

## Drought-induced photosynthetic inhibition and autumn recovery in two Mediterranean oak species (*Quercus ilex* and *Quercus suber*)

M. VAZ,<sup>1,2</sup> J.S. PEREIRA,<sup>3</sup> L.C. GAZARINI,<sup>1</sup> T.S. DAVID,<sup>4</sup> J.S. DAVID,<sup>3</sup> A. RODRIGUES,<sup>5</sup> J. MAROCO<sup>6</sup> and M.M. CHAVES<sup>3,5</sup>

<sup>1</sup> Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)/Dep. Biologia, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal

<sup>2</sup> Corresponding author (mvaz@uevora.pt)

<sup>3</sup> Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>4</sup> Instituto Nacional de Recursos Biológicos—INRB, I.P., Quinta do Marquês, 2780-159 Oeiras, Portugal

<sup>5</sup> Instituto Tecnologia Química e Biológica (ITQB-UNL), Av. da República, 2780-157 Oeiras, Portugal

<sup>6</sup> Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco, 34, 1149-041 Lisboa, Portugal

Received April 14, 2009; accepted April 18, 2010; published online June 22, 2010

**Summary** Responses of leaf water relations and photosynthesis to summer drought and autumn rewetting were studied in two evergreen Mediterranean oak species, *Quercus ilex* spp. *rotundifolia* and *Quercus suber*. The predawn leaf water potential ( $\Psi_{PD}$ ), stomatal conductance ( $g_s$ ) and photosynthetic rate ( $A$ ) at ambient conditions were measured seasonally over a 3-year period. We also measured the photosynthetic response to light and to intercellular  $CO_2$  ( $A/PPFD$  and  $A/C_i$  response curves) under water stress (summer) and after recovery due to autumn rainfall. Photosynthetic parameters,  $V_{c_{max}}$ ,  $J_{max}$  and triose phosphate utilization (TPU) rate, were estimated using the Farquhar model. RuBisCo activity, leaf chlorophyll, leaf nitrogen concentration and leaf carbohydrate concentration were also measured. All measurements were performed in the spring leaves of the current year. In both species, the predawn leaf water potential, stomatal conductance and photosynthetic rate peaked in spring, progressively declined throughout the summer and recovered upon autumn rainfall. During the drought period, *Q. ilex* maintained a higher predawn leaf water potential and stomatal conductance than *Q. suber*. During this period, we found that photosynthesis was not only limited by stomatal closure, but was also downregulated as a consequence of a decrease in the maximum carboxylation rate ( $V_{c_{max}}$ ) and the light-saturated rate of photosynthetic electron transport ( $J_{max}$ ) in both species. The  $V_{c_{max}}$  and  $J_{max}$  increased after the first autumnal rains and this increase was related to RuBisCo activity, leaf nitrogen concentration and chlorophyll concentration. In addition, an increase in the TPU rate and in soluble leaf sugar concentration was observed in this period. The results obtained indicate a high resilience of the photosynthetic apparatus to summer drought as well as good recovery in the following autumn rains of these evergreen oak species.

**Keywords:** biochemical parameters, drought, Mediterranean, photosynthesis, *Quercus ilex*, *Quercus suber*, recovery, water relations.

### Introduction

In the south-western Iberian Peninsula with a Mediterranean-type climate, the landscape is dominated by evergreen oak savannas, exploited as multipurpose silvopastoral systems. Covering a large area (2–2.5 million hectares), the main tree species are *Quercus ilex* spp. *rotundifolia* (syn. *Quercus rotundifolia*; syn. *Quercus ilex* spp. *ballota*) (holm oak) and *Quercus suber* (cork oak). Although both species often coexist, *Q. ilex* mainly occurs in the drier, inland regions, whereas *Q. suber* dominates in the wetter, western coastal areas. In these ecosystems, trees must withstand a long hot and dry summer season when water deficits are associated with high light intensity and temperatures, which impose restrictions on carbon assimilation (Faria et al. 1998, Chaves et al. 2002, Pereira et al. 2004, 2007). Water deficits are alleviated by the autumn rains.

The Mediterranean climate has traditionally been highly variable with a considerable frequency of extreme years, for example, in rainfall regime (Xoplaki et al. 2004). This may aggravate in the future. According to most climate change scenarios for the region, the severity of the summer drought may increase as well as the frequency of severe droughts (Miranda et al. 2002, Giorgi and Lionello 2008). Therefore, the capacity to overcome a water limitation period and the capacity for a rapid recovery after rainfall may be adaptive features of these Mediterranean evergreen trees

(Galmés et al. 2007a). Indeed, a comparison between deciduous and evergreen oak trees in California (*Quercus douglasii*) and Portugal (*Q. ilex* ssp. *rotundifolia*) under similar climatic conditions showed that the annual photosynthetic assimilation was the same. As evergreens have leaves with a lower photosynthetic capacity to assimilate as much carbon as the corresponding deciduous trees over the year, they have to achieve a longer growing season (Baldocchi et al. 2010). To a certain extent, this requires a recovery of leaf photosynthetic capacity upon rehydration in the autumn.

The Mediterranean evergreen oaks are mostly isohydric, i.e., they effectively control tissue dehydration via stomatal closure and deep rooting to avoid drought-induced hydraulic failure (David et al. 2007). Several studies have addressed different aspects of photosynthetic limitations during water stress imposition in Mediterranean species. However, analyses of photosynthetic recovery in Mediterranean evergreens after water stress are still scarce (Flexas et al. 2006, Galmés et al. 2007a). The responses of photosynthesis to soil water depletion comprise two distinct phases: a first stage characterized by a decrease in stomatal conductance (Faria et al. 1998, Chaves et al. 2002, 2003, Centritto et al. 2003, Flexas et al. 2006, 2007) and a decrease in mesophyll conductance (Flexas et al. 2006, 2007, Galmés et al. 2007a, 2007b) and a second stage in which further decreases in stomatal conductance may coincide with metabolic impairment (Flexas et al. 2006). Under field conditions when water stress develops gradually over periods comprising weeks or months, changes in leaf biochemistry that result in the downregulation of photosynthetic metabolism may occur in response to lowered carbon substrates (Chaves and Oliveira 2004, Flexas et al. 2007) caused by stomatal restriction. The majority of studies about photosynthesis recovery in Mediterranean plants focused on seedlings (e.g., Galmés et al. 2007a), with scarce information for trees under field conditions. The process of recovery is rather complex as the factors involved seems to be multiple and to strongly depend on the species and conditions analysed (Chaves et al. 2009). In a recent study on Mediterranean species, Galmés et al. (2007a) demonstrated that the recovery capacity was large in herbs, intermediate in semi-deciduous species and the least in evergreens. The main objectives of this work were to study (i) the photosynthetic resilience of these Mediterranean evergreen oaks to seasonal drought and (ii) whether the reversibility of drought-induced downregulation of the photosynthetic machinery occurs upon the first autumn rains, and if it does, what underlying processes are involved?

## Materials and methods

### The experimental site and plant material

The experiment was conducted between 1999 and 2002, in a mixed *montado* located at Herdade da Alfarrobeira (38°32' 26" N, 8°00'01" W, 220–250 m a.s.l.), near Évora (southern

Portugal). A more detailed description of the site is given in David et al. (2007) and Pereira et al. (2007). Tree cover consists of sparse holm oak trees with scattered patches of cork oak trees. The average stand density and tree crown cover are about 30 trees per hectare and 21%, respectively. The understory consists of natural pasture invaded by shrubs, mainly *Cistus salvifolius* L. The climate is Mediterranean with dry, hot summers and rainy, mild winters. The long-term (1951–80) mean annual rainfall is 665 mm, mainly concentrated from autumn to early spring (90%) in <75 days of rain per year (Figure 1). The mean annual open water evaporation is 1760 mm<sup>-1</sup>. The mean annual air temperature is about 15 °C, ranging from 8.6 °C in January to 23.1 °C in August (INMG 1991). The soil is a Dystric Cambisol with a maximum soil depth of around 1 m and a low water retention capacity, overlying a granite rock.

