



HAL
open science

Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species and during drought

O Roupsard, P Gross, E Dreyer

► **To cite this version:**

O Roupsard, P Gross, E Dreyer. Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species and during drought. *Annales des sciences forestières*, 1996, 53 (2-3), pp.243-254. hal-00883047

HAL Id: hal-00883047

<https://hal.science/hal-00883047>

Submitted on 11 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Limitation of photosynthetic activity by CO₂ availability in the chloroplasts of oak leaves from different species and during drought

O Roupsard, P Gross, E Dreyer*

Équipe bioclimatologie et écophysiologie, unité d'écophysiologie forestière, Centre de Nancy, Inra, 54280 Champenoux, France

(Received 2 November 1994; accepted 26 June 1995)

Summary — It has recently been suggested that the low photosynthesis rates in tree species as compared to highly productive crops is at least partly due to resistances opposing the CO₂ fluxes in the mesophyll of tree leaves. To validate this assertion, values of CO₂ mole fractions in the chloroplasts of leaves from *Quercus petraea*, *Q. robur*, *Q. ilex* and *Populus* sp were estimated on the basis of the analysis of the partitioning of light driven electron flow between fractions used for the carboxylation or the oxygenation of RuBP by Rubisco. The procedure used included: i) a measure of total light driven electron flows derived from the chlorophyll a fluorescence ratio $\Delta F/F_m'$, which is proportional to the photochemical efficiency of PS II, multiplied by incident irradiance and a calibration coefficient; ii) an estimation of the electron flux devoted to carboxylation obtained from net CO₂ assimilation and respiration rate measurement, and using the known electron requirements (four electrons for CO₂ or O₂ fixation); iii) the derivation of the CO₂ mole fraction in the chloroplasts from the specificity factor of Rubisco, and the ratio of carboxylation/oxygenation of RuBP. Results showed that in the absence of drought stress, the mole fraction of CO₂ in the chloroplasts (35–45% of the atmospheric one) was much lower than the calculated substomatal one (60–70% of the atmospheric) in all species. Moreover, lowest values were

* Correspondence and reprints: dreyer@nancy.inra.fr

Abbreviations: *A*: net CO₂ assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *A*_{1%}: net CO₂ assimilation under nonphotorespiratory (1% O₂) conditions; *R*_q: nonphotorespiratory respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *g*_{s+b}: leaf conductance to CO₂ ($\text{mmol m}^{-2} \text{s}^{-1}$); *g*_s: stomatal conductance to CO₂ ($\text{mmol m}^{-2} \text{s}^{-1}$); *c*_a, *c*_i, *c*_c: mole fractions of CO₂ in the free atmosphere, in the substomatal spaces and in the chloroplast stroma, respectively ($\mu\text{mol mol}^{-1}$); *c*_{cl} and *c*_{ci}: liquid phase concentrations of CO₂ and O₂ in the chloroplast stroma ($\mu\text{mol l}^{-1}$); *g*_m: mesophyll conductance to CO₂ (ie, from the substomatal spaces to the chloroplast stroma, $\text{mmol m}^{-2} \text{s}^{-1}$); *F*_m' and *F*: maximal and steady-state fluorescence in the presence of actinic light; Φ_{II} : photochemical efficiency of PS II; Φ_{e} : apparent quantum yield of light-driven electron flow; *PPFD*: incident photosynthetic photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *J*_T: total light driven electron flow ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *J*_C and *J*_O: electron flows devoted to RuBP carboxylation and oxygenation, respectively ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *S*: specificity factor of Rubisco; α and α_c : leaf absorbance in the PAR (adaxial surface) measured with an integrating sphere or computed from fluorescence data, respectively.

recorded in the species with lowest assimilation rates, suggesting that the differences in the net CO₂ assimilation rate between species are linked to the CO₂ availability in the chloroplasts. Finally, the CO₂ availability decreased with increasing drought in the soil, stressing the importance of reduced influx of CO₂ as an important factor for drought-induced declines of photosynthesis. These results are discussed with respect to the occurrence of significant resistances in the leaf mesophyll, in addition to the stomatal resistances.

oaks / drought / stomatal conductance / CO₂ diffusion / chloroplasts / mesophyll conductance / chlorophyll fluorescence

Résumé — Limitation de l'activité photosynthétique par la disponibilité en CO₂ dans les chloroplastes de feuilles de différentes espèces de chênes, et au cours d'une sécheresse. Des travaux récents suggèrent que les faibles niveaux d'assimilation de CO₂ souvent observés chez les ligneux, en comparaison avec ceux d'autres plantes cultivées, seraient au moins partiellement dus à des limitations d'origine mésophyllienne, de l'entrée de CO₂ dans les chloroplastes. Ces limitations s'additionneraient aux limitations d'origine stomatique. Nous avons testé cette hypothèse en déterminant les fractions molaires de CO₂ dans les chloroplastes de feuilles de différentes espèces de chênes (*Quercus petraea*, *Q. robur*, *Q. ilex*) et comparé les résultats avec ceux d'un ligneux hautement productif (*Populus euramericana*). La procédure mise en œuvre vise à estimer les fractions molaires de CO₂ dans les chloroplastes à partir d'une analyse de la partition des flux d'électrons photosynthétique entre la carboxylation et l'oxygénation du RuBP par la Rubisco. Les étapes essentielles consistent : i) en une détermination des flux d'électrons à l'aide du rapport de fluorescence $\Delta F/F_m'$ proportionnel à l'efficacité quantique de la conversion de l'énergie lumineuse par le PS II; ii) en une estimation de la fraction de ce flux utilisé pour la carboxylation de RuBP, par le biais des mesures d'assimilation nette de CO₂ et de respiration; iii) en la dérivation des fractions molaires de CO₂ dans les chloroplastes à partir du coefficient de spécificité de la Rubisco et du rapport des flux d'électrons utilisés pour la carboxylation et l'oxygénation du RuBP. Les résultats montrent que la fraction molaire de CO₂ dans les chloroplastes ainsi déterminée représentait 35 à 45 % de celle de l'atmosphère, et était beaucoup plus faible que celle qui est estimée dans les espaces intercellulaires (60 à 70 % de celle de l'atmosphère). De plus, elle était d'autant plus faible que l'assimilation nette de CO₂ était faible, suggérant ainsi que cette dernière pourrait être partiellement limitée par la disponibilité en CO₂ aux sites de carboxylation. De plus, elle a fortement baissé lors d'une contrainte hydrique, suggérant que la disponibilité en CO₂ est le principal facteur induisant la baisse de l'assimilation nette dans ces conditions. Ces résultats sont discutés en termes de contribution du mésophylle aux résistances à l'influx de CO₂ vers les chloroplastes.

