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Limitation of photosynthetic activity by CO_2 availability in the chloroplasts of oak leaves from different species and during drought

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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 CO_2 fluxes in the mesophyll of tree leaves. To validate this assertion, values of CO_2 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 CO_2 assimilation and respiration rate measurement, and using the known electron requirements (four electrons for CO_2 or O_2 fixation); iii) the derivation of the CO_2 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 CO_2 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

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Abbreviations: A: net CO_2 assimilation rate (μmol m^{-2} s^{-1}); $A_{1\%}$: net CO_2 assimilation under nonphotorespiratory (1% O_2) conditions; R_d: nonphotorespiratory respiration (μmol m^{-2} s^{-1}); g_{s+b}: leaf conductance to CO_2 (mmol m^{-2} s^{-1}); g_s: stomatal conductance to CO_2 (mmol m^{-2} s^{-1}); c_a, c_i, c_c: mole fractions of CO_2 in the free atmosphere, in the substomatal spaces and in the chloroplast stroma, respectively (μmol mol^{-1}); c_{cl} and o_{cl}: liquid phase concentrations of CO_2 and O_2 in the chloroplast stroma (μmol l^{-1}); g_m: mesophyll conductance to CO_2 (ie, from the substomatal spaces to the chloroplast stroma, mmol m^{-2} 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; *PFD*: incident photosynthetic photon flux density (μmol m^{-2} s^{-1}); J_T: total light driven electron flow (μmol m^{-2} s^{-1}); J_C and J_O: electron flows devoted to RuBP carboxylation and oxygenation, respectively (μmol m^{-2} s^{-1}); S: specificity factor of Rubisco; α and α_c: leaf absorptance 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 CO_2 assimilation rate between species are linked to the CO_2 availability in the chloroplasts. Finally, the CO_2 availability decreased with increasing drought in the soil, stressing the importance of reduced influx of CO_2 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 / CO_2 diffusion / chloroplasts / mesophyll conductance / chlorophyll fluorescence

Résumé — Limitation de l'activité photosynthétique par la disponibilité en CO_2 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 CO_2 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 CO_2 dans les chloroplastes. Ces limitations s'additionneraient aux limitations d'origine stomatique. Nous avons testé cette hypothèse en déterminant les fractions molaires de CO_2 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 CO_2 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'efficience 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 CO_2 et de respiration ; iii) en la dérivation des fractions molaires de CO_2 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 CO_2 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 CO_2 était faible, suggérant ainsi que cette dernière pourrait être partiellement limitée par la disponibilité en CO_2 aux sites de carboxylation. De plus, elle a fortement baissé lors d'une contrainte hydrique, suggérant que la disponibilité en CO_2 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 CO_2 vers les chloroplastes.*

chêne / sécheresse / conductance stomatique / chloroplaste / diffusion du CO_2 / conductance mésophyllienne / fluorescence de la chlorophylle

INTRODUCTION

The influx of atmospheric CO_2 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 CO_2 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 CO_2 influx other

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

Estimates of the CO_2 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 CO_2 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 $^{18}O_2$-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 CO_2 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 CO_2 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 CO_2 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 C_3 species. However, we have only limited information about the respective role of stomata and of internal resistances to CO_2 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 CO_2 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

CO_2 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:

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

where A = net CO_2 influx; g_{s+b} = leaf conductance to CO_2; g_m = mesophyll conductance to CO_2; c_a, c_i, c_c = gas phase mole fractions of CO_2 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 CO_2 (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:

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

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:

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

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:

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

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

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

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):

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

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

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:

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

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 CO_2 in water. Partitioning coefficients between air and water for CO_2 (K_{hCO2}) and O_2 (K_{hO2}) 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 polynomes were used for calculations of temperature dependent *(t)* coefficients:

$$K_{hCO2}\ (\text{mol l}^{-1}\ \text{bar}^{-1}) = 78.5\ 10^{-3} - 2.89\ 10^{-3}\ t + 54.7\ 10^{-6}\ t^2 - 0.417\ 10^{-6}\ t^3$$

$$K_{hO2}\ (\text{mol l}^{-1}\ \text{bar}^{-1}) = 2.10\ 10^{-3} - 57.1\ 10^{-6}\ t + 1.024\ 10^{-6}\ t^2 - 7.503\ 10^{-9}\ t^3$$

which yields values of 0.03636 and 0.00125 mol l^{-1} bar^{-1} at 22.5 °C for K_{hCO2} and K_{hO2}, respectively.

