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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

D Epron, E Dreyer *

INRA-Nancy, Laboratoire de Bioclimatologie et d'Écophysiologie Forestières, Station de Sylviculture et Production, Champenoux, F-54280 Seichamps, France

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Summary — Net CO₂ assimilation (A), stomatal conductance for CO₂ (g), intercellular mole fraction of CO₂ (Ci), kinetics of chlorophyll a fluorescence, and their half decay time (t₁/₂), their ratio of fluorescence decrease (Rfd), 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 Ci representations for photosynthesis data revealed an apparent impairment of mesophyll photosynthesis, together with reduced CO₂ supply to mesophyll due to stomatal closure. But in all species chlorophyll a fluorescence kinetics displayed very similar shapes, constant t₁/₂ and stable Rfd 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 Ci 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

* Correspondence and reprints.

Abbreviations : A = net CO₂ assimilation rate; A_max = A at saturating Ci; A_p = adaptative index; Ci = intercellular CO₂ molar fraction; dA/dCi = carboxylation efficiency; F_p and F_t = maximal and terminal fluorescence levels; g = stomatal conductance for CO₂; LWC = leaf water content; P_i = inorganic phosphate; PPFD = photosynthetic photon flux density; PSI and PSII = photosystem II and I; Rfd = ratio of fluorescence decrease; t₁/₂ = 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
European oak species grow in habitats differing widely in the frequency 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 CO₂ 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 CO₂, and CO₂ 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 CO₂ mole fraction (C𝑖); in almost all stress situations, reductions seemed to occur at fairly constant C𝑖 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 C𝑖 calculation (Terashima et al, 1988).

In order to test potential limitations induced by water stress on carbon assimila-
tion of leaves \textit{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 \textit{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 ($R_{Iq}$; Lichtenthaler \textit{et al}, 1986) and an adaptive index reflecting the degree of integrity of photosynthetic membranes ($A_p$; Strasser \textit{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.

\textbf{MATERIAL AND METHODS}

\textbf{Plant material and growth conditions}

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

Three-year-old (\textit{Q pubescens} and \textit{Q ilex}) or 4-year-old (\textit{Q petraea}) saplings were grown in 7-I 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.

\textbf{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 \textit{Q pubescens}, June 1989 for \textit{Q petraea} and July 1989 for \textit{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 ($\alpha$) 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 \textit{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 ($\psi_{wp}$) 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 ($\Delta w$) 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 $\Delta w$, 8 mmol mol$^{-1}$. During the establishment of ($A$, $C_a$) response curves, PPFD 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 ($dA/dC_i$) was calculated as an estimate of carboxylation efficiency. Apparent quantum yield of photosynthesis ($\alpha$) was computed as the initial slope of the ($A$, PPFD) response, obtained in a 1% O$_2$ air mixture to limit photorespiration.

Chlorophyll a fluorescence 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, $\lambda$ = 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, $\lambda_{\text{max}}$ 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 the 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 ($R_{1d} = (F_p - F_t)/F_t$) and stress adaptive index ($A_p = 1 - [(1 +...
RESULTS

Plant water status

Predawn leaf water potential ($\psi_{wp}$) of all plants decreased rapidly after approximately 1 wk of water deprivation. Small amounts of water were added to maintain $\psi_{wp}$ between $-2.0$ and $-4.0$ MPa. $\psi_{wp}$ 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 $\psi_{wp}$ 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 $\psi_{wp}$ (fig 2). The high interindividual variability observed at high $\psi_{wp}$ 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 $\psi_{wp}$ 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 initial slopes (S) of these relationships, which give an estimate of instant water use
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,Cᵢ)* 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*ₚₑ, but the demand functions were also modified, which could indicate that both stomatal and non-stomatal factors contributed to the
drought induced decline in $A$. The maximal CO$_2$ assimilation rate ($A_{\text{max}}$) decreased first, as soon as $A$ and $g$ were inhibited. In contrast, the initial slope of the ($A$, $C$) response curves ($dA/dC_i$) remained constant until $\psi_{wp}$ values fell to below $\approx -2.0$ 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 $R_{id}$ (4–5), $A_p$ (= 0.25) and higher $t_{1/2}$ (30 s instead of $\approx 15$ 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 cuticles 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 $\psi_{wp}$ 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 $R_{id}$ 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, i.e., in 1 case at $\psi_{wp} < -5.0$ MPa for both $Q$ petraea and $Q$ pubescens, and in 3 cases $<-4.0$ MPa for $Q$ ilex, $t_{1/2}$ increased strongly while $R_{id}$ 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 ($F_s$).