chêne / sécheresse / conductance stomatique / chloroplaste / diffusion du CO₂ / conductance mésophyllienne / fluorescence de la chlorophylle

INTRODUCTION

The influx of atmospheric CO₂ to the chloroplasts is an important limiting step for the photosynthetic activity of leaves, under optimal as well as under stress conditions. Stomata play an essential part in this limitation and the response of photosynthesis to drought stress is mainly mediated by stomatal closure as it has been abundantly documented in oaks and in numerous other

species (see review by Cornic, 1994; Epron and Dreyer, 1993).

The diffusion path from substomatal spaces to the sites of carboxylation in the chloroplast stroma has very often been considered to oppose only a weak resistance to CO₂ fluxes and has been neglected in many descriptive models developed in the 1970s and early 1980s (Gaastra, 1959; Farquhar and Sharkey, 1982). Only in the last decade have limitations in CO₂ influx other

than by stomata or leaf boundary layer received increasing attention (review by Parkhurst, 1994).

Estimates of the CO₂ mole fraction in the chloroplast stroma (c_c) which would have made it possible to test for the importance of such limitations were not available until recently. Two groups of techniques developed in the last years allow us now to address this question: i) Models based on carbon isotope discrimination have been shown to gain accuracy when taking into account a discrimination step due to diffusion and transport of CO₂ in the mesophyll (Evans et al, 1986; Lloyd et al, 1992). ii) An analysis of the relative rates of carboxylation and oxygenation of RuBP in the chloroplasts yielded indirect estimates of c_c . Rates of oxygenation were computed using either ¹⁸O₂-enriched air (Renou et al, 1990; Tourneux and Peltier, 1994), or with simultaneous measurements of gas exchange and chlorophyll a fluorescence (Peterson, 1989; Di Marco et al, 1990; Cornic and Briantais, 1991).

The use of these techniques already yielded important results. The concentrations of CO₂ in the chloroplasts have been shown to be significantly lower than the calculated substomatal concentrations (Di Marco et al, 1990; Lloyd et al, 1992; Loreto et al, 1992). The contributions of stomata (+ boundary layer) and of mesophyll transport to the overall limitation of CO₂ influx have been shown to be of the same order of magnitude in many cases (Lloyd et al, 1992; Loreto et al, 1992). Moreover, it has been hypothesized that a high mesophyll resistance may be a discriminating factor between highly productive crops (with low resistances) and less productive species (as, for instance, tree species). It has also been observed that the concentration of CO₂ in the chloroplasts (c_c) decreased during drought stress (Renou et al, 1990; Cornic and Briantais, 1991; Tourneux and Peltier, 1994).

We now have much evidence that in oak trees submitted to drought, the photosynthetic process is very resistant to short-term dehydration (Epron and Dreyer, 1993), similarly to what had been described for many other C₃ species. However, we have only limited information about the respective role of stomata and of internal resistances to CO₂ influx in the limitations of net assimilation rates during water stress. Moreover, oak species display very different leaf anatomies, ranging from deciduous to strongly sclerophyllous; all of them are heterobaric. We therefore used combined measurements of gas exchange and chlorophyll fluorescence to estimate the availability of CO₂ in the chloroplasts of different species of oaks compared to values observed in a rapidly growing, and amphistomatous species (*Populus* sp). We also tested the hypothesis that drought induced a decline in c_c , which was the cause of the decrease in assimilation rates during water stress.

Theory

CO₂ influx into leaves may be described by a model derived directly from Gaastra (1959) and Von Caemmerer and Farquhar (1981), which may be written in the simplified form of:

$$A = g_{s+b} (c_a - c_i) = g_m (c_i - c_c) \quad [1]$$

where A = net CO₂ influx; g_{s+b} = leaf conductance to CO₂; g_m = mesophyll conductance to CO₂; c_a , c_i , c_c = gas phase mole fractions of CO₂ in the atmosphere, in the substomatal spaces and in the chloroplast stroma, respectively.

A , g_{s+b} , c_a were measured directly in the gas exchange chamber, c_i was computed from the preceding, and c_c was estimated as described later. Computations use a correction for mass efflux of water vapour limiting the inward diffusion of CO₂ (Von Caemmerer and Farquhar, 1981). The mes-

ophyll conductance as defined here results from a combination of gas phase diffusion in the intercellular spaces and from liquid phase transport across the membranes to the chloroplast stroma. Its computation is based on the determination of the mole fraction of air in equilibrium with the chloroplast stroma (c_c) rather than with liquid phase concentrations, for the sake of unit coherence (see details later).

