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# Thermal optima of photosynthetic functions and thermostability of photochemistry in cork oak seedlings

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**Summary** Temperature effects on photosynthesis were studied in seedlings of evergreen Mediterranean cork oak (*Quercus suber* L.). Responses to changes in temperature and the temperature optima of maximal carboxylation rate ($V_{cmax}$) and maximal light-driven electron flux ($J_{max}$) were estimated from gas exchange measurements and a leaf-level photosynthesis model. The estimated temperature optima were approximately 34 and 33 °C for $V_{cmax}$ and $J_{max}$, respectively, which fall within the lower range of temperature optima previously observed in deciduous tree species. The thermostability of the photosynthetic apparatus was estimated according to the temperature at which basal chlorophyll a fluorescence begins to increase ($T_c$). The $T_c$ was highly variable, increasing from 42 to 51 °C when ambient temperature rose from 10 to 40 °C, and increasing from 44 to 54 °C with decreasing soil water availability while net $CO_2$ assimilation rate dropped to almost zero. When a heat shock was imposed, an additional small increase in $T_c$ was observed in drought-stressed and control seedlings. Maximal $T_c$ values following heat shock were about 56 °C, which, to our knowledge, are the highest values that have been observed in tree species. In conclusion, the intrinsic temperature responses of cork oak did not differ from those of other species (similar $T_c$ under ambient temperature and water availability, and relatively low thermal optima for photosynthetic capacity in seedlings grown at cool temperatures). However, the large ability of cork oak to acclimate to drought and elevated temperature may be an important factor in the tolerance of this evergreen Mediterranean species to summer drought and high temperatures.

*Keywords: chlorophyll fluorescence, gas exchange, maximal carboxylation rate, maximal light-driven electron flow, regeneration.*

## Introduction

Cork oak (*Quercus suber* L.) is a sclerophyllous, evergreen oak species growing in the Western Mediterranean Basin, with high economic potential as a cork producer. In Northern Africa, many cork oak stands are aging, and as a result, trees die and cork production declines. The regeneration of cork oak stands from natural seeds, which is the most widely used regeneration procedure for oak stands, faces serious difficulties in many locations. Usually, following mast years with massive acorn production, large numbers of seedlings germinate and then grow for 1 to 2 years, but die rapidly thereafter. The severe mortality recorded among seedlings may be caused by a combination of factors: over-grazing by cattle; pests and disease; and severe drought and high temperatures. The extent of seedling mortality may threaten the survival of many Mediterranean cork oak stands, and thus adequate stand management enabling regeneration from seedlings is of central importance for cork oak forestry (Hasnaoui 1992, Merouani et al. 1998).

The Mediterranean climate, particularly in Northern Africa, is characterized by long periods of water shortage during summer, with high peak irradiances (above 2000 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD)) and temperatures (40–45 °C in the shade). Such high temperatures and irradiances may cause stress in seedlings, particularly when stomata are closed and transpiration is reduced. Nevertheless, there is little information available about high temperature responses of tree species, and even less about the acclimation processes that may occur under such conditions.

Photosynthesis is considered to be one of the most high-temperature-sensitive functions in the leaf (Berry and Björkman 1980). Photosynthetic responses to temperature may be analyzed with a biochemical model of leaf photosynthesis (Farquhar et al. 1980, von Caemmerer and Farquhar 1981) that describes leaf photosynthetic capacity according to three parameters: the maximal ribulose bisphosphate (RuBP) carboxylation rate ($V_{cmax}$); the maximal light-driven electron flux ($J_{max}$) used to regenerate RuBP; and the rate of triose phosphate utilization (TPU), which is often neglected because of its

limited extent under normal conditions. The temperature responses of the two first parameters have been described several times (Leuning 1997, 2002, Dreyer et al. 2001, Medlyn et al. 2002*a*, 2002*b*); they usually display a large increase with increasing temperature, and temperature optima of about 33–35 °C for $J_{max}$ and 38 °C for $V_{cmax}$. Some degree of interspecific variability has been detected among broad-leaved tree species, particularly for $J_{max}$, which differed by a few degrees among the tested species (Dreyer et al. 2001, Medlyn et al. 2002*a*, 2002*b*). Above their respective temperature optima, both parameters decline rapidly.

Tolerance of the photosynthetic apparatus to high temperatures is thought to be mainly related to the thermostability of Photosystem II (PSII) photochemistry, which seems to be one of the most sensitive components of the electron transport chain in the thylakoids (Schreiber and Berry 1977, Berry and Björkman 1980, Havaux 1992). Photosystem I photochemistry has been described as less sensitive to heat stress than PSII photochemistry (Havaux et al. 1991). Increased membrane fluidity and protein denaturation at high temperatures may set an upper limit to PSII functionality (Berry and Björkman 1980, Yordanov et al. 1986). This critical temperature, $T_c$, can be estimated as the point at which basal chlorophyll a fluorescence begins to increase under gradually increasing temperatures (Schreiber and Berry 1977, Bilger et al. 1984). The $T_c$ depends on both species and genotype (Epron 1997, Knight and Ackerly 2002), but is also modulated to a large extent by climate. It increases after short periods of exposure to higher temperatures (Berry and Björkman 1980, Havaux 1993*b*) and in response to drought stress (Havaux 1992, Epron 1997, Ladjal et al. 2000), abscisic acid application (Ivanov et al. 1992), or growth in high $CO_2$ concentration (Taub et al. 2000).