Four cork oak (*Q. suber* L.) and four holm oak (*Q. ilex* ssp. *rotundifolia* syn. *Quercus rotundifolia* Lam.) trees about 80 years old were selected in nearby plots (~150 m apart). The trunk diameter at breast height (DBH), crown projected area and height of the sampled trees ranged from 0.45 to 0.53 m, from 91.13 to 150.19 m<sup>2</sup> and from 8.5 to 9.5 m in *Q. suber* and from 0.33 to 0.43 m, from 47.71 to 89.99 m<sup>2</sup> and from 7 to 8 m in *Q. ilex*, respectively. The mean stand DBH was 0.42 ± 0.18 and 0.34 ± 0.17 m in cork and holm oaks, respectively. In both oak species, new leaves emerge in spring ('spring leaves') and may display a weak flushing in autumn ('autumn leaves'). However, all measurements reported in this work were performed in fully expanded, current-year leaves, i.e., those that emerged in the spring of each year.

### Predawn leaf water potential

Predawn leaf water potential ( $\Psi_{IPD}$ ) was measured on a monthly basis, from January 1999 to December 2001, with a Scholander pressure chamber (PMS 1000; PMS Instru-

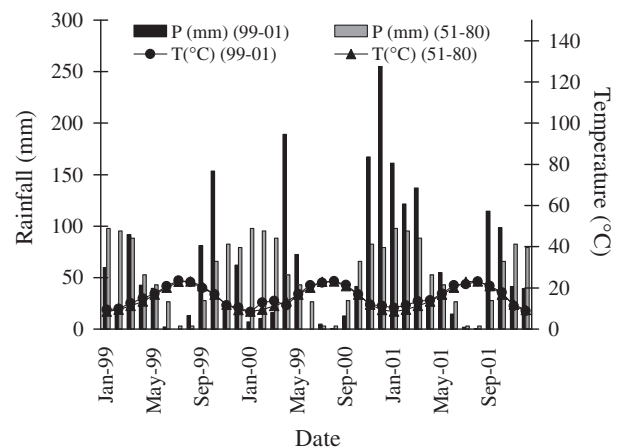


Figure 1. Monthly values of total rainfall (bars) and mean air temperature (lines) observed at the experimental site (1999–2001) and long-term averages (1951–80).

ments, Corvallis, OR). Three to four leaves were sampled in the south-facing side of the crown of each tree, just prior to sunrise. Samples were taken at similar heights above ground to avoid variability due to hydrostatic water potential and placed into a plastic bag immediately after cutting to prevent further transpiration.

#### Leaf gas exchange measurements

Gas exchange measurements were done with a portable steady-state photosynthetic system (Li-6400; Li-Cor, Lincoln, NE) in 1999 (22 March, 16 July, 10 September, 13 October and 8 November), 2000 (19 May and 22 August) and 2001 (15 March, 15 June, 25 August and 30 October) on fully expanded leaves from the current-year spring flushing. The photosynthetic rate under natural conditions ( $A$ ) and stomatal conductance ( $g_s$ ) were measured in the morning periods (8.00–10.00 h) ( $A_{\max}$  and  $g_{s\max}$ ) throughout the experiment in four leaves per tree. Two sets of photosynthesis ( $A$ ) measurements were conducted: the responses of leaf photosynthesis to  $\text{CO}_2$  concentration in intercellular spaces ( $A/C_i$ ) and photosynthetic photon flux density ( $A/\text{PPFD}$ ) in two seasons (summer and autumn) of 1999 and 2000. The  $A/C_i$  curves were produced under saturating light intensity ( $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), leaf temperature of around  $25 \pm 2 \text{ }^\circ\text{C}$  and a relative humidity of around 50%. The  $\text{CO}_2$  concentration ( $C_i$ ) in the leaf chamber was raised from 5, 10, 20, 35, 60, 100 to 120 Pa. The  $A/\text{PPFD}$  curves were produced under ambient  $\text{CO}_2$  concentration (35 Pa), leaf temperature of around  $25 \pm 2 \text{ }^\circ\text{C}$  and a relative humidity of around 50%. The light intensity was raised from 50, 100, 200, 500, 1000, 1500 to  $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The leaves were allowed to equilibrate for 8–10 min before logging data in  $A/C_i$  and  $A/\text{PPFD}$  curves. All leaf gas exchange measurements (for the  $A/C_i$  and  $A/\text{PPFD}$ ) were conducted between early and mid-morning (8.00–10.00 h) to avoid afternoon stomatal closure (Pereira et al. 1987).

#### Analysis of light response curves

The response of leaf photosynthesis to photosynthetic photon flux density ( $A/\text{PPFD}$ ) can be described by the following function (Harley et al. 1986):

$$A = \Phi \text{PPFD} / \sqrt{(1 + \Phi^2 \text{PPFD}^2 / A_{\max}^2)} - \text{Rd}$$

where  $A$  is the net photosynthetic rate, PPFD is the incident photon flux density,  $A_{\max}$  is the light-saturated photosynthetic rate,  $\text{Rd}$  is the rate of respiration in the light, exclusive of photorespiration, and  $\Phi$  is the initial slope (quantum yield). The parameters  $\Phi$  and  $A_{\max}$  were obtained from five individual curves.

#### Biochemical and structural parameters

After gas exchange measurements, the leaves were collected for analysis of RuBisCo total activity, nitrogen concentration,

chlorophyll concentration, soluble sugars and starch and specific leaf area (SLA). For RuBisCo total activity analysis, four discs per leaf (0.6 cm in diameter taken from the same leaves used in gas exchange measurements) were harvested in the light, frozen in liquid  $\text{N}_2$  and stored at  $-80 \text{ }^\circ\text{C}$  until the assay. Enzyme activity was measured spectrophotometrically following the oxidation of NADH at 340 nm and  $21 \text{ }^\circ\text{C}$  as described by Leegood (1993). The leaf nitrogen concentration was determined by the standard Kjeldahl method in a composite leaf sample. Leaves were oven dried at  $70 \text{ }^\circ\text{C}$  during 48 h and ground for analysis. The chlorophyll concentration was measured using a spectrophotometer (Perkin-Elmer 124, Waltham, MA), as described by Arnon (1949), in four discs (0.6 cm in diameter) per leaf. The discs were obtained from two leaves close to those used in gas exchange measurements. For non-structural carbohydrate determination, four discs per leaf (0.6 cm in diameter) were analysed from four leaves per tree. The leaf samples were collected at midday (12.00–13.00 h), immediately frozen in liquid nitrogen and stored at  $-80 \text{ }^\circ\text{C}$  until analysis. The soluble sugars were extracted in ethanol (70%, v/v) and the residue was incubated in HCl (1.1%, v/v) for 30 min at  $95 \text{ }^\circ\text{C}$  for starch extraction. Both soluble sugars and starch were determined colorimetrically at 625 nm with an anthrone reagent (Robyt and White 1990). For determination of SLA (leaf area per unit of dry weight), 30 leaves were collected and transported to the laboratory in refrigerated bags to avoid weight loss by respiration. The leaf area was measured, after petiole removal, with an area meter (LI-3000A; Li-Cor). The leaves were then oven dried at  $80 \text{ }^\circ\text{C}$  for at least 48 h and the dry mass was determined (Mettler-Toledo, PB 3002, Columbus, OH).