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 $t_{1/2}$ did not increase significantly (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 $R_{id}$, and a severe water loss was necessary to induce $R_{id}$ decrease.
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 (\(\alpha\)). Well-watered plants of *Q petraea* displayed a larger decrease than *Q pubescens* and *Q ilex*. But, when drought was imposed, \(\alpha\) was strongly reduced (> 70%) in *Q petraea* and *Q pubescens*. In contrast, *Q ilex* water-stressed plants exhibited approximately the same reduction in \(\alpha\) 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
rate (A) and stomatal conductance for CO2 (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 ψwp. In Q ilex, decreases in A and g were steep, with higher initial values, but the overall evolution was 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 ψwp 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 (dA/dg) for Q ilex than for Q petraea or Q pubescens under limited water supply.

Alteration of (A, Cj) relationships showed that apparently both stomatal and non stomatal factors contributed to the limitation of A. The maximal rate of net CO2 assimilation at high Ci (Amax) 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 (RUP2) which could be limited by reduced photophosphorylation associa-

**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_{t0.5} and R_{t12}) after photoinhibitory treatment on control and water-stressed (ψwp = -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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>α (%)</th>
<th>R_{t0.5} (%)</th>
<th>R_{t12} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q petraea</td>
<td>Control</td>
<td>42</td>
<td>34</td>
<td>78</td>
</tr>
<tr>
<td>Q petraea</td>
<td>Stressed</td>
<td>27</td>
<td>38</td>
<td>62</td>
</tr>
<tr>
<td>Q pubescens</td>
<td>Control</td>
<td>68</td>
<td>36</td>
<td>69</td>
</tr>
<tr>
<td>Q pubescens</td>
<td>Stressed</td>
<td>28</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>Q ilex</td>
<td>Control</td>
<td>66</td>
<td>60</td>
<td>94</td>
</tr>
<tr>
<td>Q ilex</td>
<td>Stressed</td>
<td>60</td>
<td>44</td>
<td>87</td>
</tr>
</tbody>
</table>
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 ($\psi_{wp}$). 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.
In order to obtain additional information on this apparent mesophyll limitation of net CO₂ assimilation, we studied the decrease of *in vivo* fluorescence during the onset of drought. Surprisingly, half decay time (t₁/₂), ratio of fluorescence decrease (Rfd) and stress adaptation index (Ap) were not affected by drought until ψwp 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 Fp levels in relation to water stress which we observed is in agreement with this view. The evolution of Rfd and Ap under leaf water stress has seldom been documented; however, Schwab et al (1989) showed the stability of Rfd in Spinacia oleracea and in resurrection plants until relative water content declined to 40%. In addition to the same Rfd stability we also observed a remarkably constant half decay time (t₁/₂). In fact, the decrease in fluorescence following the Fp peak results both from photochemical quenching (Qp) and non photochemical quenching (Qnp). 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 de-excitation 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 Rfd and t₁/₂ observed in our experiments could be an argument in favour of a stability of both Qp and Qnp. This hy-

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**Fig 7.** Fluorescence half decay time from Fp to Ft level (t₁/₂) 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 (ψwp). 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.
hypothesis 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 (i.e., 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.

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 dehydration in air. After large water losses, $F_0$ level and $F_p$ to $F_0$ half decay time ($t_{1/2}$) increased. However, $F_p$ levels were not affected by a rapid in vitro dehydration 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 ecophysiological range of $\psi_{wp}$ (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,
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 cuticle thickness). Clearly, as differences in susceptibility to photo inhibition associated with water stress may play a major role as an adaptive 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 photo inhibition, even during water shortage.

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