We have studied the temperature responses of cork oak seedlings by: (1) estimating the temperature responses and thermal optima of $V_{cmax}$ and $J_{max}$; (2) estimating the thermostability of PSII photochemistry by means of $T_c$ data; (3) characterizing the potential for acclimation of $T_c$ to increasing temperature and to water stress; and (4) assessing the response of $T_c$ to heat shock.

## Materials and methods

### *Experiment 1: temperature responses of photosynthesis and thermostability of PSII*

Cork oak seedlings were obtained from acorns collected in November 2000 at Bellif, Northern Tunisia and grown in a greenhouse at Nancy (Northeastern France) in 5-l pots filled with a 2:1 (v/v) sand:blond peat mixture, beginning in April 2001. Twenty grams of slow-release fertilizer (Nutricote 100, N,P,K 13:13:13 + oligo elements) were added to each pot. An automated drip irrigation system provided water twice daily to each pot.

At the age of 4 months, five seedlings were transferred to a climate chamber (air temperature = 25 °C; relative humidity = 60–80%; photosynthetically active radiation = 250 µmol $m^{-2}$ $s^{-1}$). After acclimation for 48 h in the chamber, irradiance and relative humidity were held constant while air temperature was reduced to 10 °C and then increased in six steps of 3 days each to 10, 18, 25, 32, 36 and 40 °C. Plants were acclimated to each temperature for at least 48 h before gas exchange and fluorescence measurements. The same leaf was used for measurements at different temperatures in order to limit variability.

### *Experiment 2: impact of drought stress on thermostability and response to heat shock*

Thirty 8-month-old cork oak seedlings were grown in the greenhouse and then transferred to the climate chamber as in Experiment 1. After 48 h of acclimation in the climate chamber, the potted seedlings were divided between two treatments: one set of seedlings was watered daily to pot capacity, whereas the other set was subjected to soil water depletion. The seedlings were kept in the climate chamber for 3 weeks. Each day, volumetric soil water content (SWC) was measured with a TDR probe (Soil Moisture, Victoria, Canada) and pots were weighed. In the drought treatment, SWC dropped rapidly to 5%. The pot weight corresponding to 5% SWC was determined and then maintained for 2 weeks by small daily additions of water. Predawn photochemical efficiency of PSII ($F_v/F_m$) and maximal net $CO_2$ assimilation rate ($A_{max}$) were measured on one leaf of four to five seedlings every second day.

The critical temperature for PSII stability was estimated every second day during the drought treatment on four leaves sampled from different seedlings in each treatment. At the end of the drought period, all seedlings were subjected to heat shock by maintaining the air temperature at 40 °C for 36 h. The $F_v/F_m$ and $A_{max}$ were measured on one leaf of four to five seedlings before and after the heat shock.

### *Maximal net $CO_2$ assimilation rate and stomatal conductance*

Leaf gas exchange was recorded with a portable open path gas exchange chamber (LI-6400, Li-Cor, Lincoln, NE) installed in the climate chamber. Leaves were inserted into the chamber and photosynthesis was induced for 25 min at 25 °C, at a $CO_2$ partial pressure ($c_a$) of 35 Pa and a PPFD of 1000 µmol $m^{-2}$ $s^{-1}$. Net assimilation rate and stomatal conductance recorded after induction were used as estimates of $A_{max}$ and maximal stomatal conductance ($g_{smax}$), respectively.

### *Photosynthetic capacity and temperature*

Responses of net $CO_2$ assimilation rate ($A$) to $c_a$ were recorded with the LI-6400 on fully induced leaves according to the following procedure: an increase in $c_a$ to 175 Pa followed by a gradual decrease to 5 Pa in 12 steps of 6 min each. At each step, $A$, stomatal conductance ($g_s$) and the computed $CO_2$ partial pressure in the substomatal cavities ($c_i$) were recorded after 4 min of stabilization. Each parameter was measured three times at 1-min intervals during each step to check for stability.

Maximal carboxylation rate and $J_{max}$ were estimated by adjusting Farquhar's model to the $A$–$c_i$ curves as described by Dreyer et al. (2001).

The temperature dependence of $V_{cmax}$ and $J_{max}$ is described by a model derived from the Arrhenius equation (Sharpe and DeMichele 1977):

$$P_T = \frac{P_{T_{ref}} e^{\frac{\Delta H_a}{RT_{ref}}\left(1 - \frac{T_{ref}}{T}\right)}}{1 + e^{\frac{T\Delta S - \Delta H_d}{RT}}} \left(1 + e^{\frac{T_{ref}\Delta S - \Delta H_d}{RT_{ref}}}\right) \quad (1)$$

where $P_{T_{ref}}$ is the parameter value at a reference temperature $T_{ref}$ (here 25 °C, or 298.16 K) in the absence of high temperature inhibition (Leuning 1997, Wohlfahrt et al. 1999), $\Delta H_a$ (J mol$^{-1}$) is the activation energy, $R$ is the gas constant (8.3143 J K$^{-1}$ mol$^{-1}$), $\Delta S$ (J K$^{-1}$ mol$^{-1}$) is an entropy term and $\Delta H_d$ (J mol$^{-1}$) is the deactivation energy of the given parameter.