#### Biochemical modelling and relative stomatal limitation

The biochemical photosynthetic model of Farquhar et al. (1980), modified according to Sharkey (1985), was used to estimate the apparent maximum carboxylation rate ( $V_{c\max}$  ( $C_i$ )), maximum electron transport rate ( $J_{\max}$ ), mitochondrial respiration ( $\text{Rd}$ ) and triose phosphate utilization (TPU) rate.  $V_{c\max}$  and  $\text{Rd}$  were estimated by fitting the model to the measured  $A/C_i$  data for  $C_i < 20$  Pa according Sharkey et al. (2007). The Michaelis–Menten constant ( $K_c$  and  $K_o$ ),  $\alpha$ ,  $\tau$  and temperature dependence of the model parameters were corrected as described by Harley et al. (1986). The temperature dependence of  $K_c$ ,  $K_o$ ,  $\alpha$  and  $\tau$  was described by an exponential function of the type: Parameter =  $\exp[c - \Delta H_a/(RT)]$  where  $c$  is a scaling constant characteristic for each parameter,  $\Delta H_a$  is the activation energy for the parameter,  $R$  is the ideal gas constant and  $T$  is the leaf temperature in Kelvin for the values of the constants used. This biochemical model considered mesophyll conductance to  $\text{CO}_2$  ( $g_m$ ) as infinite. This assumption could induce underestimation of  $V_{c\max}$  values, as shown in Ethier and Livingston (2004). As  $g_m$  was not estimated in the present work, only apparent values of maximum carboxylation rates ( $V_{c\max}$  ( $C_i$ )) are displayed. However, to quantify the magnitude of  $V_{c\max}$  underestimation in the present study, a

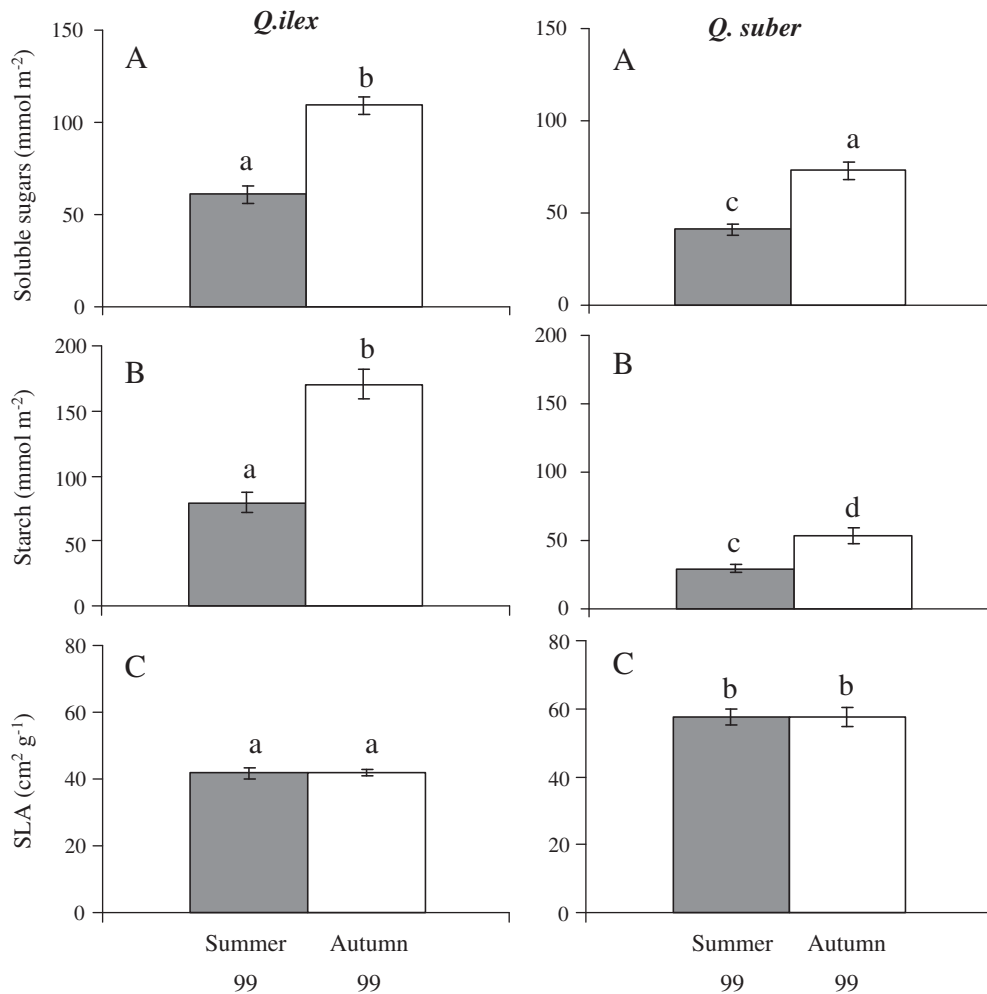


Figure 2. Soluble sugar content, starch content and SLA in *Q. ilex* and *Q. suber*. Values are presented as the means  $\pm$  SE. Different letters denote statistically significant differences at the 5% level.

sensitivity analysis was performed using  $g_m$  in the range 0.037, 0.050, 0.100, 0.150 and 0.200 mol m<sup>-2</sup> s<sup>-1</sup> for *Q. ilex* in autumn (1999 and 2000) and actual values of maximum carboxylation ( $V_{c_{max}}$  ( $C_c$ )) were estimated. A  $g_m$  of 0.037 mol m<sup>-2</sup> s<sup>-1</sup> was reported in the literature for mature trees of *Q. ilex* in similar ambient conditions (Niinemets et al. 2006).

#### Statistical analysis

All statistical analyses were performed using the SPSS software package (Statistical Package for the Social Sciences for Windows 11 (2001), Chicago, IL). One-way analysis of variance (ANOVA) was used to test the differences between means of predawn leaf water potential ( $\Psi_{IPD}$ ), leaf gas exchange ( $g_{s_{max}}$  and  $A_{max}$ ) and biochemical and leaf structural parameters. Significant differences between the means were evaluated with Tukey's HSD test with  $P = 0.05$ . Descriptive statistics and ANOVA (post hoc Student–Newman–Keuls test at  $P = 0.05$ ) were performed in order to assess the significance of the differences observed in the physiological para-

meters. Two-way factorial ANOVA (season: summer and autumn and species: *Q. ilex* and *Q. suber*) were used to test the main effects and factor interactions on  $V_{c_{max}}$ ,  $J_{max}$ , Rd, TPU,  $A_{maxc}$  (light-saturated and ambient CO<sub>2</sub> concentration) and quantum yield ( $\Phi$ ). All measurements are shown as means  $\pm$  SE. Before applying the ANOVA test, the data were tested for homogeneity of variance by using Levene and Bartlett tests, at the significance level of  $P = 0.05$ . When variances across groups were unequal, i.e., the usual ANOVA assumptions were not satisfied, the Welch ANOVA test was applied (significance level of  $P = 0.05$ ).

## Results

#### Environmental conditions

Figure 1 shows the monthly values of rainfall and average air temperatures from January 1999 to December 2001 and the long-term regional averages (1951–80). The annual rainfall was lower than average in 1999 (586.4 mm) and always