Estimation and partitioning of light driven electron fluxes: The ratio $(F_m' - F) / F_m'$ (F_m' = maximal and F = steady-state fluorescence under actinic irradiance) has been shown by Genty et al (1989) to be a good estimate of the quantum yield of energy conversion by PS II (Φ_{II}) and to be linearly related to the apparent quantum yield of light driven electron flow estimated as:

$$\Phi_{e^-} = 4 (A_{1\%} + R_d) / PFD \quad [2]$$

where $A_{1\%}$ = net CO_2 assimilation under nonphotorespiratory conditions; R_d = nonphotorespiratory respiration; and PFD = incident photosynthetic photon flux density (Genty et al, 1989; Epron et al, 1994; Valentini et al, 1995).

R_d was assumed to be equal to the respiration measured under darkness before illumination. Data obtained under these conditions allow the calibration of the relationship between Φ_{II} and Φ_{e^-} as:

$$\Phi_{e^-} = \Phi_{II} / k + b \quad [3]$$

Usually, b is very close to 0, and $1/k$ depends on leaf absorptance (α) and distribution of light between the two photosystems, which was assumed to be uniform. In this case:

$$1/k = 0.5 \alpha_c \quad [4]$$

Under ambient concentrations of O_2 , the total light driven electron flow (J_T) may be computed under any given condition from:

$$J_T = (\Phi_{II} / k + b) PFD \quad [5]$$

J_T may be fractionated into two components used for carboxylation (J_C) and for oxygenation of RuBP (J_O) (Peterson, 1989; Di Marco et al, 1990; Cornic and Briantais, 1991) using the equations developed by Valentini et al (1995):

$$J_C = 1/3 [J_T + 8 (A + R_d)] \quad [6]$$

$$J_O = 2/3 [J_T - 4 (A + R_d)] \quad [7]$$

These equations are based on the assumption that respired CO_2 is recycled through carboxylation, and that carboxylation and oxygenation of RuBP are the only significant sinks of electrons. This latter assumption is supported by the observations of Loreto et al (1994), who checked that leaves fed with glyceraldehyde (that is, when RuBP regeneration and consequently when RuBP carboxylation and oxygenation were inhibited) presented only a very limited residual electron transport rate. Observations made in our laboratory on leaves in a CO_2 -free and 1% O_2 atmosphere yielded similar low levels (Dreyer and Huber, unpublished report).

c_c was computed from the model describing the kinetic properties of Rubisco (Farquhar et al, 1980) as:

$$c_{cl} = (o_{cl} / S) (J_C / J_O) \quad [8]$$

where S = specificity factor of Rubisco; c_{cl} and o_{cl} = liquid phase concentrations of CO_2 and O_2 in the chloroplast stroma, the latter being taken equal to the atmospheric concentration after correction for solubility in water. S has been shown to be close to 96 at 22 °C (Balaguer et al, 1996), which is within the range of values reported for other C_3 plants (Jordan and Ögren, 1984; Kane et al, 1994).

The gas phase balance mole fraction c_c is computed after correcting c_{cl} for the sol-

ubility of CO₂ in water. Partitioning coefficients between air and water for CO₂ (K_{hCO_2}) and O₂ (K_{hO_2}) have been derived from Umbreit et al (1972, in Edwards and Walker, 1983); pH-related changes in the partitioning coefficients were assumed to be only very limited. The following third-order polynomials were used for calculations of temperature dependent (t) coefficients:

$$K_{hCO_2} \text{ (mol l}^{-1} \text{ bar}^{-1}\text{)} = 78.5 \cdot 10^{-3} - 2.89 \cdot 10^{-3} t + 54.7 \cdot 10^{-6} t^2 - 0.417 \cdot 10^{-6} t^3$$

$$K_{hO_2} \text{ (mol l}^{-1} \text{ bar}^{-1}\text{)} = 2.10 \cdot 10^{-3} - 57.1 \cdot 10^{-6} t + 1.024 \cdot 10^{-6} t^2 - 7.503 \cdot 10^{-9} t^3$$

which yields values of 0.03636 and 0.00125 mol l⁻¹ bar⁻¹ at 22.5 °C for K_{hCO_2} and K_{hO_2} , respectively.

Equation [8] may then be rewritten as:

$$c_c = (J_c/J_o) / S (K_{hO_2}/K_{hCO_2}) O \quad [9]$$

where O = the mole fraction of O₂ in the air, assuming an atmospheric pressure of 1 000 hPa.

MATERIALS AND METHODS

Gas exchange and chlorophyll a fluorescence were measured on leaves enclosed in a small (10 cm²) aluminium gas exchange chamber (LSC-2, ADC, Hoddesdon, UK) located in a climate cabinet. Temperature in the chamber was controlled with a flow of water provided by a thermostatic water bath. Gas exchange monitoring was realized with a differential system based on a Binos infrared analyser for CO₂ and H₂O (Leybold Heraeus, Germany). CO₂ concentration in the air was controlled with an absolute ADC analyser (Mark II, ADC, Hoddesdon, UK). Mass flow controllers (FC 260, Tylan, USA) were used for precise regulation of air influx and of CO₂ injection into the chamber. A Peltier-regulated cold water trap was used to regulate the vapour pressure deficit in the chamber. Gas pressures in the different compartments of the measuring system were continuously recorded with pressure trans-

ducers (FGP Instruments, France). All primary parameters were recorded with an IBM Personal Computer AT3, connected to a data-logger (SAM80, AOIP, France), with a software developed in the laboratory allowing on line calculation of gas exchange, and digital control of chamber functions (technical details available on request). Actinic irradiance was provided by a slide projector (Kindermann 250 SL) and a 250 W halogen lamp. Irradiance levels were adjusted using neutral density filters to the desired incident value, and controlled with a Li-Cor quantum sensor. Maximal and steady-state fluorescence were recorded with a Pulse Amplitude Modulated fluorometer (PAM 101, Walz, Effeltrich, Germany; frequency 100 KHz), with the fibre optics at 45° over the window of the leaf chamber. The intensity of the saturating pulse, provided by a halogen lamp (KL 1500 Schott, Germany) was set so as to saturate fluorescence (700 ms, approximately 4 000 μmol m⁻² s⁻¹). Fluorescence signals and lamp settings were controlled with a software developed in the laboratory (IBM PC + data acquisition card).