A simple derivation shows that the optimal temperature ($T_{opt}$) can be computed as:

$$T_{opt} = \frac{-\Delta H_d}{R \ln \frac{-\Delta H_a}{\Delta H_a - \Delta H_d} - \Delta S} \quad (2)$$

Further details as well as the values of the different parameters may be found in Dreyer et al. (2001). Briefly, dark respiration ($R_d$), $J_{max}$ and $V_{cmax}$ were estimated for each leaf and at each temperature using the secant method (NLIN Procedure, SAS Institute, 1990). The temperature responses of estimated $V_{cmax}$ and $J_{max}$ were fitted using Equation 1. All leaves were assumed to display similar $\Delta H_a$, $\Delta H_d$ and $\Delta S$ values but different $V_{cmax(T_{ref})}$ and $J_{max(T_{ref})}$ values because of the known variability of these parameters in relation to leaf characteristics. Four dummy variables $F_1$ to $F_4$ were used for referencing each measured leaf and the following model was fitted on estimates of $V_{cmax}$ or $J_{max}$:

$$P = (P_{5(T_{ref})} + \alpha_1 F_1 + \alpha_2 F_2 + \alpha_3 F_3 + \alpha_4 F_4) u(T) \quad (3)$$

where $P$ is the parameter $V_{cmax}$ or $J_{max}$, $P_{5(T_{ref})}$ is the value of parameter $P$ in the absence of inhibition at $T_{ref}$ for leaf number 5; dummy variable $F_i = 1$ if the leaf is leaf $i$, otherwise $F_i = 0$; $\alpha_i$ is the difference between the value of the parameter in the absence of inhibition at $T_{ref}$ for leaf $i$ and $P_{5(T_{ref})}$; and $u(T)$ corresponds to Equation 1.

In addition, $\Delta S$ in $u(T)$ was replaced by the expression derived from Equation 2:

$$\Delta S = \frac{\Delta H_d}{T_{opt}} - R \ln \frac{\Delta H_a}{\Delta H_d - \Delta H_a} \quad (4)$$

and $\Delta H_d$ was replaced by $\Delta H_a + \Delta H_d'$, with $\Delta H_d' > 0$ to force $\Delta H_d$ to be higher than $\Delta H_a$ and to obtain a well defined peak at $T_{opt}$.

Eight parameters ($P_{5(T_{ref})}$, $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_4$, $T_{opt}$, $\Delta H_a$ and $\Delta H_d'$) were estimated to describe the temperature response. This procedure, as well as the sequence of acclimation temperatures, were identical to those used by Dreyer et al. (2001) and Robakowski et al. (2002), thus enabling direct comparisons of estimated $T_{opt}$.

*Photochemical efficiency*

Chlorophyll a fluorescence was measured with a Mini-PAM fluorometer (Walz, Effeltrich, Germany) and the maximal quantum yield of photochemistry of dark-acclimated leaves (8 h in the chamber) was calculated as $F_v/F_m = 1 - F_o/F_m$ (Genty et al. 1989), where $F_v$, $F_o$ and $F_m$ are variable, non-variable (ground) and maximal fluorescence, respectively.

*Thermostability of PSII*

Photosystem II thermostability was estimated from $T_c$ data (Bilger et al. 1984, Ivanov et al. 1992) as described in Robakowski et al. (2002).

Leaves were collected and placed on wet filter paper at 20 °C in the dark for 15 min. A leaf disk was punched out and inserted into a device that exposes the disk to a target temperature (± 0.1 °C) that gradually increases from 20 to 60 °C at a constant rate of 1 °C min$^{-1}$. A detailed description of this device may be obtained from P. Gross (INRA, Nancy Research Centre, Champenoux, France; Gross@nancy.inra.fr). Ground fluorescence of the treated leaf disk was monitored continuously in complete darkness with a PAM-2000 fluorometer (Walz) with fiberoptics inserted into the aluminum body just over the leaf sample (red modulated light, irradiance < 1 µmol m$^{-2}$ s$^{-1}$). The temperature inducing a steep increase in $F_o$ ($T_c$) was determined from the fluorescence traces. Means of values from four to five different leaves were computed and compared by ANOVA and a Sheffé test at $P < 0.05$.

## Results

*Experiment 1*

*Temperature responses of $V_{cmax}$ and $J_{max}$* Response curves of $A$ as a function of $c_i$ are shown in Figure 1. These curves were used to adjust $V_{cmax}$ and $J_{max}$ as demonstrated in Figure 1b. The curves obtained at low temperatures were relatively flat at high $CO_2$ concentrations, possibly as a result of a limitation of TPU (Sharkey et al. 1986). The last values at the highest $CO_2$ concentrations were therefore excluded from the adjustment procedure in order to limit the impact of TPU on estimates of $J_{max}$; nevertheless, the resulting estimates must be used with caution.

The response of $V_{cmax}$ to temperature followed already described patterns, with a large increase with increasing temperature up to 34 °C, followed by a steep decrease (Figures 2a and 3). Maximal light-driven electron flux followed a similar pattern, with a smaller increase to 33 °C and a steep decrease thereafter. As a result, the ratio $J_{max}/V_{cmax}$ decreased almost linearly with increasing temperature from 2.4 at 10 °C to 0.78 at 40 °C (Figure 2b). At a reference temperature of 25 °C, this ratio was about 1.6. A synthesis of the parameters adjusted according to the temperature responses is given in Table 1.