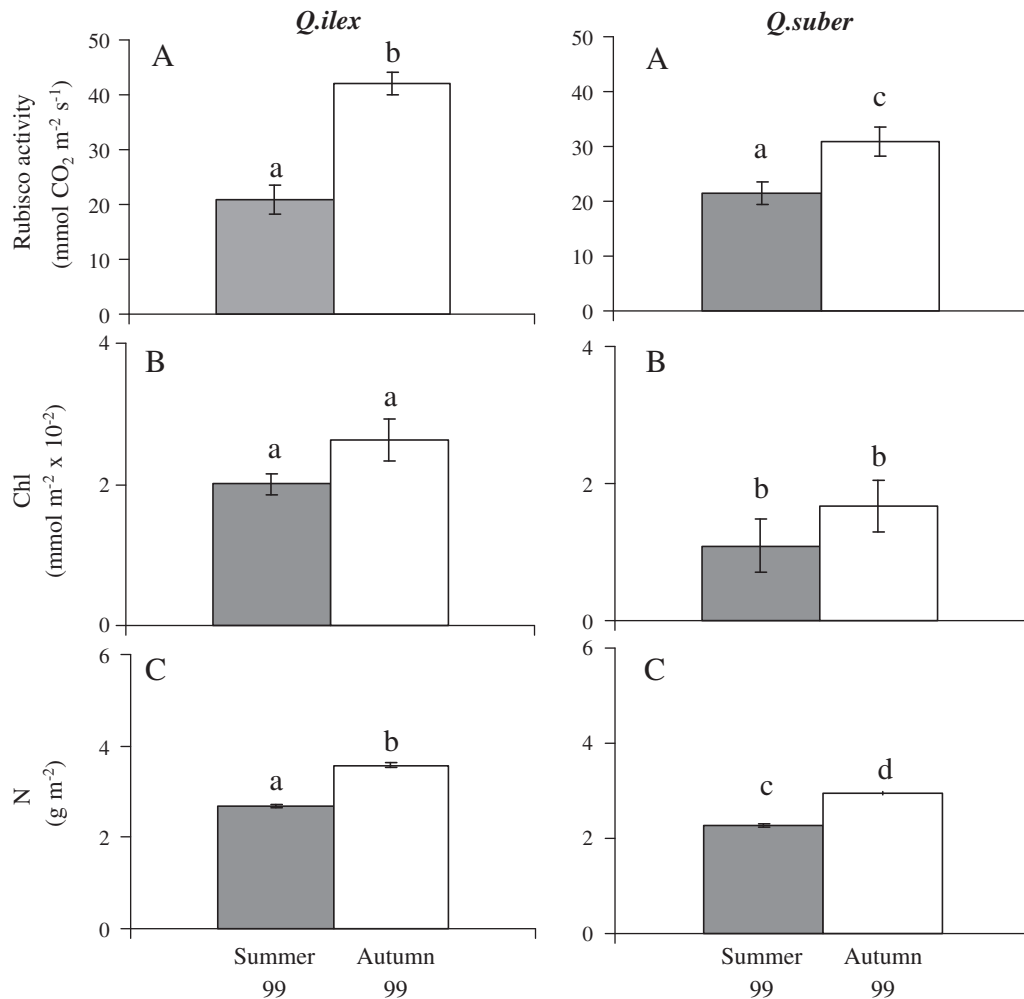


Figure 3. In vitro activities of ribulose-1-5-bisphosphate carboxylase oxygenase (RuBisCo), total leaf chlorophyll content (Chl) and leaf N contents (N) in *Q. ilex* and *Q. suber*. Values are presented as the means  $\pm$  SE. Different letters denote statistically significant differences at the 5% level.

scarce during summer periods, ranging from 0.0 to 14.6 mm in June, from 0.0 to 4.8 mm in July, from 0.2 to 13 mm in August and from 12.8 to 114.6 mm in September. The spring (March–June) of 1999 and the summer of 2000 were drier

than average. The mean air temperature closely followed the pattern of the 30-year average. During the experimental period, the monthly average temperature ranged from 11.5 to 17.2 °C in spring, from 20.9 to 23.6 °C in summer and

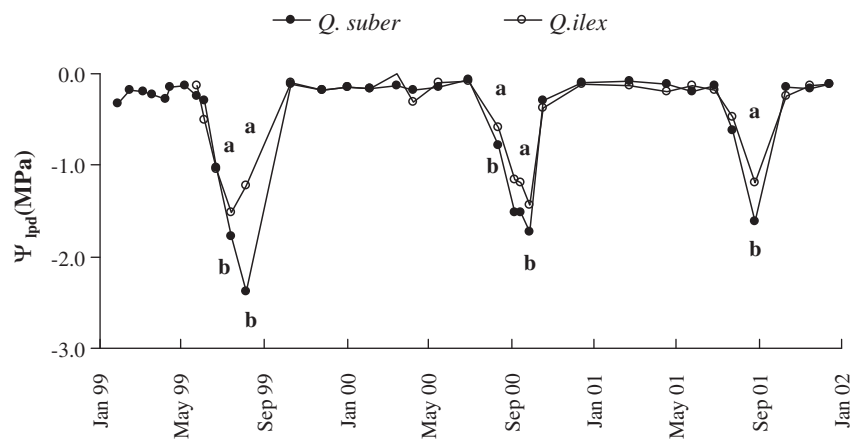


Figure 4. Seasonal evolution of predawn leaf water potential ( $\Psi_{pd}$ ) in *Q. ilex* and *Q. suber* (1999–2001). Values are presented as the means  $\pm$  SE. Different letters denote statistically significant differences at the 5% level.



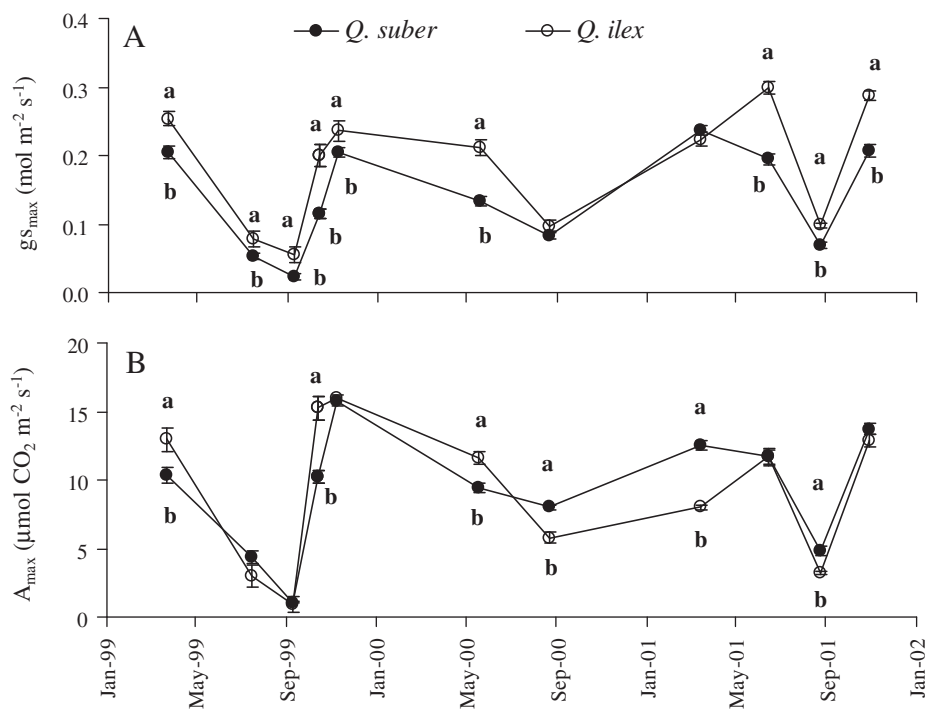


Figure 5. Seasonal trends of morning values of (A) stomatal conductance ( $g_{s_{max}}$ ) and (B) photosynthesis ( $A_{max}$ ) in *Q. ilex* and *Q. suber* (1999–2001). Values are presented as the means  $\pm$  SE. Different letters denote statistically significant differences at the 5% level.

from 11.8 to 16.9 °C in autumn. The average maximum temperature reached 30.5 °C in July.

#### Leaf structure and composition

The leaves of *Q. ilex* had a lower SLA than those of *Q. suber* ( $P < 0.001$ ) (Figure 2). The lower SLA in *Q. ilex* is related to its higher degree of sclerophylly and tissue density. The differences in SLA between summer and autumn were not significant for both oak species. Total soluble sugars and starch leaf concentrations were lower in summer than in autumn for *Q. ilex* and *Q. suber* ( $P < 0.001$ ) (Figure 2). Leaves of *Q. ilex* had higher total soluble sugar and starch concentrations per unit of leaf area than *Q. suber* ( $P < 0.001$ ). The leaf RuBisCo activity and total leaf nitrogen concentration per unit of leaf area were also lower in summer than autumn for both *Q. ilex* and *Q. suber* ( $P < 0.001$ ) (Figure 3). The chlorophyll concentration per unit of leaf area showed no significant differences in both species, from summer to autumn (Figure 3). The total leaf nitrogen concentration, the chlorophyll concentration and the RuBisCo activity, expressed per unit of leaf area, were higher for *Q. ilex* than *Q. suber* due to the thicker leaves of the former.

#### Seasonal changes in tree water status

The predawn leaf water potential ( $\Psi_{IPD}$ ) was high in spring (approximately  $-0.15$  MPa) and declined during the summer drought in both species (Figure 4). After the autumn rains,  $\Psi_{IPD}$  recovered to pre-summer drought values in *Q. ilex* and *Q. suber*. Differences in  $\Psi_{IPD}$  between species were particu-

larly marked in late summer (September) when  $\Psi_{IPD}$  remained higher in *Q. ilex* ( $-1.23$ ,  $-1.44$  and  $-1.18$  MPa in 1999, 2000 and 2001, respectively) than in *Q. suber* ( $-2.38$ ,  $-1.73$  and  $-1.61$  MPa in 1999, 2000 and 2001, respectively). The differences in  $\Psi_{IPD}$  between species at the end of the summer drought were statistically significant ( $P < 0.001$ ).

