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Original article

Stomatal and non stomatal limitation of photosynthesis by leaf water deficits in three oak species: a comparison of gas exchange and chlorophyll a fluorescence data

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Summary — Net CO_2 assimilation *(A)*, stomatal conductance for CO_2 *(g)*, intercellular mole fraction of CO_2 *(C_i)*, kinetics of chlorophyll a fluorescence, and their half decay time *($t_{1/2}$)*, their ratio of fluorescence decrease (R_{fd}), and their adaptive index *(A_p)* have been monitored on potted trees from 3 oak species (*Quercus petraea*, *Q pubescens* and *Q ilex*) grown in a climate chamber and submitted to drought. Use of *A vs* C_i representations for photosynthesis data revealed an apparent impairment of mesophyll photosynthesis, together with reduced CO_2 supply to mesophyll due to stomatal closure. But in all species chlorophyll a fluorescence kinetics displayed very similar shapes, constant $t_{1/2}$ and stable R_{fd} and A_p values until predawn leaf water potential dropped below –4.0 MPa. These observations led to the conclusion that photochemical energy conversion and photosynthetic carbon reduction cycle could be very resistant to leaf water deficits, and that observed decreases in mesophyll photosynthesis had to be attributed to a possible artefact in C_i calculation. On the other hand, the susceptibility of leaves to photoinhibition increased as a consequence of water shortage, especially in *Q petraea* and *Q pubescens*. Differences in drought adaptation between the studied species could probably be related to susceptibility to photoinhibition rather than to a direct sensitivity of photosynthesis to leaf water deficits, at least in the range of stress intensities of ecophysiological significance.

photosynthesis / water stress / chlorophyll a fluorescence / oak / stomatal conductance / drought / photoinhibition

Résumé — Limitation d'origine stomatique et non stomatique de la photosynthèse de trois espèces de chêne soumises à la sécheresse : comparaison de mesures d'échanges gazeux et de fluorescence de la chlorophylle. *Les échanges gazeux foliaires et la fluorescence de la*

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Abbreviations : *A* = net CO_2 assimilation rate; A_{max} = A at saturating Ci; A_p = adaptative index; C_i = intercellular CO_2 molar fraction; *dA/dCi* = carboxylation efficiency; F_p and F_t = maximal and terminal fluorescence levels; *g* = stomatal conductance for CO_2; *LWC* = leaf water content; P_i = inorganic phosphate; *PPFD* = photosynthetic photon flux density; *PSII* and *PSI* = photosystem II and I; R_{fd} = ratio of fluorescence decrease; $t_{1/2}$ = fluorescence half-decay time; α = apparent quantum yield of photosynthesis; ψ_{wp} = predawn leaf water potential; Δw : leaf to air water vapour molar fraction difference

chlorophylle ont été étudiés lors d'une sécheresse édaphique imposée en conditions contrôlées, sur de jeunes plants de Quercus patraea, Q pubescens *et* Q ilex. *L'analyse des relations entre assimilation nette de CO_2 (A) et fraction molaire intercellulaire calculée de CO_2 (C_i) semble indiquer que l'inhibition de A a résulté à la fois d'une fermeture des stomates, mais aussi d'une altération des processus mésophylliens de la photosynthèse. Par contre, la forme des cinétiques de fluorescence de la chlorophylle réalisées* in vivo *ainsi que les valeurs de $t_{1/2}$ (temps de demi décroissance), R_{fd} (rapport de décroissance de fluorescence) ou de A_p (index d'adaptation) n'ont pas été affectées tant que le déficit hydrique foliaire n'avait pas atteint un niveau élevé (potentiel hydrique de base inférieur à –4,0 MPa). Ceci semble indiquer une grande résistance de l'appareil photosynthétique au déficit hydrique foliaire. Par contre, l'étude de la réaction de la photosynthèse aux forts éclairements a révélé une sensibilité accrue à la photo-inhibition chez* Q petraea *et* Q pubescens *lors d'une sécheresse édaphique, contrairement à ce qui a été observé pour* Q ilex. *Les différences d'adaptation à la sécheresse existant en conditions naturelles entre ces 3 espèces pourraient être due à une sensibilité accrue à la photo-inhibition plutôt qu'à une sensibilité directe de l'appareil photosynthétique au déssèchement foliaire, du moins dans la gamme des déssèchements les plus fréquemment rencontrés en conditions naturelles.*

photosynthèse / stress hydrique / fluorescence / chêne / conductance stomatique / sécheresse / photo-inhibition

INTRODUCTION

European oak species grow in habitats differing widely in the frequence of drought occurrence. *Quercus petraea* (subgenus *Lepidobalanus* section *robur*), as a mesophytic mid European species is rather sensitive to water shortage, while *Q pubescens* (subgenus *Lepidobalanus* section *robur*) grows in much drier soils. *Q ilex* (*subgenus Lepidobalanus* section *ilex*), a Mediterranean sclerophyllous xerophyte, is sometimes submitted to long periods of water deficits accompanied by high levels of solar irradiance.

Differences in drought tolerance between species may be partly due to differential sensitivities of photosynthetic processes in leaves to tissue dehydration. But it is still unclear whether water shortage and resulting leaf water deficits have direct effect on the mesophyll processes of photosynthesis (photochemical energy conversion and/or carbon metabolism), or only indirect effects *via* stomatal closure and subsequent limitations of CO_2 diffusion to chloroplasts.

Some studies with chloroplastic suspensions or enzyme extracts have reported the occurrence of both reductions in photochemical processes (Boyer, 1976) and in ribulose-biphosphate carboxylase-oxygenase activity (Vu *et al,* 1987).

Leaf gas exchange measurements and analysis using diffusion models (Jones, 1973, 1985; Farquhar and Sharkey, 1982) have frequently led to the result that leaf water deficits impair both mesophyll ability to assimilate CO_2, and CO_2 diffusion to chloroplasts (Jones and Fanjul, 1983; Teskey *et al,* 1986; Cornic *et al,* 1987; Grieu *et al,* 1988). In these studies, net assimilation was analysed as a function of calculated intercellular CO_2 mole fraction (C_i); in almost all stress situations, reductions seemed to occur at fairly constant C_i values, therefore displaying both diffusional and biochemical limitations of photosynthesis (Jones, 1973, 1985; Cornic *et al,* 1983). However, recent results suggest that this model may be misleading, due to artefacts in C_i calculation (Terashima *et al,* 1988).