*Photochemical efficiency and thermostability of PSII* Values of $F_v/F_m$ were optimal (about 0.82) between 10 and 25 °C, be-

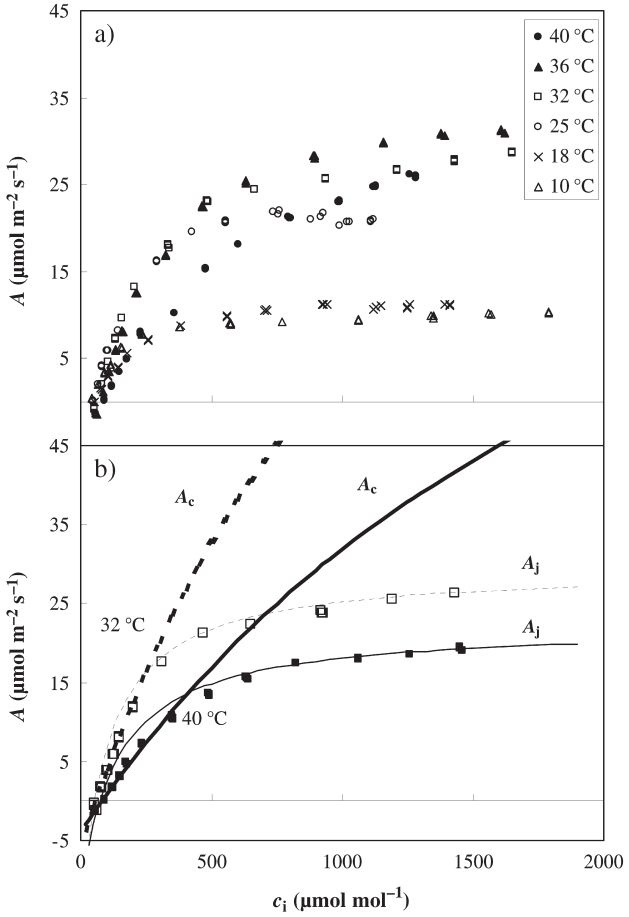



Figure 1. Examples of response curves of net $CO_2$ assimilation rate ($A$) to substomatal $CO_2$ mole fraction ($c_i$) in young cork oak (*Quercus suber*) seedlings. (a) Response curves at increasing temperatures. (b) Adjustment of $CO_2$-limited ($A_c$) and ribulose bisphosphate-limited ($A_j$) functions allowing estimation of maximal carboxylation rate ($V_{cmax}$) and maximal light-driven electron flux ($J_{max}$), respectively.

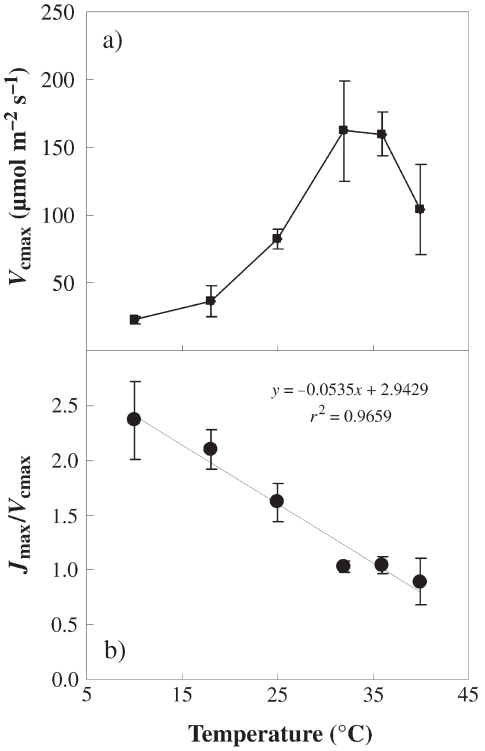



Figure 2. (a) Mean values of maximal carboxylation rate ($V_{cmax}$) and (b) the ratio of maximal carboxylation rate to maximal light-driven electron flux ($J_{max}/V_{cmax}$) measured in young cork oak seedlings as a function of temperature (means ± CI, $n = 5$, $P = 0.05$).

gan to decrease at 32 °C and fell below 0.7 at temperatures above 36 °C (Figure 4a). The critical temperature for PSII stability was about 42 °C in plants acclimated to 10 °C for 48 h, but increased to 51 °C in plants acclimated to 40 °C. The relationship between $T_c$ and acclimation temperature was almost linear (Figure 4b).

*Experiment 2*

*Impact of drought and heat shock* After 9 days of drought treatment, soil volumetric water content decreased from 25% (field capacity) to 5%; thereafter, it was maintained at 5% through partial rewatering every day (Figure 5a). This low soil water content (SWC) resulted in low predawn leaf water potentials (about –3 MPa at the end of the drought stress). Maximal net $CO_2$ assimilation rates under conditions of ambient $CO_2$ and saturating irradiance ($A_{max}$) were about 10 µmol $m^{-2}$ $s^{-1}$ in seedlings in the control treatment, but gradually decreased in seedlings in the drought treatment (Figure 5b). At 5% SWC, $A_{max}$ was nil, and some leaves released $CO_2$. The decrease in $A_{max}$ was accompanied by (and was probably a result of) stomatal closure (data not shown). In both control and drought-stressed seedlings, predawn $F_v/F_m$ values were close to the optimum value (0.83) during the entire drought treatment, revealing the lack of permanent drought-induced damage to photochemistry (Figure 5c).