#### Seasonal evolution of gas exchange

In spring periods (March to May), the new fully expanded leaves had high values of  $g_{s_{max}}$  ( $g_{s_{max}} = g_s$  measured in the morning, between 8.00 and 10.00 h), ranging from 0.237 to 0.254  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. ilex* and from 0.204 to 0.224  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. suber* (Figure 5). During the dry summer period,  $g_{s_{max}}$  declined and was higher ( $P < 0.001$ ) in *Q. ilex* than in *Q. suber* (0.055–0.098  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. ilex* and 0.023–0.069  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. suber*). The  $g_{s_{max}}$  reached the lowest values for both species in the summer of 1999 as it was the driest year of the experimental period. At the end of the drought period (September),  $g_{s_{max}}$  decreased by 80% in *Q. ilex* and by 90% in *Q. suber* in relation to previous spring values. After the first autumn rains (October to December),  $g_{s_{max}}$ , measured in the same spring and summer leaves, recovered to pre-drought values (0.236–0.287  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. ilex* and 0.205–0.207  $\text{mol m}^{-2} \text{s}^{-1}$  in *Q. suber*). Minimum values of  $A_{max}$  also occurred in summer for both species (0.89–5.78  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$  in *Q. ilex* and 1.09–7.99  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$  in *Q. suber*) (Figure 5). The  $A_{max}$  decreased by 93% in *Q. ilex* and by 89% in *Q. suber* in relation to previous spring values. In autumn, upon rewetting,

Table 1. Estimated model parameters ( $V_{c_{max}}(C_i)$ ,  $J_{max}$ , TPU, Rd) and RSL for *Q. ilex* and *Q. suber* in the summer and autumn of 1999 and 2000. Values are presented as the mean ( $n = 12$ )  $\pm$  SE. Different letters show statistically significant differences ( $P < 0.05$ ).

	$V_{c_{max}}(C_i)$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$J_{max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	TPU ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Rd ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	RSL (%)
<i>Q. ilex</i>					
Summer 1999	31.96 $\pm$ 2.27 a	100.80 $\pm$ 6.33 a	2.68 $\pm$ 0.29 a	2.34 $\pm$ 0.36 a	39.5 $\pm$ 1.3 a
Summer 2000	40.18 $\pm$ 1.26 b	113.60 $\pm$ 7.37 a	3.73 $\pm$ 1.03 a	2.02 $\pm$ 0.24 a	32.5 $\pm$ 1.9 b
Autumn 1999	47.91 $\pm$ 1.27 c	125.20 $\pm$ 7.61 a	6.41 $\pm$ 0.11 b	0.63 $\pm$ 0.17 b	25.2 $\pm$ 2.2 c
Autumn 2000	52.48 $\pm$ 1.08 d	137.42 $\pm$ 6.41 a	7.56 $\pm$ 1.19 b	0.76 $\pm$ 0.13 b	19.9 $\pm$ 1.2 c
<i>Q. suber</i>					
Summer 1999	40.63 $\pm$ 2.61 b	103.14 $\pm$ 12.46 a	2.27 $\pm$ 0.27 a	2.27 $\pm$ 0.27 a	39.7 $\pm$ 1.5 a
Summer 2000	45.49 $\pm$ 2.97 c	119.06 $\pm$ 14.23 a	3.28 $\pm$ 0.69 a	2.06 $\pm$ 0.45 a	35.5 $\pm$ 2.3 b
Autumn 1999	54.64 $\pm$ 3.93 d	125.82 $\pm$ 8.65 a	6.20 $\pm$ 0.30 b	1.26 $\pm$ 0.34 b	29.9 $\pm$ 2.4 b
Autumn 2000	61.26 $\pm$ 3.01 e	144.63 $\pm$ 9.62 b	8.32 $\pm$ 0.46 b	1.35 $\pm$ 0.39 b	27.6 $\pm$ 1.2 b

carbon assimilation recovered from water deficits in both species (Figures 1 and 5). The  $A_{max}$  increased from 0.89 to 15.92  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in *Q. ilex* and from 1.09 to 15.24  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in *Q. suber*.

#### $A/C_i$ and $A/PPFD$ response curves

Measurements of PPFD-saturated  $A$  plotted in relation to  $C_i$  were used to assess the biochemical limitations of photosynthesis under summer and autumn conditions. Values for the photosynthetic parameters  $J_{max}$  (maximum electron transport rate),  $V_{c_{max}}$  (the maximum carboxylation rate), TPU (triose phosphate utilization rate) and Rd (mitochondrial respiration) were obtained by fitting the Farquhar et al. (1980) model of leaf photosynthesis to individual  $A/C_i$  response curves (Table 1). The relative stomatal limitation (RSL) was also estimated from the  $A/C_i$  response curves (Table 1).

The two-way ANOVA test revealed a significant effect ( $P < 0.01$ ) of season (summer and autumn) on all photosynthetic parameters estimated. The  $J_{max}$ ,  $V_{c_{max}}$  and TPU parameters were low under summer drought conditions (1999 and 2000) when low predawn leaf water potential values occurred

in both oak species. Both *Quercus* species showed a remarkable capacity of photosynthetic recovery after a drought period. In fact, the photosynthetic parameter values increased after the autumn rains when the trees had a more favourable water status, as indicated by the higher predawn leaf water potentials (Figure 4). In *Q. ilex*, the  $V_{c_{max}}$  increased from 31 to 50%, the  $J_{max}$  from 24 to 30% and the TPU from 103 to 139% from summer to autumn. In *Q. suber*, the  $V_{c_{max}}$  increased from 35 to 40%, the  $J_{max}$  from 22 to 35% and the TPU from 154 to 173% for the same periods. The  $J_{max}/V_{c_{max}}$  ratio, a parameter that gives an indication of the nitrogen partitioning between thylakoid (photochemistry) and soluble (Calvin cycle) proteins, was not significantly influenced by season. The  $J_{max}/V_{c_{max}}$  ratio varied from 2.6 to 3.2 in *Q. ilex* and from 2.3 to 2.6 in *Q. suber*. Mitochondrial respiration (Rd) was, for both species and dates, higher in summer than in autumn due to the higher air temperature. The  $V_{c_{max}}$  was related to nitrogen leaf content (Figure 6) and the  $J_{max}$  was related to total leaf chlorophyll content (Figure 7) in both *Quercus* species. The  $A_{maxc}$  (maximum assimilation rate, estimated from the  $A/PPFD$  curves in light-saturated and ambient  $\text{CO}_2$  concentrations) was lower ( $P < 0.01$ ) in summer than in autumn in both *Quercus* species (Table 2). The two-way

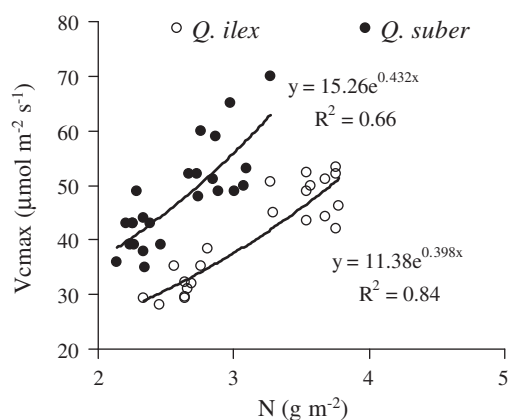


Figure 6. Relationship between maximum RuBisCo carboxylation ( $V_{c_{max}}$ ) and leaf N content (N) in *Q. ilex* and *Q. suber* in the summer and autumn of 1999.

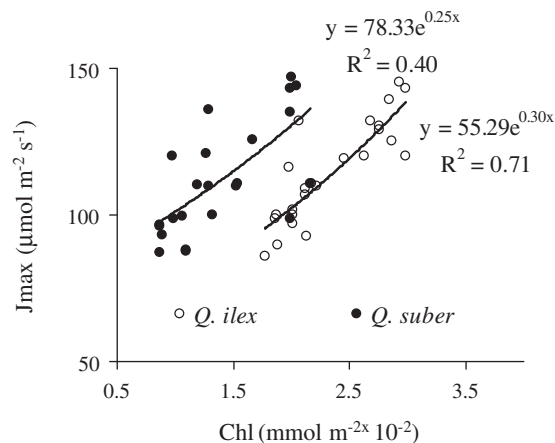


Figure 7. Relationship between maximum rate of electron transport ( $J_{max}$ ) and leaf chlorophyll content (Chl) in *Q. ilex* and *Q. suber* in the summer and autumn of 1999.