In order to test potential limitations induced by water stress on carbon assimila-

tion of leaves *in vivo* on our 3 oak species, we compared the results obtained with gas exchange measurements and with chlorophyll a fluorescence kinetics.

Chlorophyll a fluorescence kinetics, based on the Kautsky effect, allow the assessment to be made of possible impairments in:

– energy conversion at PSII level (variable fluorescence); and

– in the transfer of electrons from the first acceptors to the photosynthetic carbon reduction cycle (fluorescence decrease) (Krause and Weis, 1984; Briantais *et al,* 1986). In this study, we analysed the shapes of fluorescence decrease which is related to the onset of both photochemical and non photochemical quenching, and calculated the half decay time $t_{1/2}$, the ratio of fluorescence decrease (R_{fd}; Lichtenthaler *et al,* 1986) and an adaptative index reflecting the degree of integrity of photosynthetic membranes (A_p; Strasser *et al,* 1987). In addition, water stress often promotes susceptibility to photoinhibition (Krause, 1984). Susceptibility to photoinhibitory damages has therefore been compared in our species and related to the level of drought tolerance.

The aims of these experiments were to give an insight into the mechanisms of stress reactions, and to compare them in the 3 tree species known for their differences in drought tolerance.

MATERIAL AND METHODS

Plant material and growth conditions

The oak species studied were *Quercus petraea* Liebl (seed origin: Forêt Domaniale d'Amance, near Nancy, France), *Q ilex* L (seed origin: Mont Ventoux, near Avignon, France) and *Q pubescens* Willd (seed origin: Mont Ventoux).

Three-year-old (*Q pubescens* and *Q ilex*) or 4-year-old *(Q petraea)* saplings were grown in 7-l plastic pots on a 1:1 (v/v) mixture of brown peat and sandy soil, in a naturally illuminated greenhouse; they were fertilised 4 times a year during the growing season with a complete nutrient solution (N,P,K; 7,6,9; Solugene), and were watered twice a week with deionized water.

Experimental time course

One week before each experiment, the plants were transferred to a growth cabinet with following day/night conditions: 16/8 h; air temperature, 22/16 °C; relative humidity, 70/95 %. Photosynthetic photon flux density (*PPFD*) at the top of the plants was maintained at 300 μmol m^{-2} s^{-1} provided by neon lamps. Ambient CO_2 molar fraction averaged 475 ± 25 μmol mol^{-1}.

Measurements were performed during May 1989 for *Q pubescens*, June 1989 for *Q petraea* and July 1989 for *Q ilex*. For each species, 2 control saplings were watered daily and 4 or 5 plants were exposed to water shortage by withholding irrigation for about 20 d. Small amounts of water were added to the pots when needed, to avoid death of plants. Predawn leaf water potential, net CO_2 assimilation rate and chlorophyll fluorescence kinetics were studied 2 d a week for the water-stressed plants and only 1 d a week for the control. At the end of the stress period, a twig of 2 control and of 2 or 3 drought-stressed plants was exposed for 4 h to a *PPFD* of 2 000 μmol m^{-2} s^{-1} provided by a sodium lamp (SON-T-400W, Philips) in order to assay susceptibility to photoinhibition. An electric fan was used to prevent thermal injury to the leaves. Apparent quantum yield of photosynthesis (α) and chlorophyll fluorescence were used to quantify possible photoinhibitory effects. To investigate the effect of rapid dehydration on chlorophyll fluorescence kinetics, 20 leaf discs were punched from a twig of a well-watered plant of *Q petraea*. Five leaf discs were kept on a wet filter paper and 15 were submitted to dehydration in air for several h. This stress treatment was imposed in darkness at room temperature (≈ 20 °C).

Water relations

Predawn leaf water potential (ψ_{wp}) was measured using a pressure chamber. Leaf water content (*LWC*) was estimated after over-drying a leaf disk during 48 h at 60 °C. Each value of *LWC* is the mean of 3 replicates.

Gas exchange measurements

Whole leaf gas exchange was measured in an open system designed in the laboratory. Net CO_2 assimilation (*A*) and transpiration (*E*) rates were monitored with a differential infra-red gas analyser for both CO_2 and water vapour (Binos, Leybold Heraeus). Two or 3 leaves (*Q pubescens* and *Q petraea*) or ≈ 10 leaves (*Q ilex*) were enclosed in a 2-l assimilation chamber, in which air temperature (T_a), leaf-to-air water vapour molar fraction difference (Δw) and ambient CO_2 molar fraction (C_a) were controlled. A gas stream of 2 l min^{-1} was provided continuously and monitored by a mass flow controller. A fan homogenized the air inside the chamber. CO_2 molar fraction of the air in the chamber (C_a) was controlled by injecting pure CO_2 into the main flux of CO_2 free air. Air with a low oxygen concentration (1% O_2) was obtained when needed, from a mixture of 5% CO_2 free air + 95% N_2. Illumination provided from the growth cabinet was increased to 400 μmol m^{-2} s^{-1} with a sodium lamp (SON-T 400W, Philips), and monitored with a quantum sensor (Li 190SB, LiCor). Regulations and data acquisition were monitored by an application stored in a computer (AT3, IBM) *via* a data logger (SAM 80 AOIP). The means of 5 successive measurements were computed and stored every 10 s. Stomatal conductance for CO_2 (*g*) and intercellular CO_2 molar fraction (C_i) were calculated according to von Caemmerer and Farquhar (1981).

The following conditions prevailed in the assimilation chamber: T_a, 22 °C and Δw, 8 mmol mol^{-1}. During the establishment of (*A*, C_a) response curves, *PPFD* was maintained at 400 μmol m^{-2} s^{-1} and C_a was changed every 15 min from 950 to 800, 650, 500, 350, 200 and 50 μmol mol^{-1}. (*A*, C_a) response curves were run 45 min after illumination, and values of *A* and *g* were recorded at the end of the period at 350 μmol mol^{-1}. During the establishment of (*A*, *PPFD*) response curves, C_a was maintained at 950 μmol mol^{-1} in a 1% O_2 air and PPFD was changed every 30 min from 0 to 100, 200, 300 μmol m^{-2} s^{-1}. (*A*, *PPFD*) response curves were run before and 30 min after the high-illumination treatment.