Equation [8] may then be rewritten as:

$$c_c = (J_C/J_O)\ /S\ (K_{hO2}/K_{hCO2})\ O \qquad [9]$$

where O = the mole fraction of O_2 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 cm^2) 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 CO_2 and H_2O (Leybold Heraeus, Germany). CO_2 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 CO_2 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 transducers (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 CO_2: 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% O_2 and 350 µmol mol^{-1} CO_2, 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} CO_2 and 1% O_2 (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 µmol mol^{-1} 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 condictions. 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,

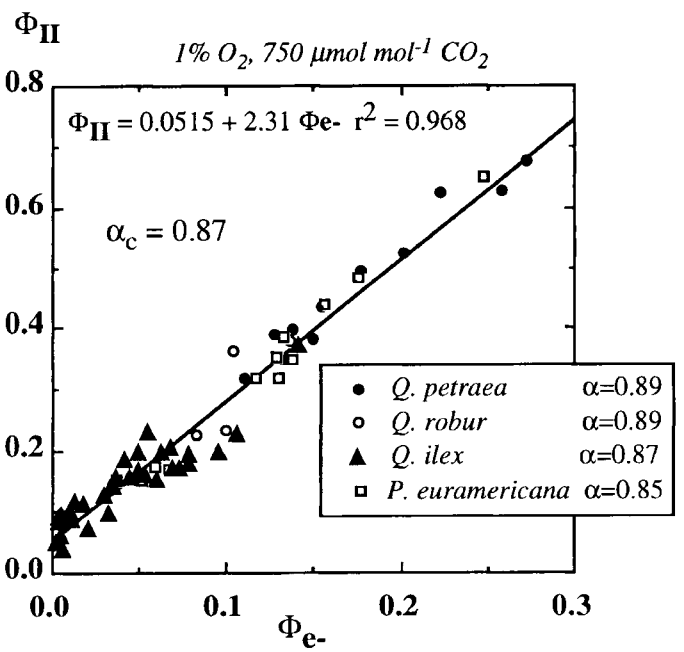

Fig 1. Relationship between the photochemical efficiency of PS II (Φ_{II}), and the apparent quantum yield of light driven electron transport (Φ_{e-}), as measured on leaves from different species at 1% O_2 and 700 µmol mol^{-1} CO_2. Each symbol represents a single measurement. Changes were obtained during the measurement of light response curves for all species, and additionnally 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).

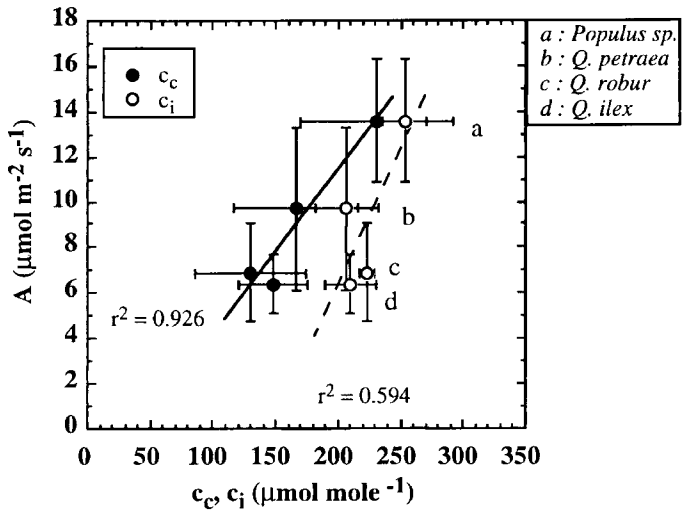

Fig 2. Mean net assimilation rates *(A)* as a function of calculated intercellular (c_i) or chloroplastic (c_c) CO_2 mole fractions in the leaves of potted seedlings from three oak and one poplar species. n = five to six leaves per species. Bars indicate ± SD. Temperature: 22 °C; c_a = 350 µmol mol^{-1}; *PFD* = 500 µmol m^{-2} 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 CO_2 assimilation *(A)* and of CO_2 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) CO_2 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