Measurement conditions in the gas exchange chamber were, unless otherwise stated: temperature: 22.5 °C, irradiance: 500 μmol m⁻² s⁻¹, atmospheric CO₂: 350 μmol mol⁻¹, leaf to air difference in vapour pressure: 10 Pa kPa⁻¹.

During initial experiments, the calibration of the relationship between Φ_{e-} and Φ_{II} was performed at 2% O₂ and 350 μmol mol⁻¹ CO₂, and by measuring A and Φ_{II} at increasing irradiances. Φ_{e-} was then calculated as in equation [2], assuming that nonphotorespiratory respiration remained constant and equal to the value measured under darkness (R_d). This procedure yielded curvilinear relationships (results not shown) similar to the ones reported by Valentini et al (1995) under natural conditions. A new set of measurements was made at 700 μmol mol⁻¹ CO₂ and 1% O₂ (three leaves per species, and five levels of irradiance per leaf).

Potted seedlings of *Quercus petraea* Matt Liebl, *Q robur* L, *Q ilex* L and cuttings of *Populus deltoides* x *nigra* L were grown in a greenhouse in 10 L pots filled with a mixture sand/blond peat (50/50 v/v) under optimal water supply and with a slow release fertilisation (Nutricote100, N/P/K 13/13/13, with trace elements). Measurements were made on fully expanded leaves in all cases.

Optical properties of the leaves were measured on three well-developed leaves per species with a portable spectroradiometer (Li-1800, Li-

Cor, USA) and an integrating sphere (Li 1800-12S, Li-Cor, USA). The leaf absorptance (α) of the adaxial surface was computed over the PAR (400–700 nm) as the difference:

$$\alpha = 1 - T - R \quad [10]$$

with T , transmittance and R , reflectance. These values were compared to the computed mean value of the tested species (α_c) derived from equation [4].

Drought was imposed by withholding irrigation on six seedlings of *Q. ilex* and *Q. petraea*, for 10 days. Drought intensity was estimated with the predawn leaf water potential (Ψ_{wp} , pressure chamber). The experiments were made in July 1993 for *Q. robur*, and October 1993, on current year leaves for *Q. ilex*. A and Φ_{II} were measured every second day on one leaf from all plants. With *Q. ilex*, each measurement under normal conditions was followed by another one under nonphotorespiratory conditions (1% O_2 and 700 $\mu\text{mol mol}^{-1} \text{CO}_2$) to test for potential drought-induced deviations from linearity in the relationship Φ_{II} versus Φ_{e-} . Results are presented as mean values of A , c_i , c_c for three (*Q. petraea*) and four (*Q. ilex*) increasing levels of drought intensity.

RESULTS

Figure 1 shows the relationship between the apparent quantum yield of the linear light driven electron flow (Φ_{e-}) calculated from gas exchange and the quantum efficiency of the photochemical conversion by PS II (Φ_{II}) derived from chlorophyll fluorescence on leaves of *Quercus petraea*, *Q. robur*, *Q. ilex* and *Populus euramericana*. This relationship was linear and identical for the four tested species. The overall regression calculated was thereafter used to compute Φ_{e-} from any given value of Φ_{II} measured under photorespiratory conditions. The values of absorptance (α) measured on leaves from the same seedlings are indicated in the insert. Interestingly, they were very close to the value computed from equation [4] (α_c). The points obtained during increasing water stress with *Q. ilex* displayed no significant deviation from linearity, confirming that even under stress conditions,

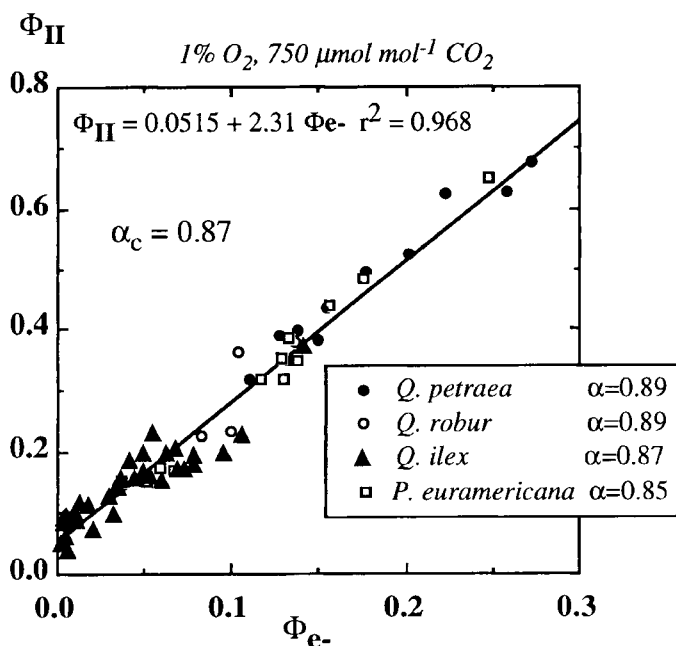
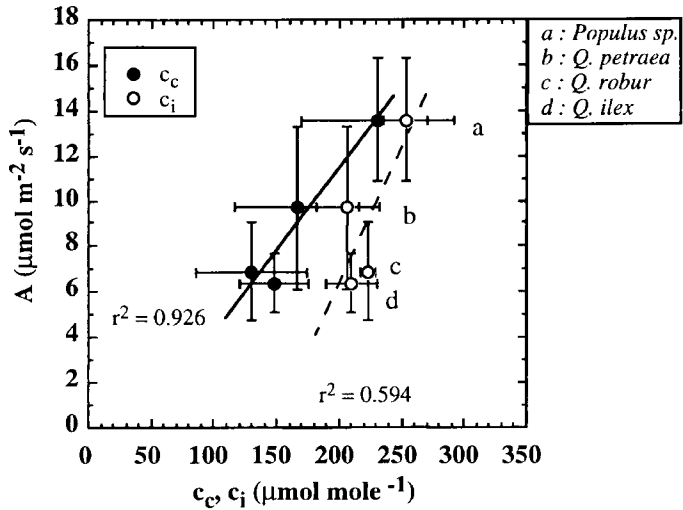