In control seedlings, $T_c$ remained stable at approximately

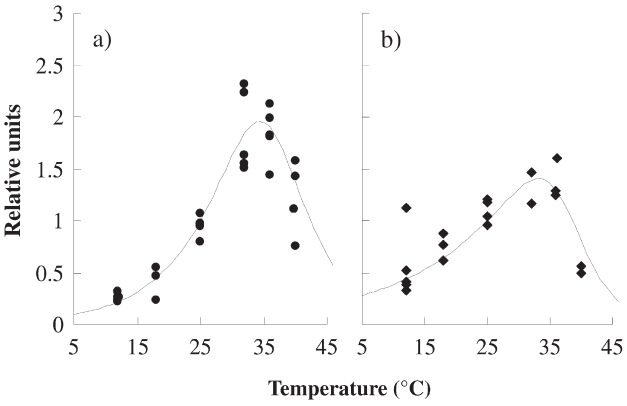



Figure 3. Adjustment of Arrhenius functions to the normalized temperature response of (a) maximal ribulose bisphosphate carboxylation rate ($V_{cmax}$) and (b) maximal light-driven electron flux ($J_{max}$) as estimated from five leaves of cork oak seedlings. The parameters describing the adjusted functions are shown in Table 1.

Table 1. Mean values of the parameters describing the response of leaf photosynthetic capacity to temperature in young cork oak seedlings grown in a greenhouse: $V_{cmax}$ = maximal ribulose bisphosphate carboxylation rate; $J_{max}$ = maximal light-driven electron flux; $T_{opt}$ = optimal temperature; $\Delta H_a$ = activation energy; $\Delta H_d$ = deactivation energy; and $\Delta S$ = entropy factor.

| Parameter | $V_{cmax}$ | $J_{max}$ |
|---|---|---|
| Value at 25 °C (μmol $m^{-2}$ $s^{-1}$) ± SE | 82.5 ± 7.4 | 134.5 ± 24.0 |
| $T_{opt}$ (°C) | 34.1 | 33 |
| $\Delta H_a$ (kJ $mol^{-1}$) | 88.2 | 47.9 |
| $\Delta H_d$ (kJ $mol^{-1}$) | 248.8 | 273.7 |
| $\Delta S$ (J $K^{-1}$ $mol^{-1}$) | 805 | 881 |

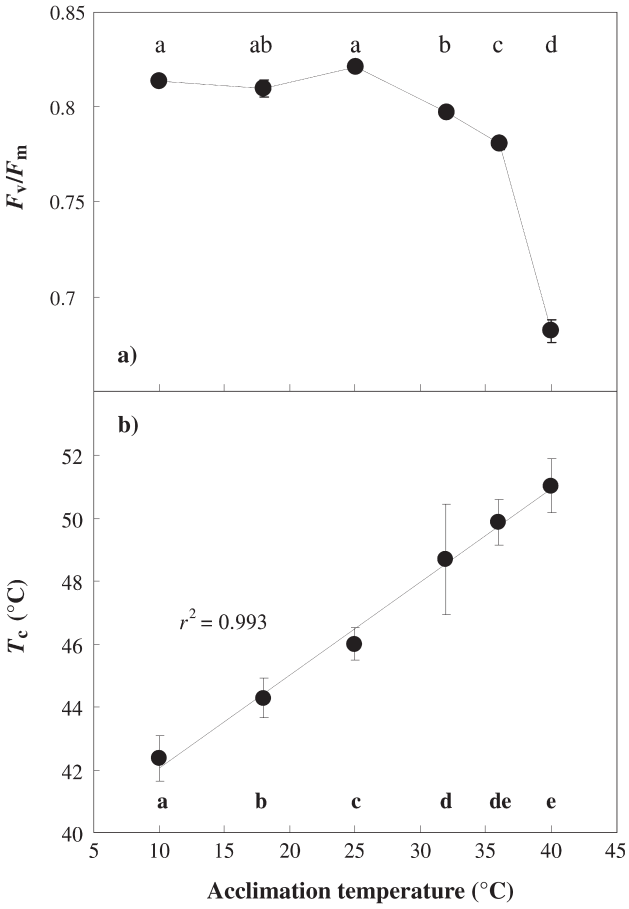



Figure 4. Relationship between acclimation temperature imposed for 48 h before measurements and (a) maximal PSII quantum yield recorded at the end of a dark period ($F_v/F_m$) and (b) critical temperature for PSII stability ($T_c$) in cork oak leaves. Different letters indicate significantly different values (means ± CI, $n$ = 5 for $F_v/F_m$ and $n$ = 3 for $T_c$; Fisher's PLSD, $P < 0.05$).