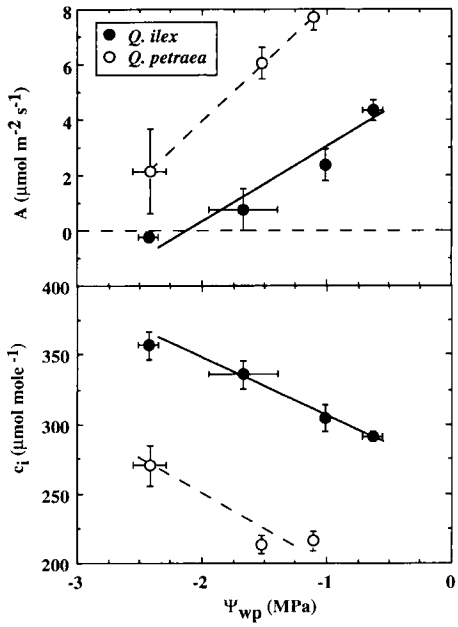

Fig 3. Relationship between predawn leaf water potential (Ψ_{wp}) and net CO_2 assimilation rates *(A)*, or calculated CO_2 mole fraction in the substomatal spaces (c_i) in leaves of potted seedlings of *Q petraea* and *Q ilex* during a gradual drought. Six seedlings were used. Each value is the mean ± SE of three to 14 individual measurements. Air temperature: 22 °C; c_a: 350 µmol mol^{-1}; *PFD:* 500 µmol m^{-2} 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 absorptance 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).

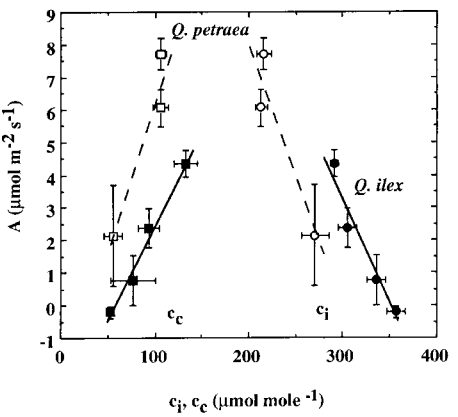

Fig 4. Relationship between intercellular (c_i) or chloroplastic (c_c) CO_2 mole fractions, and net assimilation rates *(A)* measured on leaves of potted seedlings of *Q petraea* and *Q ilex* during a drought-induced decline of *A*. Each value is the mean ± SE of three to 14 individual measurements.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

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).

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 C_3 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 CO_2 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 CO_2 in the chloroplast stroma (c_c) than the calculated substomatal mole fraction (c_i). In the absence of water stress, the c_c/c_a ratio was around 0.35–0.45 in oaks, depending on species, which is within the range of values published for other C_3 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 C_3 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 CO_2 availability in the chloroplasts limiting net assimilation rates? Changes in CO_2 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 C_3 species may also be partly explained by variable CO_2 availability (Loreto et al, 1992; Epron et al, 1995) rather than solely by the biochemical limitations put forward by Wullschleger (1993). Nevertheless, a colimitation by c_c and biochemical factors cannot be ruled out, and additionnal data are needed to clarify this point.

Second, what is the reason for such a large drop of CO_2 between substomatal spaces and the chloroplastic stroma? This can be addressed by the straightforward application of the unidirectionnal diffusion model to compute a mesophyll (or internal) conductance (g_m) to CO_2 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 CO_2. This leads to the assumption that internal resistances may play an important role in limiting CO_2 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 CO_2 has been suspected; however, recent evidence suggests that its role in photosynthesis is only minor in C_3 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 CO_2 availability in the chloroplasts during drought.

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