Table 2. Light-saturated photosynthesis ( $A_{\max c}$ ) and quantum yield ( $\Phi$ ) in *Q. ilex* and *Q. suber* in summer and autumn (1999). Different letters denote statistically differences ( $P < 0.05$ ) at the 5% level.

		$\Phi$ ( $\mu\text{mol CO}_2/\mu\text{mol photons}$ )	$A_{\max c}$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )
<i>Q. ilex</i>	Summer	0.056 $\pm$ 0.003 a	11.46 $\pm$ 1.78 a
	Autumn	0.069 $\pm$ 0.005 b	21.97 $\pm$ 1.75 b
<i>Q. suber</i>	Summer	0.075 $\pm$ 0.009 c	7.31 $\pm$ 0.81 c
	Autumn	0.084 $\pm$ 0.006 d	23.02 $\pm$ 1.73 b

Table 3.  $V_{\text{c}_{\max}}$  estimated by the model of Farquhar et al. (1980) ( $g_m = \infty$ ) ( $V_{\text{c}_{\max}}(C_i)$ ) and by the model of Ethier and Livingston (2004) ( $g_m = 0.037, 0.050, 0.100, 0.150$  and  $0.200 \text{ mol m}^{-2} \text{ s}^{-1}$ ) ( $V_{\text{c}_{\max}}(C_c)$ ) for *Q. ilex* in the autumn of 1999 and 2000. Values are presented as the mean ( $n = 12$ )  $\pm$  SE. Different letters show statistically significant differences ( $P < 0.05$ ).

		$V_{\text{c}_{\max}}(C_i)$ , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$			$V_{\text{c}_{\max}}(C_c)$ , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$		
Autumn	$g_m = \infty$ $\text{mol m}^{-2} \text{ s}^{-1}$	$g_m = 0.037^a$ $\text{mol m}^{-2} \text{ s}^{-1}$	$g_m = 0.050$ $\text{mol m}^{-2} \text{ s}^{-1}$	$g_m = 0.100$ $\text{mol m}^{-2} \text{ s}^{-1}$	$g_m = 0.150$ $\text{mol m}^{-2} \text{ s}^{-1}$	$g_m = 0.200$ $\text{mol m}^{-2} \text{ s}^{-1}$	
	1999	47.91 $\pm$ 1.27 a	58.03 $\pm$ 1.30 c	57.75 $\pm$ 1.32 c	57.42 $\pm$ 1.33 c	57.31 $\pm$ 1.29 c	57.25 $\pm$ 1.39 c
2000	52.48 $\pm$ 1.08 b	63.98 $\pm$ 1.21 d	63.26 $\pm$ 1.26 d	62.90 $\pm$ 1.29 d	62.78 $\pm$ 1.10 d	62.72 $\pm$ 1.13 d	

<sup>a</sup> $g_m$  value for *Q. ilex* trees in autumn in leaves (1 year) in Niinemets et al. (2006).

ANOVA test revealed no significant effect ( $P > 0.01$ ) of date or species on  $\Phi$  (quantum yield). The sensitivity analysis showed that a significant ( $P > 0.001$ ) underestimation in  $V_{\text{c}_{\max}}(C_i)$  occurs when  $g_m$  is not considered ( $g_m$  is infinite,  $g_m = \infty$ ) (Table 3) in relation to  $V_{\text{c}_{\max}}(C_c)$  values. However, we did not find significant differences in the  $V_{\text{c}_{\max}}(C_c)$  values when the  $g_m$  ranged from 0.037 to 0.200  $\text{mol m}^{-2} \text{ s}^{-1}$ .

## Discussion

### Seasonal variations and drought effects on water potential, stomatal conductance and photosynthesis rate

The maximum stomatal conductance ( $g_{s_{\max}}$ ) and predawn leaf water potential ( $\Psi_{\text{IPD}}$ ) showed similar patterns in response to water availability in both species. High values of  $g_{s_{\max}}$  and  $\Psi_{\text{IPD}}$  occurred in spring, declined progressively during the summer drought and recovered in response to soil rewetting in autumn. However, the ability to maintain the stomata open under water stress differed between the two species. In summer (August and September) of the 3 years of this study, predawn leaf water potential and stomatal conductance were higher in *Q. ilex* than in *Q. suber*. These results are in agreement with those reported by David et al. (2007) for the same site and species. According to these authors, the better water status of *Q. ilex* during the summer drought can be ascribed to a deeper or more effective root system of *Q. ilex* compared with that of *Q. suber*, since tree density, climate, soil water and groundwater levels were similar in the experimental plots of the two species.

The maximum rate of photosynthesis ( $A_{\max}$ ) varied seasonally in both species, showing high values in spring and autumn. Over the course of the summer, as a consequence of

the multiple stresses (drought, high temperature and high light intensity), the observed decrease in  $\text{CO}_2$  assimilation was associated with a decrease in stomatal conductance and the apparent maximum carboxylation rate ( $V_{\text{c}_{\max}}(C_i)$ ).

The gradual decrease of  $g_s$  and  $A$  with increasing water deficit is a characteristic response of plants and one of the primary physiological responses to drought. The response of photosynthesis to drought in *Quercus* trees growing under natural conditions was reported in many studies (e.g., Faria et al. 1996, 1998, Salleo and Lo Gullo 1990, Damesin and Rambal 1995, Corcuera et al. 2002). A close relationship between  $A$  and  $g_s$ , as observed in this study, was also documented by others in the same species (Faria et al. 1998, Penñelas et al. 1998) and in other *Quercus* species (Damesin and Rambal 1995), reinforcing the importance of stomatal limitation to photosynthesis in these species.

The autumn recovery of  $A_{\max}$  that promoted high values of leaf soluble sugars, starch and TPU compared with summer shows the relevance of the evergreen habit for whole plant carbon assimilation following the summer drought and, therefore, for the accumulation of reserves in roots and stems.

### Maximum carboxylation rate ( $V_{\text{c}_{\max}}$ ) in drought and recovery

The analyses of sensitivity of  $V_{\text{c}_{\max}}$  in relation to  $g_m$  revealed that the major consequence of assuming an infinite  $g_m$  is that  $V_{\text{c}_{\max}}$  estimates from  $A/C_i$  curves (apparent  $V_{\text{c}_{\max}}$  or  $V_{\text{c}_{\max}}(C_i)$ ) are always lower than those estimated from  $A/C_c$  curves (actual  $V_{\text{c}_{\max}}$  or  $V_{\text{c}_{\max}}(C_c)$ ). To the best of our knowledge, there are no  $g_m$  values for leaves of mature tree of *Q. ilex* sp. *rotundifolia* or *Q. suber* under drought conditions in the literature. Therefore, estimates of actual  $V_{\text{c}_{\max}}$  for these cases were not possible. This is a crucial point to be studied in the



future, as the discrepancies between apparent and actual  $V_{c_{\max}}$  are larger with decreasing  $g_m$  (Niinemets et al. 2009). Moreover, recent studies in Mediterranean plants (Niinemets et al. 2005, 2009, Galmés et al. 2007a, 2007b) highlight the importance of  $g_m$  as a limiting factor in photosynthesis, particularly under water stress conditions. The  $V_{c_{\max}}$  ( $C_c$ ) values we estimated for *Q. ilex* (using  $g_m = 0.05 \text{ mol m}^{-2} \text{ s}^{-1}$ ) were in the same range as those obtained by Niinemets et al. (2005) for Mediterranean species such as *Q. ilex*, *Olea europea* and *Laurus nobilis* in leaves of 1.5-year-old trees. Besides, in the present study, the apparent values of maximum carboxylation rates ( $V_{c_{\max}}$  ( $C_c$ )), around  $50\text{--}60 \mu\text{mol m}^{-2} \text{ s}^{-1}$  for summer and autumn, were in the same range as those referred to for numerous sclerophyllous species ( $V_{c_{\max}}$ ,  $35\text{--}71 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ; see review by Wullschleger 1993). These include some reported for the same genus (*Quercus rubra* L. and *Quercus prinus* adult trees) with  $V_{c_{\max}}$  ranging from  $44$  to  $57 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , in Turnbull et al. (2002), or  $V_{c_{\max}}$  from  $30$  to  $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$  in *Q. suber* potted plants, in Maroco et al. (2002). Nevertheless, other published values for the same genus are much higher: Xu and Baldocchi (2003), for example, observed a maximum  $V_{c_{\max}}$  of  $127.3 \mu\text{mol m}^{-2} \text{ s}^{-1}$  in adult trees of blue oak (*Q. douglasii*) under environmental conditions similar to ours (see Baldocchi et al. 2010). In temperate-zone deciduous oaks, Dreyer et al. (2001) reported  $V_{c_{\max}}$  of  $87.7$  and  $90.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$  in *Quercus petraea* and *Quercus robur*, respectively, and Harley and Baldocchi (1995) reported  $78 \mu\text{mol m}^{-2} \text{ s}^{-1}$  in chestnut oak (*Q. prinus*).