As defined by Jones (1973, 1985), (*A*, C_i) response curves outline the mesophyll photosynthetic capacity (demand functions). The supply functions, defined as the lines with an x-axis intercept equal to $C_a[1 - E/(g + E/2)]$ and a negative slope equal to $-(g + E/2)$ (Guehl and Aussenac, 1987), give an estimate of diffusive limitations to CO_2 assimilation. Stomatal and mesophyll components of *A* limitation can be evaluated by considering the displacement of those 2 functions on the same (*A*, C_i) graph. The initial slope of the (*A*, C_i) response curve ($dA/d C_i$) was calculated as an estimate of carboxylation efficiency. Apparent quantum yield of photosynthesis (α) was computed as the initial slope of the (*A*, *PPFD*) response, obtained in a 1% O_2 air mixture to limit photorespiration.

Chlorophyll a fluoresence measurements

The slow induction transients of *in vivo* chlorophyll fluorescence were measured at room temperature with the apparatus described by Lichtenthaler and Rinderle (1988). Fluorescence of 30-min dark -adapted leaf disks was excited by an He-Ne laser (215, Spectra Physics; 5 mW, λ = 632.8 nm) using 1 arm of a 3-arm glass-fibre optic, and guided by the other arms to detecting photodiodes (SD 444-41-11-261, Silicon Detector Corp). The exciting red light at leaf surface amounted to ≈ 400 μmol m^{-2} s^{-1} (80 W m^{-2}). A red cut-off filter (Schott RG 665) was used to exclude excitation light and interference filters (Schott DAL, λmax 691 nm or 732.9 nm) were applied to sense the fluorescence induction kinetics simultaneously in the 690 or 735 nm spectral regions. Both fluorescence kinetics were recorded with a-2-channel recorder (BS316 W + W, Electronic Inc).

Fluorescence decrease was analysed using following indices: half decay time ($t_{1/2}$, *eg* the time needed to reach the level $(F_p - F_t)/2$, ratio of fluorescence decrease ($R_{fd} = (F_p - F_t)/F_t$) and stress adaptative index ($A_p = 1 - [(1 +$

R_{fd}735)/(1 + R_{fd} 690)]). All of these were computed from manual measurements on chart recordings. During drought stress each measurement was replicated 3 times, and made before onset of illumination. For the photoinhibition study, 2 chlorophyll fluorescence kinetics were recorded for each twig before high illumination treatment, 30 min after and 1 night later.

RESULTS

Plant water status

Predawn leaf water potential (ψ_{wp}) of all plants decreased rapidly after approximately 1 wk of water deprivation. Small amounts of water were added to maintain ψ_{wp} between –2.0 and –4.0 MPa. ψ_{wp} time-course was similar for *Q petraea* or *Q pubescens*, but displayed a steeper decrease for *Q ilex* (fig 1).

Leaf water content *(LWC)* was lower (45% approximately) in *Q ilex* leaves than in *Q petraea* or *Q pubescens* (60 and 55% respectively). Because of a high interindividual variability, no significant reduction in *LWC* could be observed during drought, excepted when ψ_{wp} decreased below –4.0 MPa. *LWC* then decreased to 45% *Q petraea* leaves, 40% in *Q pubescens* and 35% in *Q ilex*.

Effects of drought on net CO_2 assimilation (A), stomatal conductance (g) and (A, Ci) relationships

Both *A* and *g* decreased in response to decreasing ψ_{wp} (fig 2). The high interindividual variability observed at high ψ_{wp} was not due to variations in water status. Stomatal closure and inhibition of *A* started between –1.0 and –2.0 MPa in all tested species. *A* and *g* reached values near to zero when ψ_{wp} attained ≈ –3.0 MPa in *Q petraea*, and ≈ –4.0 MPa in *Q pubescens* and *Q ilex*.

During drought, *A* and *g* decreased in parallel, which led to a linear relationship and was an indication of a close coupling between both parameters (fig 3). But in well watered *Q ilex* and *Q pubescens* plants, this relationship did not remain linear at high conductances; in this case *A* was probably limited by other factors. The intial slopes *(S)* of these relationships, which give an estimate of instant water use

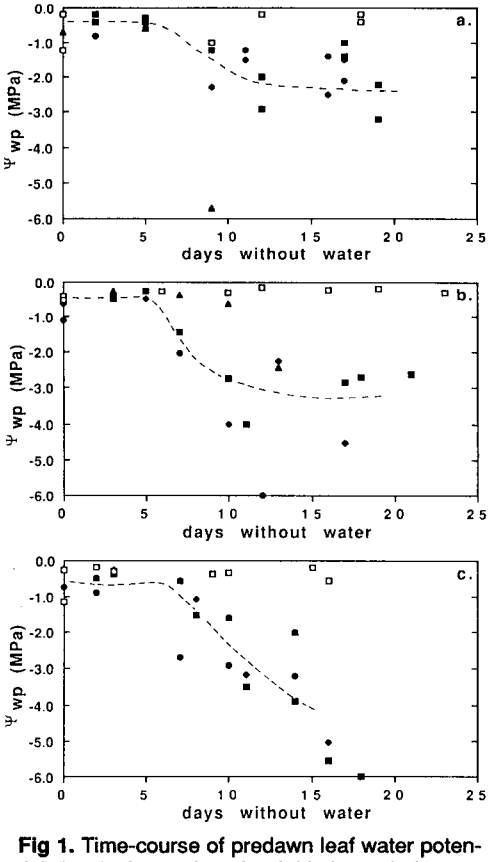

Fig 1. Time-course of predawn leaf water potential (ψ_{wp}) of 4–5 droughted (dark symbols; one for each individual) and 2 control (open symbols) saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c) during drought treatment (lines were eye–fitted).

