
<td>Pot</td>
<td>Spain</td>
<td>43° N 4° E</td>
<td>200–500</td>
<td>1265</td>
<td>7.6</td>
<td>18.8</td>
<td>12.9</td>
</tr>
<tr>
<td>El Pardo</td>
<td>Par</td>
<td>Spain</td>
<td>40° N 3° W</td>
<td>700</td>
<td>455</td>
<td>5.4</td>
<td>24.0</td>
<td>13.9</td>
</tr>
<tr>
<td>Haza de Lino</td>
<td>Haz</td>
<td>Spain</td>
<td>36° N 3° W</td>
<td>1300</td>
<td>742</td>
<td>6.3</td>
<td>21.9</td>
<td>13</td>
</tr>
<tr>
<td>San Brás de Alportel</td>
<td>Alp</td>
<td>Portugal</td>
<td>37° N 7° W</td>
<td>440–485</td>
<td>874</td>
<td>10</td>
<td>23.2</td>
<td>15.9</td>
</tr>
<tr>
<td>Sicília</td>
<td>Sic</td>
<td>Italy</td>
<td>37° N 14° W</td>
<td>170</td>
<td>448</td>
<td>11.1</td>
<td>26.2</td>
<td>17.7</td>
</tr>
<tr>
<td>Oulmés</td>
<td>Oul</td>
<td>Morocco</td>
<td>33° N 4° W</td>
<td>1100</td>
<td>673</td>
<td>10</td>
<td>20.8</td>
<td>15.4</td>
</tr>
<tr>
<td>Ain Rami</td>
<td>Ain</td>
<td>Morocco</td>
<td>35° N 5° W</td>
<td>300</td>
<td>1482</td>
<td>–</td>
<td>–</td>
<td>19.0</td>
</tr>
</tbody>
</table>
Linear regression analysis was performed to test the relationship between photoinhibition and mean annual temperature of populations. The Pearson coefficient of determination was used to establish the strength of the relationship (Statgraphics 5.1, Statistical Graphics).

**Results**

*Microclimatic conditions*

Over the 2-year study, the annual course of temperature was typical of continental areas in the Mediterranean basin (Figure 1a). The lowest daily mean temperature in the winter months was usually above 0 °C; however, there was an exceptionally cold period from November 2001 to January 2002, with an absolute minimum temperature around –12 °C, and for 15 days temperatures remained at or below –5 °C. Throughout this period, the mean daily temperature was below 0 °C and cold nights were followed by sunny days. Maximum temperatures and minimum rainfall (data not shown) were recorded in the summer months.

*Maximum quantum efficiency of PSII at dawn*

Just after leaf unfolding in June, $F_v/F_m$ values peaked at slightly above 0.8 in both years. Thereafter, the $F_v/F_m$ ratio progressively decreased until the end of the growing season in both years (Figure 1b). Minimum summer values between 0.65 and 0.7 were reached in September of each year. The first exposure to subfreezing temperatures in autumn caused an acute depression in $F_v/F_m$, which declined again as a consequence of new frost events, reaching minimal values close to 0.55 in the winter months of 2001, and 0.2–0.3 in winter 2002.

During the summer months, differences in $F_v/F_m$ among populations were nonsignificant ($P > 0.05$). However, these differences became statistically significant during the winter of 2000–2001, and highly significant in the winter of 2001–2002. During the winter, the percent of variance attributable to population was highest, reaching a value of 10%, and the total variance was also at a maximum (Figure 2).

A positive relationship was found between the mean photoinhibition assessed in the coldest period and the mean annual temperature at the place of origin of the populations (Figure 3). The warmer the population source, the greater the winter drop in $F_v/F_m$ and the greater the sensitivity to cold stress as measured by maximum photoinhibition. Populations from northern Spain (Potes) or from continental areas (El Pardo) showed the smallest winter decline in $F_v/F_m$, whereas the population from southern Italy (Sicilia) showed the largest.

**Discussion**

*Seasonal influence on maximum quantum yield of PSII*

Photoinhibition caused by low winter temperatures is one of the main factors limiting survival of evergreen species at high latitudes or altitudes (Adams et al. 2002, Williams et al. 2003). The effect of low temperatures on vegetation from low latitudes or Mediterranean areas has received little attention because summer drought is considered to be the major environmental constraint (Werner et al. 2002, but see Larcher 2000).
In the present study, we observed seasonal variation in \( F_v/F_m \) in cork oak seedlings, with low winter temperatures having a greater impact than high temperatures and drought during the summer. In all populations, \( F_v/F_m \) decreased in summer and winter months. However, the depression in maximum photochemical efficiency of PSII was more noticeable in response to the first autumn frosts and a cumulative effect of cycles with low temperature (below 0 °C) followed by cloudless days (e.g., the 2001–2002 winter). Permanent depression of photochemistry was indicated by the lack of nocturnal recovery of \( F_v/F_m \) measured at dawn. Thus, the extremely suppressed predawn values of \( F_v/F_m \) during the winter months imply permanent photoinhibition; recovery to values close to optimum occurred only after temperatures rose during spring. Photodamage in leaves has previously been interpreted to reflect the result of the combined effects of low temperatures and excess light (Greer 1990). However, high stability of PSII to dehydration and high temperature in oaks has been reported (Faria et al. 1996, Ghouil et al. 2003). This pattern has also been described in other evergreen sclerophyllous species (García-Plazaola et al. 1997, Karavatas and Manetas 1999, Oliveira and Peñuelas 2000, Ogaya and Peñuelas 2003), confirming the hypothesis that Mediterranean species are particularly sensitive to moderately low temperatures (cf. Kyparissis et al. 2000). Although cork oak shows high susceptibility to both cold and water stress—both disturbances impair photochemistry of leaves (García-Plazaola et al. 1997, 1999a)—in the present study, all populations showed a greater sensitivity to low temperatures than to summer drought.

**Differences in cold tolerance among populations of cork oak**

The drop in maximum efficiency of photosynthetic energy conversion of PSII in response to winter cold has been described for many species (Boorse et al. 1998, Verhoeven et al. 1999). However, inter-population differences in the photochemical response to low temperatures and summer drought have been analyzed less frequently. Differences among cork oak populations in the photochemical efficiency of PSII were significant during periods of unusually low temperatures that occurred during the winter of 2001–2002 (see Figure 1). Dates when total and population variances were highest corresponded with dates on which frost caused the greatest reduction in \( F_v/F_m \). Populations native to cold sites were less sensitive to cold stress than populations native to warm sites, reinforcing the roles of extreme events as selective factors (Gurvich et al. 2002, Gutschick and BassiriRad 2003), which in cork oak populations are related to a high sensitivity of PSII to cold temperatures.

In summary, cork oak showed high variability in the seasonal pattern of maximum photochemical efficiency of PSII at
dawn. The high sensitivity of cork oak to low temperatures may explain its well-known displacement in continental areas by deciduous trees or other, more cold-tolerant evergreens such as *Quercus ilex* L. This would represent an important constraint for the species if a shift in the frequency or timing of extreme frost events occurred in the Mediterranean zone as consequence of climatic change. The problem may be more acute in continental areas or at high altitudes. Intraspecific differences in cold tolerance at the population level were observed, with populations from regions with lower temperatures sustaining a less pronounced decrease in the maximum photochemical efficiency of PSII in response to low temperatures.

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References