Fig 1. Relationship between the photochemical efficiency of PS II (Φ_{II}), and the apparent quantum yield of light driven electron transport (Φ_{e-}), as measured on leaves from different species at 1% O_2 and 700 $\mu\text{mol mol}^{-1} \text{CO}_2$. Each symbol represents a single measurement. Changes were obtained during the measurement of light response curves for all species, and additionally during a drought cycle on *Q. ilex*. The theoretical leaf absorptance calculated from the relationship (see eq [4] in the text) (α_c) is reported, as well as the values measured on the adaxial surface of leaves of each species with a spectroradiometer (α).

Fig 2. Mean net assimilation rates (A) as a function of calculated intercellular (c_i) or chloroplastic (c_c) CO₂ mole fractions in the leaves of potted seedlings from three oak and one poplar species. $n =$ five to six leaves per species. Bars indicate \pm SD. Temperature: 22 °C; $c_a = 350$ $\mu\text{mol mol}^{-1}$; $PFD = 500$ $\mu\text{mol m}^{-2} \text{s}^{-1}$.



the alternate sinks for light driven electron flow remained low and negligible.

Figure 2 shows a close relationship between mean values of net CO₂ assimilation (A) and of CO₂ mole fraction in the chloroplasts (c_c) determined in the four species. The values of c_c were much lower than the atmospheric (c_a) and the substomatal (c_i) CO₂ mole fractions; c_c/c_a was 0.37, 0.42 and 0.47, and c_i/c_a , 0.64, 0.59 and 0.60 for *Q. petraea*, *Q. ilex* and *Q. robur*, respectively. These values were lower than the 0.66 and 0.72, respectively, observed in *Populus*.

Drought induced a decrease of A in seedlings of *Q. petraea* and *Q. ilex*, as shown by the relationships with predawn leaf water potential Ψ_{wp} (fig 3). *Q. robur* displayed higher A and lower c_i than *Q. ilex* at all stress intensities. Drought resulted in a reduction of A down to 0 at Ψ_{wp} close to -2.5 MPa in *Q. petraea* and -1.5 MPa in *Q. ilex*. The low values of A and the high sensitivity to water stress in the evergreen *Q. ilex* were unexpected, but probably due to the fact that greenhouse-grown and old leaves were used. In both species, c_i increased significantly with drought. In contrast, the decline

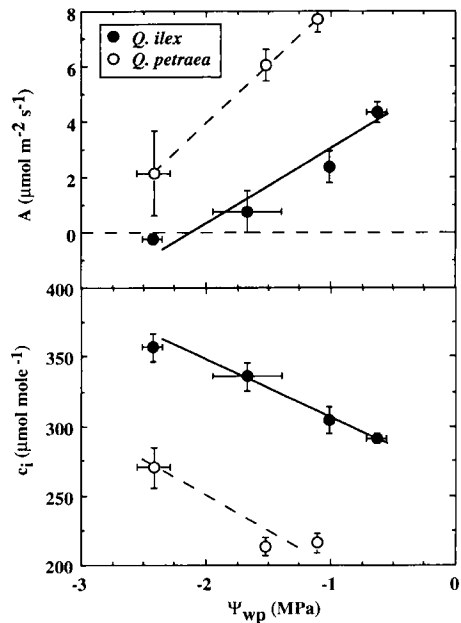


Fig 3. Relationship between predawn leaf water potential (Ψ_{wp}) and net CO₂ assimilation rates (A), or calculated CO₂ mole fraction in the substomatal spaces (c_i) in leaves of potted seedlings of *Q. petraea* and *Q. ilex* during a gradual drought. Six seedlings were used. Each value is the mean \pm SE of three to 14 individual measurements. Air temperature: 22 °C; $c_a = 350$ $\mu\text{mol mol}^{-1}$; $PFD = 500$ $\mu\text{mol m}^{-2} \text{s}^{-1}$.

of A was accompanied by a significant decrease of c_c , as shown in figure 4.