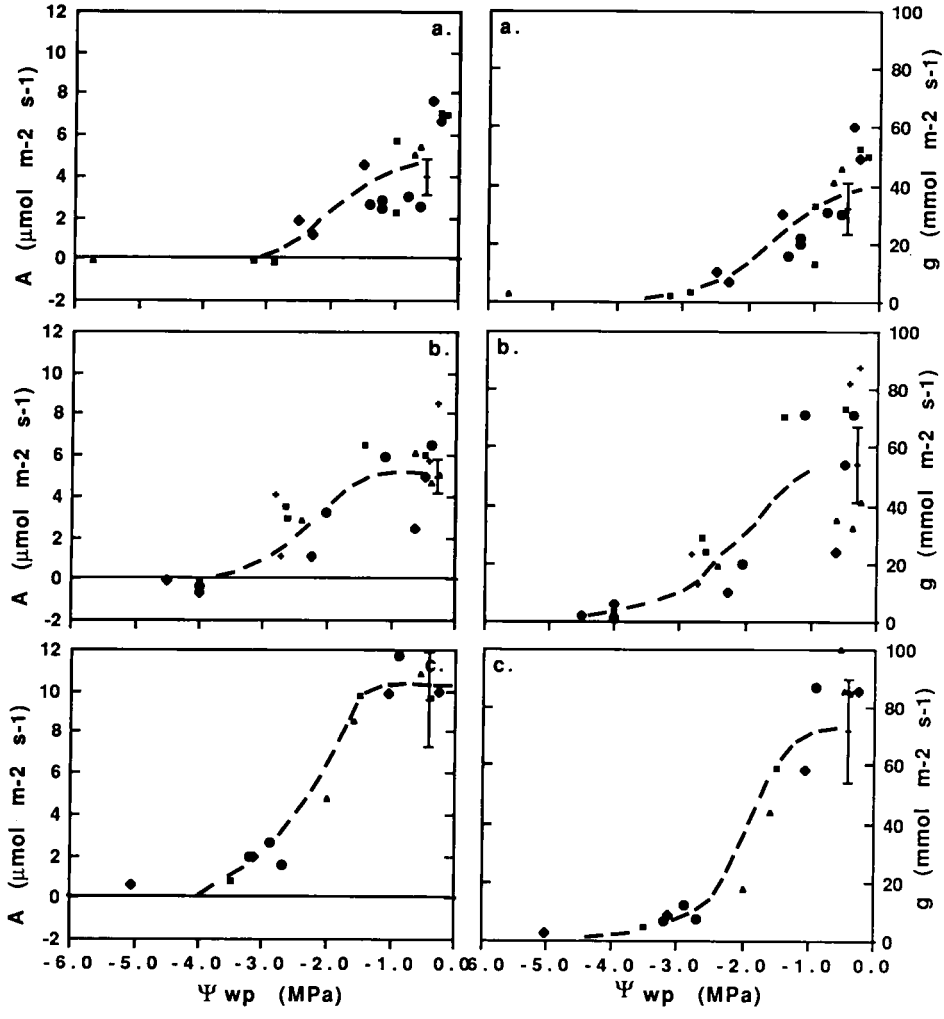

Fig 2. Net CO_2 assimilation rate (A) and stomatal conductance to CO_2 *(g)* of 4–5 droughted saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c), as a function of predawn leaf water potential *(ψ_{wp})*. Vertical bars indicate standard deviation of the mean of 5–6 replicates obtained with 2 control saplings (lines were eye–fitted).

efficiency under water shortage (Schulze and Hall, 1982), were 0.24 µmol•mmol^{-1} in *Q ilex*, and 0.13 and 0.15 in *Q petraea* and *Q pubescens*.

An example of *(A,C_i)* response curves obtained during drought development on *Q petraea* is shown in figure 4. Slopes of the supply functions were reduced due to stomatal closure with declining ψ_{wp}, but the demand functions were also modified, which could indicate that both stomatal and non stomatal factors contributed to the

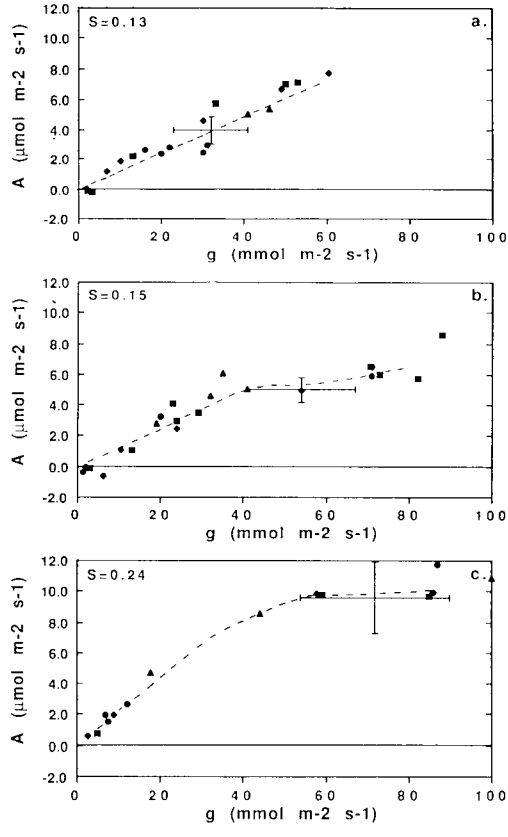

Fig 3. Relationship between net CO_2 assimilation rate (A) and stomatal conductance to CO_2 (g) during drought progression for 4–5 droughted saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c). Vertical and horizontal bars indicate standard deviation of the mean of 5 or 6 replicates obtained with 2 control saplings. Values of initial slopes of regression (S, µmol CO_2 $mmol^{-1}$ H_2O) are displayed (lines were eye-fitted).

drought induced decline in *A*. The maximal CO_2 assimilation rate (A_{max}) decreased first, as soon as *A* and *g* were inhibited. In contrast, the initial slope of the (A, C_i) response curves (dA/dC_i) remained constant until ψ_{wp} values fell to below ≈ –2.0 to –3.0 MPa. Nevertheless, we observed a close relationship between *A* at 350 µmol mol^{-1} and dA/dC_i during drought (fig 5).

Effects of drought on chlorophyll a fluorescence

All tested species displayed similar shapes for chlorophyll a fluorescence kinetics while well watered, with a fairly large inter-individual variability; *Q ilex* alone showed slightly lower values for R_{fd} (4–5), A_p (≈ 0.25) and higher $t_{1/2}$ (30 s instead of ≈ 15 s for both *Q petraea* and *Q robur*; see figs 6 and 7). These differences are probably related to the optical properties of the leaves; in fact, *Q ilex* leaves exhibit thicker cuticules and mesophyll tissues. For all 3 species, no effect of water stress could be observed on $t_{1/2}$, R_{fd} or A_p for ψ_{wp} values > –3.0 MPa for *Q petraea*, and –4.0 MPa for *Q pubescens*. With *Q ilex* a slight decrease was observed till –3.5 MPa for R_{fd} and A_p, but $t_{1/2}$ did not increase significantly with the exception of one case (figs 6 and 7). When stress became extremely severe, *ie* in 1 case at ψ_{wp} < –5.0 MPa for both *Q petraea* and *Q pubescens*, and in 3 cases < –4.0 MPa for *Q ilex*, $t_{1/2}$ increased strongly while R_{fd} decreased markedly, and A_p seemed less affected. Chlorophyll fluorescence kinetics as exemplified in figure 8a then displayed both a decrease in peak fluorescence (F_p) and an increase in steady state fluorescence (F_t).

