Original article

Drought and photosystem II activity in two Mediterranean oaks

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Summary — The responses of photosystem II (PS II) to drought were analysed on two Mediterranean oak species, *Quercus ilex* and *Q pubescens,* using the chlorophyll fluorescence pulse-amplitude-modulation technique. The maximal PS II photochemical efficiency (F_v/F_m) of the evergreen *Q ilex* and the deciduous *Q pubescens* oaks was only affected when leaf predawn water potential was lower than −4 MPa. This value is rarely observed on mature trees growing in the field, but can be undergone by young seedlings during drought periods, hence confirming the stability of PS II. Whatever the irradiance, drought resulted, in both species, in lower values of PS II photochemical efficiency in a light-adapted state ($\Delta F/F_{m'}$), due to stomatal closure and/or a direct inhibition of the dark reactions of photosynthesis. Diurnal decreases of F_v/F_m of 30 min dark-adapted leaves were greater for lower predawn water potential; a recovery was observed in the late afternoon. The reversible decreases in the diurnal time-courses of maximal fluorescence led us to assume the onset of protective mechanisms from permanent photodamages in *Q ilex* and, to a lesser extent, in *Q pubescens.*

fluorescence / photoinhibition / photosystem II / *Quercus* / water stress

Résumé — Sécheresse et activité du photosystème II chez deux chênes méditerranéens. *La réponse du photosystème II (PS II) à la sécheresse a été analysée sur deux espèces de chênes méditerranéens,* Quercus ilex *et* Q pubescens *par la technique de la fluorescence chlorophyllienne pulsée. L'efficacité photochimique maximale du PS II (*F_v/F_m*) de* Q ilex *(sempervirent) et de* Q pubescens *(caducifolié) n'a été affectée que pour un potentiel hydrique de base inférieur à −4 MPa. Une telle valeur n'est pratiquement jamais atteinte en conditions naturelles sur des arbres adultes, mais peut être subie par de jeunes plantules lors des sécheresses estivales, confirmant ainsi la résistance du PS II.*

Quel que soit l'éclairement, la sécheresse a induit, chez les deux espèces, des valeurs plus faibles de l'efficacité photochimique du PS II à la lumière ($\Delta F/F_{m'}$), dues à une fermeture des stomates et (ou) une inhibition directe des réactions sombres de la photosynthèse. Les décroissances journalières de F_v/F_m après 30 minutes d'adaptation des feuilles à l'obscurité, étaient plus prononcées pour les potentiels de base les plus faibles ; une récupération a été observée en fin d'après-midi. Les diminutions diurnes, reversibles, de la fluorescence maximale traduisent la mise en place de mécanismes de protection contre les dommages résultant de niveaux lumineux élevés chez Q ilex *et, à un degré moindre, chez* Q pubescens.

fluorescence / photoinhibition / photosytème II / Quercus / stress hydrique

INTRODUCTION

In the Mediterranean-type climate, trees must fairly often survive and grow under conditions of low water availability. Evergreen and deciduous cooccurent oak species such as *Quercus ilex* L and *Q pubescens* Willd are abundant in landscapes of the Mediterranean Basin and constitute large woodlands. Water conditions affect their area of distribution which is often related to pluviothermic factors (see Emberger, 1955) and to the average of the minimal temperatures of the coldest month. Moreover, due to climatic change, their photosynthetic system will have to run, sometimes all year round, in a thermal, hydric and atmospheric environment which will be different from today. Water deficiency may increasingly become a stress factor of widespread occurrence. Some changes in distribution may likely occur.

The functioning of photosystem II (PS II) of dark-adapted leaves of tree species differing in their tolerance to drought has been shown to be highly stable during a rapid leaf dehydration (Schreiber and Bilger, 1987; Dreyer et al, 1992). Stomatal closure associated with water stress restricts carbon assimilation. Absorption of light energy may be in excess of that required for carbon fixation and may result in damage to this photosystem (see eg, Björkman and Powles' results [1984] on the Mediterranean evergreen shrub *Nerium oleander*).

Chlorophyll a fluorescence has been used successfully for analysing PS II activity in vivo with (Epron et al, 1992) or without (Janda et al, 1994; Jefferies, 1994) further net CO_2 assimilation measurements. Maximal and actual photochemical efficiencies of PS II can be assessed (Genty et al, 1989). Sharkey et al (1988) showed that electron transport estimated by fluorescence was directly comparable (nearly 1:1) with electron transport estimated by CO_2 assimilation, assuming that only four electrons transported by PS II are used per CO_2 fixed.