In our study, the apparent maximum carboxylation rate ( $V_{c_{\max}}$ ) values were lowest in the driest summer of 1999 compared with 2000. The lower  $V_{c_{\max}}$  values that we found are in agreement with a reduced RuBisCo activity and leaf nitrogen concentration. The negative effect of prolonged drought conditions on RuBisCo activity has been reported by different authors in different species. These include work by Xu and Baldocchi (2003) in blue oak (*Q. douglasii*) and Maroco et al. (2002) in grapevine (*Vitis vinifera*). The increased biochemical limitation of the photosynthetic rate by drought may be ascribed to several other mechanisms besides a decrease in RuBisCo activity (Bota et al. 2004, Flexas et al. 2006, Chaves et al. 2009). These could include the inactivation of other Calvin cycle enzymes or a decrease in ATP and RuBP synthesis, although there is uncertainty as to which process is the most sensitive to stress (Lawlor 2002, Parry et al. 2002). Summer stress also induced a reduction in maximum electron transport capacity ( $J_{\max}$ ) in *Q. ilex* and *Q. suber*, as reported by Xu and Baldocchi (2003) for *Q. douglasii* and by Maroco et al. (2002) for *V. vinifera*.

In summer, leaves experienced high temperatures (they could attain values close to  $45 \text{ }^\circ\text{C}$ ) due to partial stomatal closure and consequently low transpiration rates. High temperatures, in addition to drought, promoted a reduction in the maximum photosynthetic rate. This occurred due to a reduction in  $V_{c_{\max}}$ ,  $J_{\max}$  and quantum yield ( $\Phi$ ) and also due to an increase in mitochondrial respiration (Rd). The same results were observed in other Mediterranean species

(Dreyer et al. 2001, Valladares and Niinemets 2007). The  $J_{\max}/V_{c_{\max}}$  ratio that we found ( $2.6\text{--}3.2$  in *Q. ilex* and  $2.3\text{--}2.6$  in *Q. suber*) suggests a tight coordination between electron transport and RuBisCo capacity, originating from a functional balance between RuBP consumption and RuBP regeneration (Wullschleger 1993, Xu and Baldocchi 2003). These  $J_{\max}/V_{c_{\max}}$  ratios are similar to those obtained in *Q. douglasii* (around 2.5) (Xu and Baldocchi 2003) and in *Q. prinus* and *Quercus alba* species (2.11 and 3.33, respectively) (Wilson et al. 2000).

The photosynthetic capacity of leaves, as estimated by  $V_{c_{\max}}$  and  $J_{\max}$ , recovered in autumn after seasonal soil rewetting to levels close to spring values. Recovery was observed in the leaves that actually displayed a decreased activity during summer; in other words, the leaves measured in the autumn belonged to the same cohort as the ones used during the previous summer. The  $V_{c_{\max}}$  is known to decline with ageing. However, in the present study, we observed an autumnal recovery of  $V_{c_{\max}}$  after the leaves had withstood summer stresses. This is likely to be related to the increase in N allocation to leaves in autumn and the increase in RuBisCo activity. In agreement with our results, Sabaté et al. (1995) reported a similar pattern in leaf nitrogen content for *Q. ilex*, with low values in summer and an increase in autumn. Bogeat-Triboulot et al. (2007) showed that recovery after water stress was accompanied by increases in some photosynthetic proteins, particularly RuBisCo and proteins of the water-splitting complex. The N allocation to the recovering leaves may result from an increase in recycling of stored N in perennial (branch) tissues (Cerasoli et al. 2004) or in older leaves (Castell et al. 1994, Cherbuy et al. 2001). Moreover, an increase in soil nitrogen availability and root uptake upon soil rewetting was observed in conditions similar to ours (Paul et al. 2003, Jarvis et al. 2007). One mechanism for the rapid increase in N uptake could be a tighter coupling between litter production and direct uptake of organic N from the litter by mycorrhizal fungi (Millard et al. 2007). The potential for mycorrhizal fungi to bypass litter decomposition by saprotrophs, by direct nutrient transfer from litter to tree roots, has been referred to (Chapman et al. 2006).

#### Concluding remarks

In summary, this study contributed to the literature by showing the capacity of survival of holm and cork oaks under Mediterranean summer drought conditions. Both *Q. ilex* and *Q. suber* exhibited a recovery of leaf water potential and stomatal conductance upon autumn rewetting after the summer drought, attaining similar values to those observed in spring, in the same cohort of leaves. Both tree species showed a remarkable capacity in recovering the photosynthetic rate. Accordingly, the activity of the carboxylation enzyme (RuBisCo) and the estimated maximum carboxylation rate ( $V_{c_{\max}}$ ) were higher in autumn than in the dry summer. The recovery of biochemical efficiency can be regarded as evidence of the high resilience of the photosynthetic apparatus.

tus under summer drought conditions in these species. In the future, it will be important to further understand the physiological features related to this recovery in these Mediterranean species, including the role played by the regulation of CO<sub>2</sub> diffusion through the leaf mesophyll.

### Acknowledgments

We thank I. Carcajeiro, J. Grilo and C. Grilo for their collaboration in the field work.

### Funding

This work was supported by the European Commission projects: MEDEFU (ENV4-CT98-0455).