Leaf discs were submitted to rapid dehydration *in vitro* in free air and obscurity (*LWC* was reduced from 70 to 30% in 5 h) to ensure that R_{fd}, A_p and $t_{1/2}$ could really be affected by strong stresses, and that the previously observed stability was not an artefact. In this case, both R_{fd} and A_p decreased markedly while $t_{1/2}$ increased strongly (fig 9). But an important difference appeared as compared to *in situ* dehydration: F_p level was not affected (fig 8b). Once again, A_p seemed to be less affected than R_{fd}, and a severe water loss was necessary to induce R_{fd} decrease.

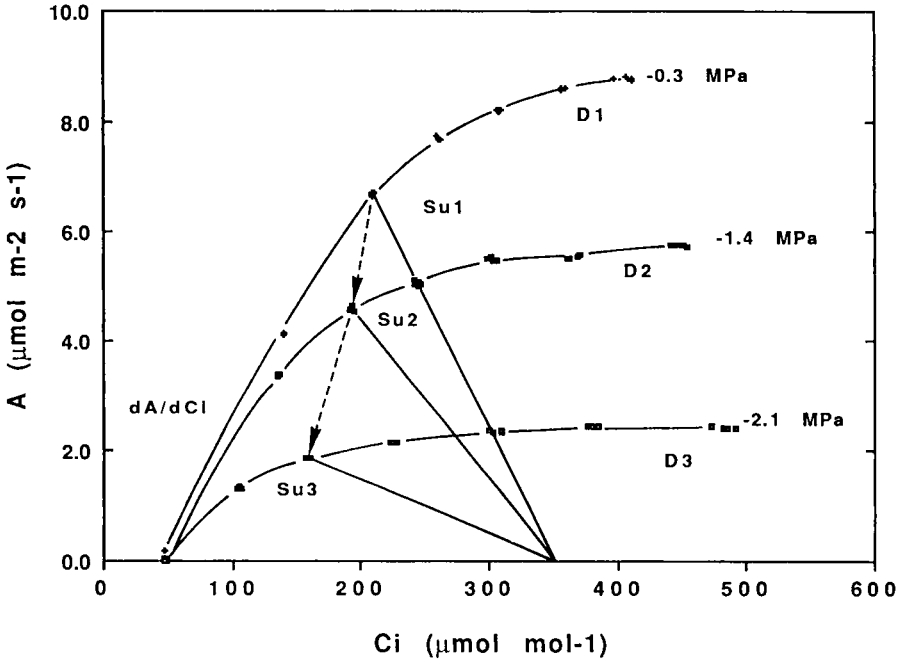

Fig 4. Net CO_2 assimilation rate (A) against intercellular CO_2 molar fraction *(C_i)* for a sapling of *Q petraea* at different levels of predawn leaf water potential (–0.3; –1.4; –2.1 MPA). $D_{(1,2,3)}$, demand functions; $Su_{(1,2,3)}$, supply functions; d*A*/d*Ci*, initial slope. Dashed arrows indicate the decrease due both to decreasing stomatal conductance and mesophyll photosynthetic capacity between 2 successive levels of ψ_{wp}.

Susceptibility to photoinhibition

Results of these experiments are presented in table I. High illumination treatments induced a decrease of the apparent quantum yield of photosynthesis (α). Well-watered plants of *Q petraea* displayed a larger decrease than *Q pubescens* and *Q ilex*. But, when drought was imposed, α was strongly reduced (> 70%) in *Q petraea* and *Q pubescens*. In contrast, *Q ilex* water-stressed plants exhibited approximately the same reduction in α as well-watered ones.

R_{fd} was strongly reduced in all species, excepted for well-watered *Q ilex*. Fluorescence kinetics exhibited a strong decrease in F_p level, but $t_{1/2}$ and the form of the fluorescence decrease were not affected (fig 8c). Recovery after 12 h of darkness following the high illumination treatment was less in water-stressed than in well-watered plants, especially in *Q pubescens*. Recovery was more pronounced in both control and stressed *Q ilex* saplings than in the other species.

DISCUSSION

Quercus ilex and *Q pubescens* exhibited similar decreases of net CO_2 assimilation

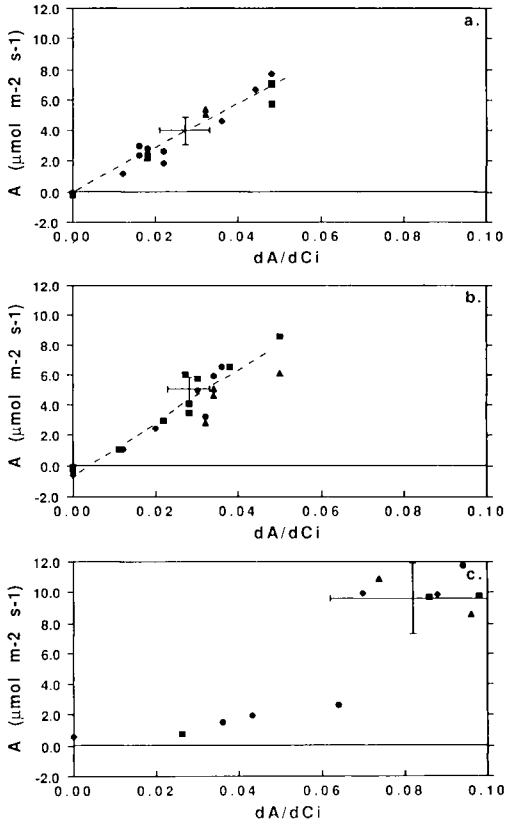

Fig 5. Relationship between the initial slope of *(A, C_i)* response curves (d*A*/d*C_i*) and net CO_2 assimilation rate at 350 µmol·mol^{-1} *(A)* during drought progression for 4–5 droughted saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c). Vertical and horizontal bars indicate standard deviation of the mean of 5–6 replicates obtained with 2 control saplings (lines were eye-fitted).