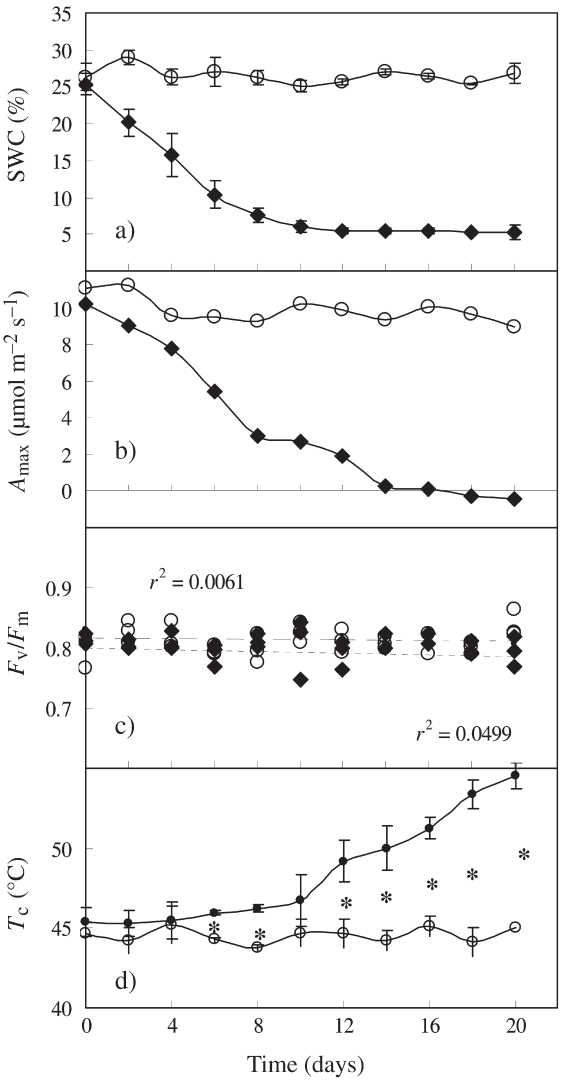



Figure 5. Time course during drought of (a) volumetric soil water content (SWC), (b) maximal net $CO_2$ assimilation rate ($A_{max}$), (c) maximal quantum yield of photochemistry of dark-acclimated leaves ($F_v/F_m$) and (d) critical temperature for PSII stability ($T_c$). Means ± CI ($P$ = 0.05), $n$ = 4 to 5. Asterisks indicate significantly different values of $T_c$ between drought-stressed (◆ or ●) and control (○) plants (Fisher's PLSD, $P < 0.05$).

45.5 °C throughout the experiment. In contrast, increases in $T_c$ were observed early in the drought treatment (Figure 5d). Significant differences were visible by Day 6, when SWC had dropped to 10% and $A_{max}$ was reduced by over 50%. The increase in $T_c$ was large, and $T_c$ reached 54.5 °C after 20 days of drought treatment. Leaf temperatures were monitored with an infrared thermometer and a maximum difference of 0.8 °C, attributable to stomatal closure, was detected between plants in the control and drought treatments. Thus the observed increase in $T_c$ in the drought treatment was probably a result of drought stress rather than increased leaf temperature.

Heat shock (40 °C for 36 h) resulted in severe decreases in $A_{max}$ and $g_s$ in both control and drought-stressed seedlings (Figure 6). The observed decreases in $A_{max}$ were around 85% in control plants and 75% in drought-stressed plants. After returning to ambient temperature (25 °C), a rapid but incomplete recovery was observed in plants in both treatments. After 2 days, $A_{max}$ remained below initial values in both treatments

(Figure 6). The heat shock also led to a significant decrease in $F_v/F_m$ in control seedlings and, to a lesser extent, in drought-stressed seedlings. Recovery after heat stress resulted in similar $F_v/F_m$ values for plants in both treatments, which were below the initial values even after 3 days of recovery (0.795 versus 0.83, $P < 0.0001$).

Values of $T_c$ before and after heat shock revealed that (i) $T_c$ was higher in drought-stressed seedlings, as already described above; (ii) no short-term reversal of the impact of drought on $T_c$ was evident in rehydrated seedlings; and (iii) $T_c$ increased in both drought-stressed and control seedlings by about 2 °C after heat shock, reaching values of about 56 °C (Figure 7).