### References

- Amon, D.I. 1949. Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24:1–15.
- Bogeat-Triboulot, M.B., M. Brosche, J. Renaut et al. 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiol.* 143:876–892.
- Baldocchi, D., S. Ma, S. Rambal, L. Misson, J.M. Ourcival, J.M. Limousin, J.S. Pereira and D. Papale. 2010. On the differential advantages of evergreenness and deciduousness in Mediterranean oak woodlands: a flux perspective. *Ecol. Appl.* (in press) doi 10.1890/08-2407.
- Bota, J., H. Medrano and J. Flexas. 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol.* 162:671–681.
- Castell, C., J. Terradas and J.D. Tenhunen. 1994. Water relations, gas exchange, and growth of sprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L. *Oecologia* 98:201–211.
- Centritto, M., F. Loreto and K. Chantzoulakis. 2003. The use of low [CO<sub>2</sub>] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant Cell Environ.* 26:585–594.
- Cerasoli, S., P. Maillard, A. Scartazza, E. Brugnoli, M.M. Chaves and J.S. Pereira. 2004. Carbon and nitrogen winter storage and remobilisation during seasonal flush growth in two-year-old cork-oak (*Quercus suber* L.) seedlings. *Ann. For. Sci.* 61:721–729.
- Chapman, S.K., J.A. Langley, S.C. Hart and G.W. Koch. 2006. Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *New Phytol.* 169:27–34.
- Chaves, M.M. and M.M. Oliveira. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J. Exp. Bot.* 55:2365–2384.
- Chaves, M.M., J.S. Pereira, J.P. Maroco, M.L. Rodrigues, C.P.P. Ricardo, M.L. Osório, I. Carvalho, T. Faria and C. Pinheiro. 2002. How plants cope with water stress in the field: photosynthesis and growth. *Ann. Bot.* 89:907–916.
- Chaves, M.M., J.P. Maroco and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. *Funct. Plant Biol.* 30:239–264.
- Chaves, M.M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103:551–560.
- Cherbuy, B., R. Joffre, D. Gillon and S. Rambal. 2001. Internal remobilization of carbohydrates, lipids, nitrogen and phosphorus in the Mediterranean evergreen oak *Quercus ilex*. *Tree Physiol.* 21:9–17.
- Corcuera, L., J.J. Camarero and E. Gil-Pelegrín. 2002. Functional groups in *Quercus* species derived from the analysis of pressure-volume curves. *Trees* 16:465–472.
- Damesin, C. and S. Rambal. 1995. Field study of leaf photosynthetic performance by a Mediterranean deciduous oak tree (*Quercus pubescens*) during a severe summer drought. *New Phytol.* 131:159–167.
- David, T.S., M.O. Henriques, C. Kruz-Besson et al. 2007. Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiol.* 27:793–803.
- Dreyer, E., X. Le Roux, P. Montpied, F.A. Daudet and F. Masson. 2001. Temperature response of leaf photosynthetic capacity in seedling from seven temperate tree species. *Tree Physiol.* 21:223–232.
- Ethier, G.J. and N.J. Livingston. 2004. On the need to incorporate sensitivity to CO<sub>2</sub> transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant Cell Environ.* 27:137–153.
- Faria, T., J.L. Garcia-Plazaola, A. Abadia, S. Cerasoli, J.S. Pereira and M.M. Chaves. 1996. Diurnal changes in photoprotective mechanisms in leaves of cork oak (*Quercus suber*) during summer. *Tree Physiol.* 16:115–123.
- Faria, T., D. Silvério, E. Breia, R. Cabral, A. Abadia, J. Abadia, J.S. Pereira and M.M. Chaves. 1998. Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species. *Physiol. Plant.* 102:419–428.
- Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta* 149:78–90.
- Flexas, J., J. Bota, J. Galmés, H. Medrano and M. Ribas-Carbo. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plant.* 127:343–352.
- Flexas, J., M. Ribas-Carbo, A. Diaz-Espejo, J. Galmes and H. Medrano. 2007. Mesophyll conductance to CO<sub>2</sub>: current knowledge and future prospects. *Plant Cell Environ.* 32:602–621.
- Galmés, J., H. Medrano and J. Flexas. 2007a. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol.* 175:81–93.
- Galmés, J., J. Flexas, R. Savé and H. Medrano. 2007b. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant Soil* 290:139–155.
- Giorgi, F. and P. Lionello. 2008. Climate change projections for the Mediterranean region. *Glob. Planet. Change* 63:90–104.
- Harley, P.C. and D.D. Baldocchi. 1995. Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. I. leaf model parameterization. *Plant Cell Environ.* 18:1146–56.
- Harley, P.C., J. Tenhunen and O.L. Lange. 1986. Use of an analytical model to study limitations on net photosynthesis in *Arbutus unedo* under field conditions. *Oecologia* 70:393–401.
- Jarvis, P.G., A. Rey, C. Petsikos et al. 2007. Drying and wetting of Mediterranean soils stimulates decomposition and carbon dioxide emission: the “Birch effect”. *Tree Physiol.* 27:929–940.
- Lawlor, D.W. 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Ann. Bot.* 89:1–15.
- Leegood, R.C. 1993. Carbon metabolism. *In* Photosynthesis and Production in a Changing Environment. Eds. D.O. Hall, J.M.O.

- Scurlock, H.R., Bolhar-Nordenkamp, R.C., Leegood and S.P. Long. Chapman and Hall, London, pp 247–267.
- Maroco, J.P., M.L. Rodrigues, C. Lopes and M.M. Chaves. 2002. Limitation to leaf photosynthesis in field-grown grapevine under drought - metabolic and modelling approaches. *Funct. Plant. Biol.* 29:451–459.
- Millard, P., M. Sommerkorn and G.A. Grelet. 2007. Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. *New Phytol.* 175:11–28.
- Miranda, P.M.A., M.A. Valente, A.R. Tomé, R. Trigo, M.F.E.S. Coelho, A. Aguiar and E.B. Azevedo. 2002. O clima de Portugal nos séculos XX e XXI. In *Alterações Climáticas em Portugal. Cenários, Impactes e Medidas de Adaptação*. Eds. F.D. Santos and P. Miranda. Gradiva, Lisboa, pp 45–113.
- Niinemets, Ü., A. Cescatti, M. Rodeghiero and T. Tosens. 2005. Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. *Plant Cell Environ.* 28:1552–1566.
- Niinemets, Ü., A. Cescatti, M. Rodeghiero and T. Tosens. 2006. Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species *Quercus ilex*. *Plant Cell Environ.* 29:1159–1178.
- Niinemets, U., A. Díaz-Espejo, J. Flexas, J. Galmés and C.R. Warren. 2009. Importance of mesophyll diffusion conductance in estimation of plant photosynthesis in the field. *J. Exp. Bot.* 60:2271–2282.
- Parry, M.A., P.J. Andraloj, S. Khan, P.J. Lea and A.J. Keys. 2002. Rubisco activity: effects of drought stress. *Ann. Bot.* 89:833–839.
- Paul, K.I., P.J. Polglase, A.M. O'Connell, J.C. Carlyle, P.J. Smethurst and P.K. Khanna. 2003. Defining the relation between soil water content and net nitrogen mineralization. *Eur. J. Soil Sci.* 54:39–48.
- Penúelas, J., I. Filella, J. Llusià, D. Siscart and J. Pinol. 1998. Comparative field study of spring and summer leaf gas exchange and photobiology of the mediterranean trees *Quercus ilex* and *Phillyrea latifolia*. *J. Exp. Bot.* 49:229–238.
- Pereira, J.S., G. Beyschlad, O.L. Lange, W. Beyschlad and J.D. Tenhunen. 1987. Comparative phenology of four mediterranean shrub species growing in Portugal. In *Plant Response to Stress: Functional Analysis in Mediterranean Ecosystems*. Eds. J.D. Tenhunen, F.M. Catarino, O.L. Lange and W.C. e Oechel. Springer-Verlag, Heidelberg, pp 503–513. NATO ASI Séries.
- Pereira, J.S., J.S. David, T.S. David, M.C. Caldeira and M.M. Chaves. 2004. Carbon and water fluxes in Mediterranean-type ecosystems: constraints and adaptations. *Prog. Bot.* 65: 467–498.
- Pereira, J.S., J.A. Mateus, L.M. Aires et al. 2007. Net ecosystem carbon exchange in three contrasting Mediterranean ecosystems. The effect of drought. *Biogeosciences* 4:791–802.
- Robyt, J.F. and B.J. White. 1990. *Biochemical techniques: theory and practice*. Waveland Press.
- Sabaté, S., A. Salla and C.A. Gracia. 1995. Nutrient content in *Quercus ilex* canopies: seasonal and spatial variation within a catchment. *Plant Soil* 168–169:297–303.
- Salleo, S. and M.A. Lo Gullo. 1990. Sclerophylly and plant water relations in three Mediterranean *Quercus* species. *Ann. Bot.* 65:259–270.
- Sharkey, T.D. 1985. Photosynthesis in intact leaves of C<sub>3</sub> plants: physics, physiology and rate limitations. *Bot. Rev.* 78:71–75.
- Sharkey, T.D., C.J. Bernacchi, G.D. Farquhar and E. Singaas. 2007. Fitting photosynthetic carbon dioxide response curves for C<sub>3</sub> leaves. *Plant Cell Environ.* 30:1035–1040.
- Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F. Schuster, K.J. Brown, V.C. Engel and K.L. Griffin. 2002. Photosynthetic characteristics in canopies of *Quercus rubra*, *Quercus prinus* and *Acer rubrum* differ in response to soil water supply. *Oecologia* 130:515–524.
- Valladares, F and Ü. Niinemets. 2007. The architecture of plant crowns: from design rules to light capture and performance. In *Handbook of Functional Plant Ecology*. Eds. FI Pugnaire and F Valladares. CRC Press, Boca Raton, FL, pp 101–149.
- Wilson, K.B., D.D. Baldocchi and P.J. Hanson. 2000. Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. *Tree Physiol.* 20:565–578.
- Wullschleger, S.D. 1993. Biochemical limitations to carbon assimilation in C<sub>3</sub> plants: a retrospective analysis of the A/ci curves from 109 species. *J. Exp. Bot.* 44:907–920.
- Xoplaki, E., J.F. Gonzalez-Rouco, J. Luterbacher and H. Wanner. 2004. Wet season Mediterranean precipitation variability: influence of large-scale dynamics and trends. *Clim. Dyn.* 23:63–78.
- Xu, L. and D.D. Baldocchi. 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiol.* 23:865.