rate *(A)* and stomatal conductance for CO_2 (g) with increasing drought. Due to a large interindividual variability, no unequivocal difference in sensitivity could be detected, even if *Q petraea* showed earlier responses to decreasing ψ_{wp}. In *Q ilex*, decreases in *A* and *g* were steep, with higher initial values, but the overall evolution was

Table I. Apparent quantum yield of photosynthesis (α) measured as the initial slope of *(A, PPFD)* curves, 30 min after photoinhibitory treatment, and ratios, of fluorescence decrease measured 0.5 and 12 h ($R_{fd\,0.5\,h}$ and $R_{fd\,12\,h}$) after photoinhibitory treatment on control and water-stressed ($\psi_{wp} \approx -2.0$ MPa in *Q petraea*, –3.0 MPa in *Q pubescens* and *Q ilex*) plants, expressed as % of their initial values. Data are means of 2 saplings (3 for *Q ilex* stressed plants). R_{fd} was recorded twice on each sapling.

Species	*Treatment*	*α (%)*	$R_{fd\,0.5\,h}$ *(%)*	$R_{fd\,12\,h}$ *(%)*
Q petraea	Control	42	34	78
	Stressed	27	38	62
Q pubescens	Control	68	36	69
	Stressed	28	37	38
Q ilex	Control	66	60	94
	Stressed	60	44	87

not very different from that of the previous species. During the entire experiment a close coupling was observed between decreases in *A* and *g*. Parallel decreases in *A* and *g* in response to decreasing ψ_{wp} have often been reported (Wong *et al*, 1985; Teskey *et al*, 1986; Di Marco *et al*, 1988). *A/g* increased during drought progression, and reached constant values with a higher water use efficiency (d*A*/d*g*) for *Q ilex* than for *Q petraea* or *Q pubescens* under limited water supply.

Alteration of *(A, C_i)* relationships showed that apparently both stomatal and non stomatal factors contributed to the limitation of *A*. The maximal rate of net CO_2 assimilation at high C_i *(A_{max})* was first affected. According to von Caemmerer and Farquhar (1981) and Farquhar and Sharkey (1982), this could mean a decrease in the rate of regeneration of ribulose 1,5 bisphosphate (RUP_2) which could be limited by reduced photophosphorylation associa-

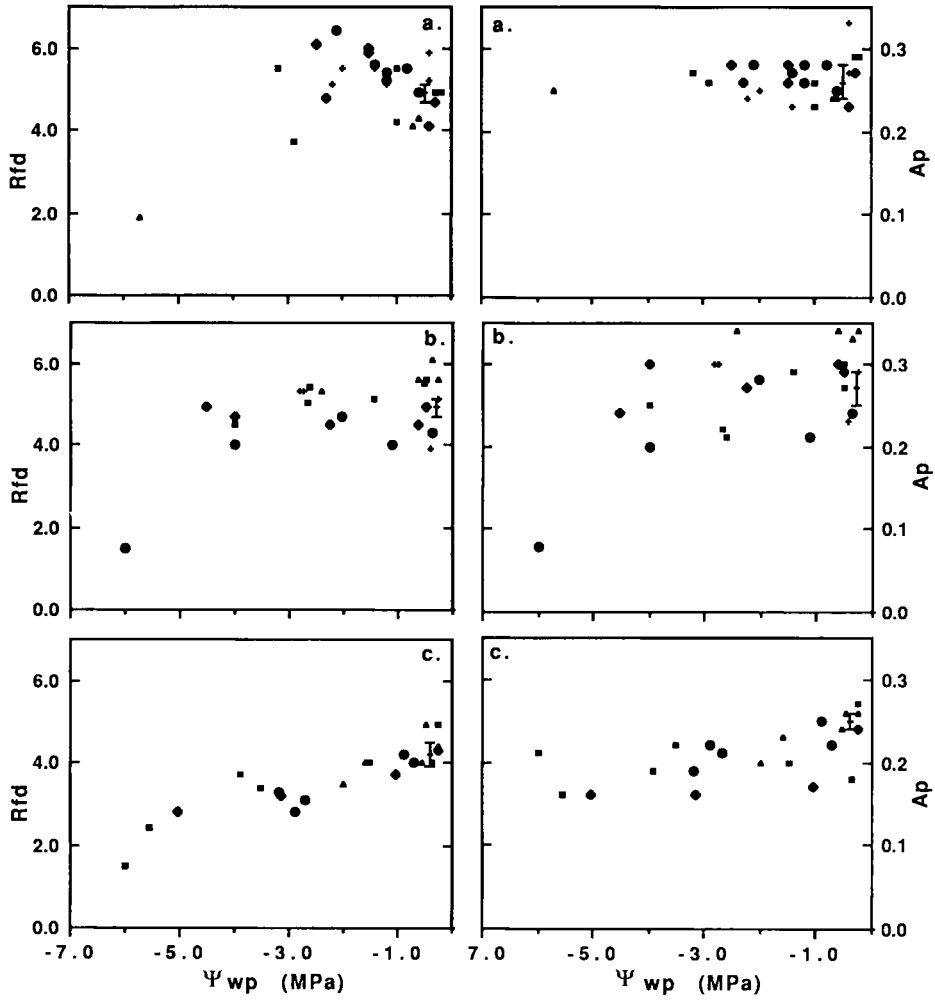

Fig 6. Ratio of fluorescence decrease *(R_{fd})* and adaptation index *(A_p)* of 4–5 droughted saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c), expressed as a function of predawn leaf water potential *(ψ_{wp})*. Each point is the mean of 3 replicates on 1 sapling and vertical bars indicate standard deviation of the mean obtained with 18–24 replicates on 2 control saplings.

ted with electron transport, or by a starvation in stromal P_i (Sharkey, 1985). The decrease in dA/dC_i could result from a decrease in carboxylation efficiency (von Caemmerer and Farquhar, 1981). Earlier results showed similar alterations in *(A, C_i)* relationships (Jones and Fanjul, 1983; Teskey *et al,* 1986; Ögren and Öquist, 1985; Kirschbaum, 1987; Cornic *et al,* 1987; Grieu *et al,* 1988). Farquhar and Sharkey (1982) have also reported that the first effects of water stress were a reduction of A_{max}, while dA/dC_i was initially unaffected.