DISCUSSION AND CONCLUSION

We evidenced a linear and unique relationship between the apparent quantum yield of the linear light driven electron flow (Φ_{e-}) calculated from gas exchange and the quantum efficiency of the photochemical conversion by PS II (Φ_{II}) derived from chlorophyll fluorescence in greenhouse-grown seedlings of *Quercus petraea*, *Q. robur*, *Q. ilex* and *Populus euramericana*. This is in accordance with the model developed by Genty et al (1989), and confirms the validity of the calculation of light driven electron flows from Φ_{II} . Similar results had already been obtained with oaks during measurements under natural conditions (Valentini et al, 1995) or grown in a greenhouse (Epron et al, 1994). We did not find the curvilinearity described by Öquist and Chow

(1992), or by Epron et al (1994a) with field-grown oaks. In fact, the lack of linearity may be sometimes due to artefacts; in particular, photorespiration has to be greatly inhibited, which may require low O_2 and high CO_2 . Earlier measurements made in the laboratory with higher O_2 (2%) resulted in curvilinearity. It should also be emphasized that the empirical fit calculated on the basis of our data was compatible with a theoretical leaf absorbance of 0.87, which has been shown to be very close to the values measured on leaves of the tested seedlings. Moreover, no drought-induced deviation from linearity could be detected, as already stated by Genty et al (1989), and confirmed by the remarkable stability of the Φ_{e-}/Φ_{II} relationship under a wide range of conditions (review by Edwards and Baker, 1993).

The computation of chloroplastic CO_2 mole fractions (c_c) from combined gas exchange/chlorophyll a fluorescence measurements depends on a series of assumptions:

i) *Absence of significant sinks for light driven electron fluxes besides RuBP carboxylation and oxygenation*: a number of potential sinks for electrons are well known; among them, the nitrite reductase operating in the chloroplasts (Huppe and Turpin, 1994); however, little evidence is available on the quantitative importance of this sink. In particular, the observation that the ratio between CO_2 fixation and PS II electron transport is largely unaffected by the level of N supply (Foyer et al, 1994), suggests a low competition with CO_2 reduction for the direct products of electron flow. Other similar sinks like the sulfate-reductase and the ferredoxin-thioredoxin reductase are probably quantitatively only very minor (Foyer et al, 1994). The Mehler reaction results in a reduction of O_2 by the PS I-associated ferredoxin, and in the production of superoxide (review by Foyer, 1994). The fraction of total electron flow devoted to this reduction has been estimated at a few percent in vivo (Foyer, 1994).

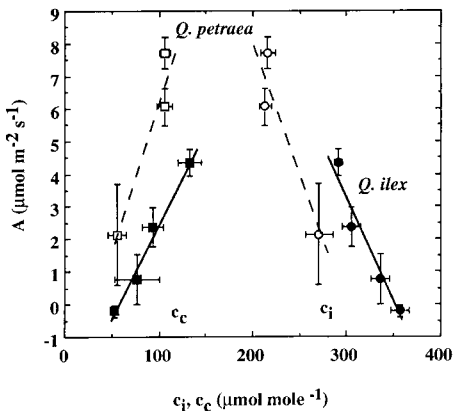


Fig 4. Relationship between intercellular (c_i) or chloroplastic (c_c) CO_2 mole fractions, and net assimilation rates (A) measured on leaves of potted seedlings of *Q. petraea* and *Q. ilex* during a drought-induced decline of A . Each value is the mean \pm SE of three to 14 individual measurements. Temperature: 22 °C; c_a : 350 $\mu mol\ mol^{-1}$; PFD: 500 $\mu mol\ m^{-2}\ s^{-1}$.

Results of Loreto et al (1994) support the view that this sink is only minor when compared to carboxylation and oxygenation of RuBP.

ii) *The specificity factor of Rubisco (S)* in tree species is close to the values measured in vitro on different crops. We used the value of 96 at 22.5 °C measured in vitro with oak leaf extracts by Balaguer et al (1996), which is close to those reported for diverse C₃ plants (Jordan and Ögren, 1984; Kent et al, 1992; Kane et al, 1994). In vivo determined apparent *S*, based on the calculated *c*_i and not *c*_c, has been shown to range from around 50 (*Fagus sylvatica* and *Castanea sativa*; Epron et al, 1995) to around 80 (*Quercus cerris*; Valentini et al, 1994). This deviation from the in vitro values has been ascribed to limitations in the CO₂ flux from substomatal spaces to chloroplasts (Epron et al, 1995). The temperature dependence of *S* is well described and may be easily modelled (decreases with increasing temperatures; Jordan and Ögren, 1984; Brooks and Farquhar, 1985). The stability of *S* during water stress has to our knowledge never been directly tested, but no evident argument opposes it.

iii) *Differences in light absorption and fluorescence profiles across the leaf do not induce significant artefacts*, like the curvilinear relationship between Φ_{e-} and Φ_{II} observed by Evans et al (1993). We observed a linearity at least up to a Φ_{e-} of 0.28, as reported also by Valentini et al (1995). Moreover, our calibration technique also integrated effects due to the light absorption profiles.

Our results showed that oak trees were operating at much lower levels of CO₂ in the chloroplast stroma (*c*_c) than the calculated substomatal mole fraction (*c*_i). In the absence of water stress, the *c*_i/*c*_a ratio was around 0.35–0.45 in oaks, depending on species, which is within the range of values published for other C₃ plants (0.25–0.35 in *Quercus ilex*, Di Marco et al, 1990;

0.35–0.50, Lloyd et al, 1992; 0.53 for *Q. rubra*, Loreto et al, 1992; 0.45 for *Solanum tuberosum*, Tourneux and Peltier, 1994; 0.60 down to 0.30 with increasing age in wheat, Loreto et al, 1994). These values are much lower than the frequently cited *c*_i/*c*_a ratio of about 0.6–0.7, which we also observed here, and also lower than values measured in poplar leaves (0.66).

In addition, our results confirmed that drought resulted in decreases of net assimilation rates associated to decreasing *c*_c, despite the apparent maintenance and even increase of *c*_i. The low intrinsic sensitivity of photosynthetic processes (photochemical energy conversion and RuBP carboxylation) to drought is now a widely accepted feature at least in C₃ plants (see review by Cornic, 1994). Our data confirm recent experiments showing that *c*_c actually decreased during water stress in several species (Renou et al, 1990; Tourneux and Peltier, 1994). Similar results have been obtained by Ridolfi and Dreyer (1995) with a poplar clone.