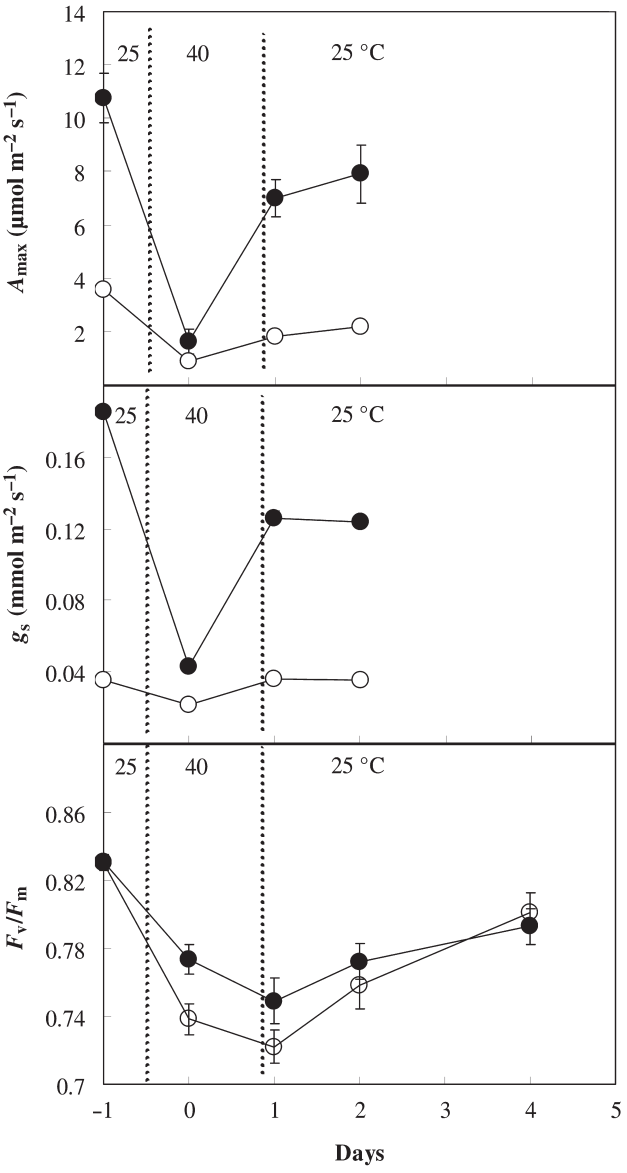



Figure 6. Time course of maximal net assimilation rate ($A_{max}$; top panel) under conditions of ambient $CO_2$ concentration and saturating irradiance, stomatal conductance to water vapor ($g_s$; middle panel) and quantum yield of photochemistry of dark-adapted leaves ($F_v/F_m$; bottom panel) of well-watered (control, ●) or drought-stressed (○) *Quercus suber* seedlings subjected to heat shock (40 °C for 36 h). The duration of the heat shock is indicated by the two dotted lines. In (c), asterisks indicate significant differences between drought-stressed and control plants (Fisher's PLSD, $P < 0.05$).

## Discussion

Photosynthetic responses to temperature are usually referred to as either reversible (within a few minutes) or degradative (i.e., irreversible over the course of several hours; Berry and Björkman 1980). Reversible responses include increases in photosynthetic processes such as $V_{cmax}$ and $J_{max}$, such as those documented in this work, although the decline in these parameters observed above the optimal temperature is probably not fully reversible. Reference values of $V_{cmax}$ at 25 °C were relatively high and close to those reported for *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. (87.7 and 90.5 mmol m$^{-2}$ s$^{-1}$, respectively; Dreyer et al. 2001). It is worth noting that the xeromorphic leaves of cork oak do not display an intrinsically lower photosynthetic capacity than those of deciduous mesophytic species. The $J_{max}/V_{cmax}$ ratio at 25 °C (1.6) that we observed is among the lowest recorded (usual values close to 2 at 25 °C; Dreyer et al. 2001). Both $V_{cmax}$ and $J_{max}$ displayed the expected temperature response, with optimum temperatures of about 34 and 33 °C for $V_{cmax}$ and $J_{max}$, respectively, which are among the lowest recorded in woody species (35 to 41 °C for $V_{cmax}$, and 30 to 38 °C for $J_{max}$; Dreyer et al. 2001, Medlyn et al. 2002*b*, Robakowski et al. 2002). However, the procedure that we used to estimate $V_{cmax}$ took no account of the mesophyll resistance to $CO_2$. This may have resulted in underestimation of $V_{cmax}$ at all temperatures (Epron et al. 1995, Piel et al. 2002). The temperature response of $V_{cmax}$ integrates intrinsic temperature responses of both Rubisco and the mesophyll resistance, which may differ from each other (Bernacchi et al. 2002). Despite this restriction, our data may be compared with the few published results obtained with similar experimental proce-

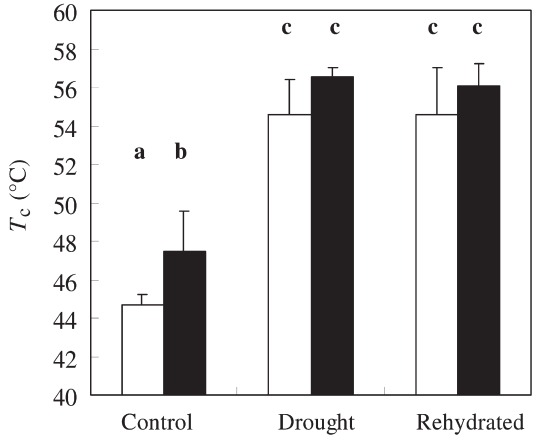



Figure 7. Critical temperature for PSII stability ($T_c$) before (open bars) and after (solid bars) a 36-h heat shock at 40 °C in control, drought-stressed or rehydrated *Quercus suber* seedlings; $n = 4–5$. Means ± CI. Effects of drought preconditioning and heat stress were significant (two-way ANOVA, $P < 0.0001$ and $P = 0.028$, respectively), whereas rehydration resulted in no change relative to drought stress.

dures. The high-temperature-induced decline in $V_{cmax}$ was probably related to the high sensitivity of Rubisco activase to moderately elevated temperatures rather than to deactivation of Rubisco itself (Feller et al. 1998, Salvucci et al. 2001, Crafts-Brandner and Salvucci 2002).