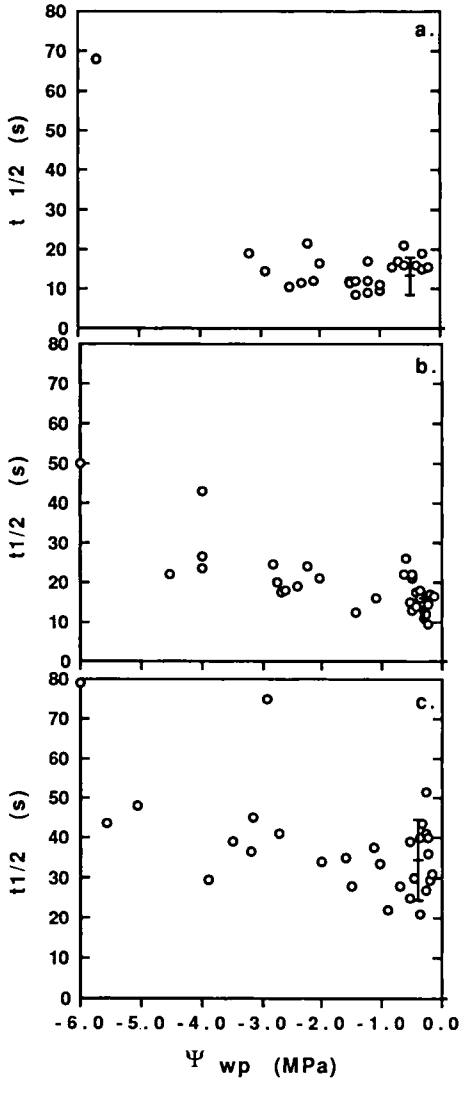

Fig 7. Fluoresence half decay time from F_p to F_t level ($t_{1/2}$) as measured on 4–5 droughted saplings of *Q petraea* (a), *Q pubescens* (b), and *Q ilex* (c), as a function of predawn leaf water potential (ψ_{wp}). Each point is the mean of 3 replicates on 1 sapling and vertical bars indicate standard deviation of the mean obtained with 18–24 replicates on 2 control saplings.

In order to obtain additional information on this apparent mesophyll limitation of net CO_2 assimilation, we studied the decrease of *in vivo* fluorescence during the onset of drought. Surprisingly, half decay time ($t_{1/2}$), ratio of fluorescence decrease (R_{fd}) and stress adaptation index (A_p) were not affected by drought until ψ_{wp} reached values < –4.0 MPa, that is well below turgor loss in these species (–2.0 in *Q petraea*, –2.8 in *Q pubescens* and –2.4 in *Q ilex* under similar conditions; Dreyer *et al*, 1990). The absence of an effect of water stress on the initial rise in fluorescence has been frequently reported (Ögren and Öquist, 1985; Genty *et al*, 1987; Toivonen and Vidauer, 1988; di Marco *et al*, 1988), indicating that PSII photochemistry is quite resistant to leaf water deficits. The absence of a decrease in F_p levels in relation to water stress which we observed is in agreement with this view. The evolution of R_{fd} and A_p under leaf water stress has seldom been documented; however, Schwab *et al* (1989) showed the stability of R_{fd} in *Spinacia oleracea* and in resurrection plants until relative water content declined to 40%. In addition to the same R_{fd} stability we also observed a remarkably constant half decay time ($t_{1/2}$). In fact, the decrease in fluorescence following the F_p peak results both from photochemica quenching (Q_p) and non photochemical quenching (Q_{np}). The former is due to reoxidation of the primary electron acceptor of PSII during the onset of carbon reduction, and the latter results largely from thermal deexcitation of PSII associated with the building up of transthylakoidal proton gradients and to a lesser extent from the transfer of excitation energy from PSII to PSI (Krause and Weis, 1984; Briantais *et al*, 1986; Krause *et al*, 1988). The remarkable stability of both R_{fd} and $t_{1/2}$ observed in our experiments could be an argument in favour of a stability of both Q_p and Q_{np}. This hy-

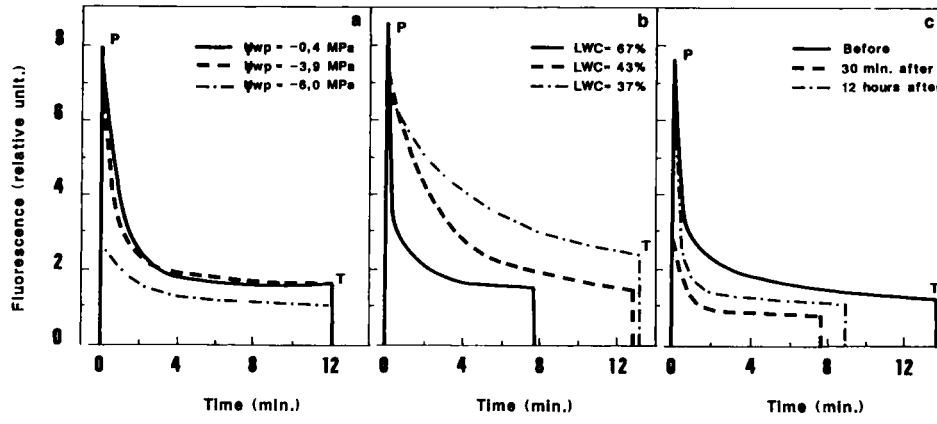

Fig 8. Chlorophyll fluorescence transients measured after 30 min of dark acclimation, at 690 nm of leaf disks; a) during *in situ* water shortage in *Q pubescens;* b) during rapid dehydration of *Q petraea* leaf disk; and c) before, 0.5 or 12 h after high light treatments in *Q pubescens.* P and T respectively indicate F_p and F_t levels.

pothesis is in agreement with the observations of Stuhlfauth *et al,* 1988 (with *Digitalis lanata*). Constancy of these parameters implies a stability of both the electron flow from PSII to the primary acceptors, and the intensity of thermal de-excitation of PSII. Electron flow could be maintained at low values of C_i through photorespiratory CO_2, recycling (Osmond *et al,* 1980; André, 1986). A reduction in the initial slope of F_p to F_t decline (*ie* an increase in $t_{1/2}$) was observed during drought with higher illuminations by Di Marco *et al,* 1988 (with *Triticum durum*); Genty *et al,* 1987 (with *Gossypium hirsutum*); Ögren and Öquist, 1985 (with *Salix* sp); Epron and Dreyer (unpublished observations with *Populus* sp). The stability we obtained with our oak species may therefore not be a general feature under different conditions and in other species.