Such results lead to two complementary questions.

First, to what extent is CO₂ availability in the chloroplasts limiting net assimilation rates? Changes in CO₂ availability in the chloroplasts (*c*_c) have now been reported several times to occur among species, or in a given species during changes with growth conditions. Ridolfi et al (1996) showed that a calcium deficiency in oak leaves induced a parallel decrease of *A* and *c*_c. Loreto et al (1994) observed a similar parallelism during senescence in wheat leaves. Differences of assimilation rates among C₃ species may also be partly explained by variable CO₂ availability (Loreto et al, 1992; Epron et al, 1995) rather than solely by the biochemical limitations put forward by Wullschlegel (1993). Nevertheless, a colimitation by *c*_c and biochemical factors cannot be ruled out, and additional data are needed to clarify this point.

Second, what is the reason for such a large drop of CO₂ between substomatal spaces and the chloroplastic stroma? This can be addressed by the straightforward application of the unidirectional diffusion model to compute a mesophyll (or internal) conductance (g_m) to CO₂ according to equation [1]. Computations made from our data yield values of 100–200 and 600 mmol m⁻² s⁻¹ in the different oak species, and in the poplars, respectively. Such values are of the same order of magnitude than the stomatal conductances to CO₂. This leads to the assumption that internal resistances may play an important role in limiting CO₂ influx from the substomatal spaces to the chloroplast stroma, as has been discussed in several works (Von Caemmerer and Evans, 1991; Lloyd et al, 1992; Loreto et al, 1992; Epron et al, 1995). The involvement in this transport process of a carbonic anhydrase favouring the interconversion between carbonate and dissolved CO₂ has been suspected; however, recent evidence suggests that its role in photosynthesis is only minor in C₃ plants (Badger and Price, 1994; Price et al, 1994). Leaf anatomy and chloroplast distributions probably play a role in this process (Nobel, 1991), but correlations between parameters like the mesophyll area/leaf area ratio and the leaf area are still weak (Loreto et al, 1992), even if Syvertsen et al (1995) revealed correlations between chloroplast distribution in leaves and g_m .

The same computation of g_m applied to the data of the water-stress experiment would result in a decrease of g_m during drought. The reality of such a decrease is very questionable. In fact, the occurrence of stomatal patchiness during drought and the resulting large artefacts in the calculation of c_i (Downton et al, 1988; Pospisilova and Santrucek, 1994) severely limit the validity of this approach. Recent evidence obtained by Genty and Meyer (1995, personal communication) with fluorescence imaging illustrated this patchiness on leaves during

drought, and showed that an accurate correction removed these artefacts. This would lead to the conclusion that stomatal closure is probably the main factor reducing CO₂ availability in the chloroplasts during drought.

ACKNOWLEDGMENT

Fruitful discussions with B Genty, D Epron and G Cornic about the use of fluorescence signals are gratefully acknowledged. Suggestions of JM Guehl and two anonymous reviewers on an earlier version of the manuscript have been very useful. We thank the French firm Eurosep (Cergy, France) who gave us access to the Li-Cor spectroradiometer for the measurements. Plants used in this experiment were grown by JM Gioria.

REFERENCES

- Badger MR, Price GD (1994) The role of carbonic anhydrase in photosynthesis. *Ann Rev Plant Physiol Plant Mol Biol* 45, 369-392
- Balaguer L, Afif D, Dizengremel P, Dreyer E (1995) Ribulose biphosphate carboxylase/oxygenase in an oak species (*Quercus robur* L): specificity and activities *Plant Physical Biochem* (in press)
- Brooks A, Farquhar GD (1985) Effect of temperature on the CO₂/O₂ specificity of Ribulose 1,5-Biphosphate Carboxylase-Oxygenase. Estimates from gas exchange measurements on spinach. *Planta* 165, 697-706
- Cornic G, Briantais JM (1991) Partitioning of photosynthetic electron flow between CO₂ and O₂ reduction in a C₃ leaf (*Phaseolus vulgaris* L) at different CO₂ concentrations and during drought stress. *Planta* 183, 178-184
- Cornic G (1994) Drought stress and high light effects on leaf photosynthesis. In: *Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field* (NR Baker, JR Bowyer, eds), Bios Scientific Pub, Oxford, UK, 297-314
- Di Marco G, Manes F, Tricoli D, Vitale E (1990) Fluorescence parameters measured concurrently with net photosynthesis to investigate chloroplastic CO₂ concentration in leaves of *Quercus ilex* L. *J Plant Physiol* 136, 538-543
- Downton WJS, Loveys BR, Grant WJR (1988) Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. *New Phytol* 110, 503-509