Increases in ambient temperature resulted in short-term changes in $V_{cmax}$ and $J_{max}$ as well as long-term modifications of PSII photochemistry. The quantum yield of photochemistry recorded after overnight recovery ($F_v/F_m$) was close to optimum (0.83) up to 32 °C and began to decrease above a temperature threshold well above the optimum for $J_{max}$, as observed in other woody species (*Cedrus atlantica* (Endl.) G. Manetti ex Carriére (Epron 1997), *Abies alba* Mill. (Robakowski et al. 2002) and mesophytic broad-leaved species (Dreyer et al. 2001)). In all cases, the decrease in $F_v/F_m$ was the result of a decline in $F_m$ with no increase in $F_o$. This observation may indicate that, up to 40 °C, the temperature-induced decreases in $F_v/F_m$ were a result of conformational changes in PSII related to thermal energy dissipation (Briantais et al. 1996, Yamane et al. 1997). Increases in $F_o$, which indicate irreversible damage to PSII and uncoupling between antenna and PSII cores, are usually recorded at much higher temperatures. Méthy et al. (1997), for example, observed increases in $F_o$ at temperatures close to 48 °C in *Quercus ilex* L. and *Pinus halepensis* Mill. The temperature at which $F_o$ began to increase, usually referred to as the critical temperature for PSII photochemistry ($T_c$; Bilger et al. 1984), was close to 45 °C in cork oak seedlings acclimated to 25 °C, which does not differ from that recorded for a range of woody seedlings under similar conditions (Dreyer et al. 2001, Knight and Ackerly 2002, Robakowski et al. 2002, F. Froux et al., unpublished data). Regardless, $T_c$ is not constant in a given genotype, but displays large plasticity in response to changes in microenvironment, and increases significantly with increases in ambient temperature (Raison et al. 1982, Havaux 1993*a*, Havaux et al. 1996). In cork oak seedlings, we observed an increase in $T_c$ of around 9 °C with a gradual increase in ambient temperature from 10 to 40 °C. To our knowledge, this is the largest extent of acclimation in $T_c$ observed in any species and supports the hypothesis that cork oak can acclimate rapidly to high temperatures through large changes in thermostability, even though it does not display unusually high values of $T_c$ at lower temperatures.

The short-term thermal treatments that we used to measure the temperature responses of primary photosynthesis parameters such as $J_{max}$ were capable of inducing large acclimation processes in the thylakoids, resulting in increased thermostability. This observation leads to the question whether such acclimation processes interact with the rate of electron transport and may have thus affected the observed temperature response of $J_{max}$. If $J_{max}$ under saturating irradiance is limited mainly by the rate of electron transport between the plastoquinol pool and the cytochrome b6f complex (i.e., between PSII and PSI; Genty and Harbinson 1996), then this may not be the case. Experimental data that could help answer this question are still missing. Nevertheless, it is important to note that the imposition of successively higher temperatures, rather than random temperatures, could result in acclimatory responses of $J_{max}$ and, to a lesser extent, of $V_{cmax}$.

Drought stress is known to induce increases in $T_c$. Havaux (1992) showed that short exposures to drought resulted in significantly increased values of $T_c$ in potato. Similarly, Epron (1997) and Ladjal et al. (2000) showed that even mild drought increased the tolerance of PSII photochemistry to heat shocks. In *Heteromeles arbutifolia* (Ait.) M. J. Roem., $T_c$ underwent diurnal cycles, and drought-stressed plants always had $T_c$ values about 1 °C higher than those of control plants (Valladares and Pearcy 1997). In cork oak seedlings, drought stress under constant ambient temperature led to an increase in $T_c$ of almost 10 °C. The increase was apparent when plants were only moderately stressed and was amplified during progression of drought. Again, this indicates large plasticity in $T_c$ in cork oak. The potential for reversal of this acclimation after cessation of drought stress is unknown. In *Cedrus atlantica*, the drought-induced increase in thermostability was stable for at least 1 month after rehydration (Ladjal et al. 2000). In our study, no short-term (i.e., few days) reversal of the increase in $T_c$ was observed after rehydration of the plants. Additional data on the long-term stability of drought-induced thermotolerance in cork oak are required.

The causes and physiological mechanisms of the high temperature- and drought-induced increases in $T_c$ are still a matter of debate. Some results indicate involvement of increased synthesis of osmotically active solutes like soluble sugars and glycine-betaine (Yang et al. 1996). Nevertheless, Ladjal et al. (2000) observed that increased thermostability could be maintained 2 months after the cessation of drought stress, whereas the osmotic adjustment induced by drought had already dissipated. Obviously, changes in thylakoid membrane composition occur, leading to lower membrane fluidity (Yordanov 1992). Such changes could include the accumulation of zeaxanthin to stabilize thylakoids (Havaux and Tardy 1996, Havaux et al. 1996).

## Conclusions

We have drawn three conclusions from our study.

(1) Thermal responses of photosynthesis—particularly maximal carboxylation rate and maximal light-driven electron flow—in cork oak seedlings were of the expected form, but the temperature optima were relatively low compared with earlier results for mesophytic deciduous trees. Similarly, thermostability of photochemistry at standard temperatures was close to 45 °C and did not differ from that previously observed with mesophytic species.

(2) Cork oak seedlings displayed large plasticity in the critical temperature for PSII stability. Acclimation to high temperatures and drought promoted increases in $T_c$ of up to 10 °C. When drought-stressed seedlings were heat shocked, $T_c$ underwent an additional increase of 2 °C, reaching values as high as 56 °C, whereas the highest values previously reported in a Mediterranean conifer (*Cedrus atlantica*) were about 52 °C (F. Froux et al., unpublished data).

(3) The large and rapid acclimation process in cork oak seedlings may have consequences for estimation of the ther-

mal optimum for $J_{max}$, although the latter may require additional synthesis of electron transport components.

Such a large degree of plasticity may contribute to the adaptation of cork oak to high temperatures and drought in Tunisia. In addition, with respect to the observed large plasticity in the thermostability of photosynthesis, and the unexpectedly high values of $T_c$ induced by moderate environmental changes, it seems unlikely that high temperature sensitivity of photosynthesis is involved in the rapid decline in the number of seedlings present in cork oak stands.

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