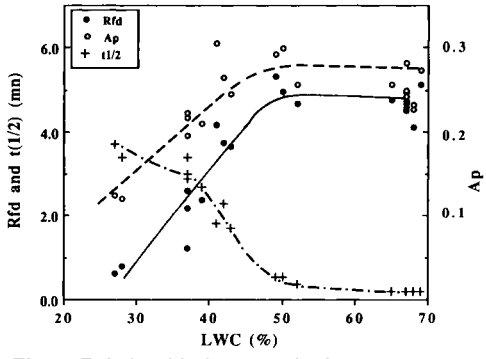

Fig 9. Relationship between leaf water content (LWC) of *in vitro* drying leaf disks of *Q petraea* and ratio of fluorescence decrease (R_{fd}, dark symbols), adaptation index (A_p, open symbols) and half decay time from F_p to F_t level ($t_{1/2}$) during rapid dehydratation in air and obscurity. Each point represents an individual value (lines were eye-fitted).

The results obtained from gas exchange and chlorophyll fluorescence studies therefore appear contradictory:

– the evolution of (A, C_i) relationship indicated the appearance of mesophyll limitations of photosynthesis during drought; and

– conversely, fluorescence data showed the absence of any major impairment in

photosynthetic apparatus during leaf water deficit. According to Terashima *et al* (1988), values of C_i could be overestimated if patchy stomatal closure occurred in water-stressed leaves. Non uniform stomatal closure has been reported in response to ABA application in *Helianthus annuus*, *Vitis vinifera* and *Vicia faba* (Downton *et al*, 1988a; Terashima *et al*, 1988) and in response to water stress in *Vitis vinifera*, *Nerium oleander*, *Eucalyptus pauciflora* and *Phaseolus vulgaris* (Downton *et al*, 1988b; Sharkey and Seeman, 1989). If C_i values were overestimated, dA/dC_i and A_{max} would be underestimated and the apparent non-stomatal inhibition of photosynthesis would be an artefact. Using another method, Kaiser (1987) and Cornic *et al* (1989) showed that apparent quantum yield and maximal rate of photosynthetic O_2 evolution measured with a CO_2 concentration of up to 5% which overcame diffusive resistance did not decline with water stress until there was a severe water loss (20–40%), indicating a high resistance of the photosynthetic apparatus. Patchy stomatal closure has not yet been studied in water-stressed oak leaves. Anyway, our results seem to indicate that the mesophyll photosynthetic capacity is rather insensitive to drought stress in the 3 oak species and that observed inhibition of net CO_2 assimilation seemed to be related mostly to stomatal closure and limitations of CO_2 diffusion into the leaves, at least during the first stages of dehydration.

When drought stress became more severe ($\psi_{wp} < -4.0$ MPa), both R_{fd} and A_p decreased and $t_{1/2}$ increased, indicating possible damage to the photosynthetic apparatus. The same results were obtained with leaf discs of *Q petraea* submitted to rapid dehydratation in air. After large water losses, F_t level and F_p to F_t half decay time ($t_{1/2}$) increased. However, F_p levels were not affected by a rapid *in vitro* dehydratation of leaf discs, while they showed strong reductions during a severe drought stress *in situ*. As high light treatments induced a decline in F_p levels (fig 8c), we suggest that photoinhibitory damage could have arisen when severe water stress was imposed on our saplings *in situ* and after carbon reduction was impaired. During leaf disc dehydration, carbon reduction was also impaired but water stress was very rapidly imposed in darkness. Kaiser (1987) has suggested that the inhibition of stromal enzymes by increasing electrolyte concentrations or by extremely high protein concentrations induced impairment of carbon reduction during severe drought stress, but that high irradiance density could be responsible for photoinhibitory damages under natural drought conditions.

Because we could not observe any alteration in the fluorescence kinetics over the entire ecophysiologically significant range of ψ_{wp} (*ie*, between 0 and –4.0 MPa), it appears that our plants did not suffer from photoinhibition during imposition of water stress under our light conditions. Powles *et al* (1984) have shown that maintenance of a minimal level of carbon reduction (by photorespiratory CO_2 recycling) prevents photoinhibition in leaves.

In leaves exposed to drought, photoinhibition of photosynthesis by high light treatments was more pronounced, especially in *Q petraea* and *Q pubescens*, as has been previously reported for *Salix* sp leaves (Ögren and Öquist, 1985). The decrease in the apparent quantum yield of net CO_2 assimilation and of F_p levels of chlorophyll fluorescence kinetics show that electron transport, and particularly PSII activity were inhibited (Powles, 1984). Recovery after photoinhibition was lower after 12 h in *Q petraea* and *Q pubescens* water-stressed leaves. As recovery from photoinhibition is known to be partly due to protein synthesis in the chloroplasts (Greer *et al*,

1986; Legouallec and Cornic, 1988), we suggest that the lesser extent of recovery in water-stressed leaves of *Q petraea* and *Q pubescens* may result from inhibition of protein synthesis during water stress. *Q ilex* leaves appeared to be less sensitive to high light treatments because they recovered even when drought stressed, perhaps because of protective mechanisms which would enhance thermal dissipation of excess light energy (Demmig *et al*, 1987; Krause, 1988). In addition, it is possible that the ratio of absorbed *PPFD* to incident *PPFD* is lower in *Q ilex* leaves because of adaptations in leaf morphology and anatomy (higher leaf and cuticule thickness). Clearly, as differences in susceptibility to photoinhibition associated with water stress may play a major role as an adaptative mechanism to drought under natural conditions in forest ecosystems, further studies are required to document their occurrence.

In conclusion, the differences in sensitivity to drought between the 3 oak species studied do not seem to rely on a direct sensitivity of the photosynthetic apparatus to leaf water deficit. There is evidence for an increase of the instantaneous water use efficiency during drought progression in *Q pubescens* and *Q ilex*, and instantaneous water use efficiency was higher in *Q ilex* both in well watered and in drought-exposed leaves. However, the better adaptation of *Q ilex* under natural drought conditions could be mainly related to its lower susceptibility to photoinhibition, even during water shortage.

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