- Edwards G, Walker DA (1983) *C3, C4. Mechanism, and cellular and environmental regulations of photosynthesis*. Blackwell Scientific Publications, Oxford, UK, 535 p
- Edwards GE, Baker NR (1993) Can CO₂ assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosyn Res* 37, 89-102
- Epron D, Dreyer E (1993) Compared effects of drought on photosynthesis of adult oak trees (*Quercus petraea* [Matt] Liebl and *Quercus robur* L.) in a natural stand. *New Phytol* 125, 381-389
- Epron D, Dreyer E, Picon C, Guehl JM (1994) Relationship between CO₂-dependent O₂ evolution and photosystem II activity in oak (*Quercus petraea* [Matt] Liebl) trees grown in the field and in seedlings grown under normal or elevated CO₂. *Tree Physiol* 14, 725-733
- Epron D, Godard D, Cornic G, Genty B (1995) Limitations of net CO₂ assimilation rates by internal resistances to CO₂ transfer in leaves of two species (*Fagus sylvatica* L. and *Castanea sativa* Mill). *Plant Cell Environ* 18, 43-51
- Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO₂ diffusion in leaves of higher plants. *Aust J Plant Physiol* 13, 281-292
- Evans JR, Jakobsen I, Ögren E (1993) Photosynthetic light-response curves. 2. Gradients of light absorption and photosynthetic capacity. *Planta* 189, 191-200
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Ann Rev Plant Physiol* 33, 317-345
- Farquhar GD, Von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ plants. *Planta* 149, 78-90
- Foyer CH, Harbinson J (1994) Oxygen metabolism and the regulation of photosynthetic electron transport. In: *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants* (CH Foyer, PM Mullineaux, eds), CRC Press, Boca Raton, USA, 2-42
- Foyer CH, Noctor G, Lelandais M, Lescure JC, Valadier MH, Boutin JP, Horton P (1994) Short-term effects of nitrate, nitrite and ammonium assimilation on photosynthesis, carbon partitioning and protein phosphorylation in maize. *Planta* 192, 211-220
- Gaastra P (1959) Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. *Medel Landbouhoog Wageningen*, 59, 1-68
- Genty B, Meyer S (1995) *Aust J Plant Physiol* 22, 277-284
- Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 990, 87-92
- Huppe HC, Turpin DH (1994) Integration of carbon and nitrogen metabolism in plant and algal cells. *Ann Rev Plant Physiol Plant Mol Biol* 45, 577-607
- Jordan DB, Ögren WL (1984) The CO₂/O₂ specificity of Ribulose 1,5-Bisphosphate Carboxylase Oxygenase. Dependence on Ribulose 1,5-Bisphosphate concentration, pH and temperature. *Planta* 161, 308-313
- Kane HJ, Viil J, Entsch B, Paul K, Morell MK, Andrews TJ (1994) An improved method for measuring the CO₂/O₂ specificity of Ribulose bisphosphate Carboxylase Oxygenase. *Aust J Plant Physiol* 21, 449-461
- Kent SS, André M, Cournac L, Farineau J (1992) An integrated model for the determination of the Rubisco specificity factor, respiration in the light and other photosynthetic parameters of C₃ plants in situ. *Plant Physiol Biochem* 30, 625-637
- Lloyd J, Syversten JP, Kriedemann PE, Farquhar GD (1992) Low conductance to CO₂ diffusion from stomata to the sites of carboxylation in the leaves of woody species. *Plant Cell Environ* 15, 873-899
- Loreto F, Harley PC, Di Marco G, Sharkey TD (1992) Estimation of mesophyll conductance to CO₂ flux by three different methods. *Plant Physiol* 98, 1437-1443
- Nobel PS (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego, CA, USA
- Öquist G, Chow WS (1992) On the relationship between the quantum yield of Photosystem II electron transport, as determined by chlorophyll fluorescence and the quantum yield of CO₂-dependent O₂ evolution. *Photosyn Res* 33, 51-62
- Parkhurst DF (1994) Diffusion of CO₂ and other gases inside leaves. *Tansley Review* 65. *New Phytol* 126, 449-479
- Peterson RB (1989) Partitioning of non-cyclic photosynthetic electron transport to O₂-dependent dissipative processes as probed by fluorescence and CO₂ exchange. *Plant Physiol* 90, 1322-1328
- Pospisilova J, Santrucek J (1994) Stomatal patchiness. *Biol Plant* 36, 481-510
- Price GD, Von Caemmerer S, Evans JR, Yu JW, Lloyd J, Oja V, Kell P, Harrison K, Gallagher A, Badger MR (1994) Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco plants has only a minor effect on photosynthetic CO₂ assimilation. *Planta* 193, 331-340
- Renou JL, Gerbaud A, Just D, André M (1990) Differing substomatal and chloroplastic CO₂ concentrations in water-stressed wheat. *Planta* 182, 415-419
- Ridolfi M, Dreyer E (1995) Photosynthesis during water stress in an ABA-unresponsive hybrid poplar (*Populus koreana* x *trichocarpa* cv Peau). In: *Photosynthesis from Light to the Biosphere* (P Mathis, ed),

- vol IV, Kluwer Academic Pub, the Netherlands, 633-636
- Ridolfi M, Rouspard O, Garrec JP, Dreyer E (1996) Effects of a calcium deficiency on stomatal conductance and photosynthetic activity of *Quercus robur* seedlings grown on a nutrient solution. *Ann Sci For* 53, 325-336
- Syvertsen JP, Lloyd J, McConchie C, Kriedemann PE, Farquhar GD (1995) On the relationship between leaf anatomy and CO₂ diffusion through the mesophyll of hypostomatous leaves. *Plant Cell Environ* 18, 149-157
- Tourneux C, Peltier G (1994) Effect of water deficit on the photosynthetic oxygen exchange measured using ¹⁸O₂ and mass spectrometry in *Solanum tuberosum*. *Planta* 195, 570-577
- Valentini R, Epron D, De Angelis P, Matteucci G, Dreyer E (1995) In situ estimation of net CO₂ assimilation, photosynthetic electron flow and photorespiration in Turkey oak (*Q cerris* L) leaves: diurnal cycles under different levels of water supply. *Plant Cell Environ* 18, 631-640
- Von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153, 376-387
- Von Caemmerer S, Evans JR (1991) Determination of the CO₂ pressure in chloroplasts from leaves of several C₃ plants. *Aust J Plant Physiol* 18, 287-305
- Wullschlegel SD (1993) Biochemical limitations to carbon assimilation in C₃ plants. A retrospective analysis of the A-ci curves from 109 species. *J Exp Bot* 44, 907-920