The objective of this study was to examine the sensitivity of PS II to drought stress in *Q ilex* and *Q pubescens*. Emphasis has been laid on drought and light level interaction.

MATERIALS AND METHODS

Plant material, growth conditions and stress application

The experiments were carried out in Montpellier, France (43°36'N, 3°53'E; 55 m elevation), on young individuals of two Mediterranean oaks: an evergreen, *Q ilex*, and a deciduous, *Q pubescens*. Trees were grown outdoors for 6 months in the experimental field of the CEFE, in pots filled with a mixture of compost (53%), mould (21%) and loam (26%). Depending on their age, 5- or 8-year-old, 10 or 35 L pots were used. At first, the plants were irrigated twice a week. Drought was imposed by withholding water supply during summer 1993. Predawn leaf water potential, moni-

tored by means of a Scholander pressure chamber, was used as an index of drought intensity. This parameter was assessed on two twigs (8-year-old trees) or on two to three leaves (5-year-old trees) of the upper part of the trees.

Chlorophyll a fluorescence measurements

The saturation pulse method associated with the pulse-amplitude-modulation technique (Schreiber et al, 1986; Schreiber and Bilger, 1987) was used for fluorescence measurements (fluorometer PAM-2000, Walz, Germany). Initial fluorescence F_0 was determined by applying a weak modulated measuring light (0.1 μmol m^{-2} s^{-1}, 655 nm) at a frequency of 600 Hz on the adaxial surface of dark-adapted leaves. Leaf clips (PEA/LC, Hansatech, UK) were used for the dark adaptation (30 min) of samples in the daytime.

At such low light intensity, all PS II reaction centers are in an open state, and therefore the rate of photochemistry is not limited. Pulses of 800 ms high intensity (up to 15 000 μmol m^{-2} s^{-1}) white light (< 710 nm) allowed the assessment of maximal fluorescence in this dark-adapted state F_m (at this stage, all PS II reaction centers are closed, due to complete reduction of primary electron acceptors Q_A) and (or) in the light-adapted state $F_{m'}$. Since F_s is the actual fluorescence intensity at any time (= steady-state value of fluorescence), emphasis was laid on:

– the quantum yield of PS II, $F_v/F_m = (F_m - F_0)/F_m$, which is a measure of PS II photochemical trapping efficiency of the dark-adapted state (Kitajima and Butler, 1975). Knowing F_0 and F_m, the Butler model (1978) makes it possible to connect the variations of F_v/F_m to the rate constants of thermal deactivation and of photochemistry. We distinguished the maximal photochemical efficiency of PS II measured predawn from the photochemical efficiency in the daytime, after 30 min of relaxation in the dark.

– the photochemical efficiency of PS II in a light-adapted state $\Delta F/F_{m'} = (F_{m'} - F_s)/F_{m'}$, which can be used to estimate changes in the quantum yield of noncyclic electron transport, according to Genty et al (1989).

The light-response curve of $\Delta F/F_{m'}$ was determined at early dawn. The related pre-programmed sequence of the fluorometer was used, giving illumination times of 5 min with the internal LED

actinic light source (up to 500 μmol m^{-2} s^{-1}). Higher light levels were delivered by a 20 W dichroic halogen lamp fitted with a short-pass interference filter (cutoff wavelength, 5% transmittance, 712 nm).

Two electrical fans were used in order to prevent heating by the halogen lamp. The tip of the fiberoptics was located 1.0 cm from and 60° to the leaf surface. Leaf temperature and the photosynthetic photon flux density *(PPFD)* were monitored by the NiCr-Ni thermocouple and the quantum sensor integrated in the leaf-clip holder of the fluorometer. Except where otherwise stated, fluorescence measurements were made on sun-exposed, attached and fully expanded, current-year leaves.

RESULTS

Drought effects on maximal photochemical efficiency of PS II

Responses of maximal photochemical efficiency of PS II (measured predawn) to declining predawn leaf water potential are shown in figure 1. F_v/F_m showed highest values up to very strong levels of drought: 0.824 ± 0.027 for *Q ilex* and 0.826 ± 0.023 for *Q pubescens*, and declined only beyond a predawn leaf water potential of about –4 MPa. For *Q ilex*, F_v/F_m could exceed 0.6 when predawn leaf water potential was close to –7 MPa.

Drought effects on light-response curves of PS II photochemical efficiency

The switching from one light quality to another did not affect the general tendencies on the fluorescence response of the leaves. Under a given *PPFD*, in both species, the quantum yield $\Delta F/F_{m'}$ decreased when predawn leaf water potential declined (fig 2). Nevertheless, in *Q ilex*, this effect was not observed at low irradiance (< 300 μmol m^{-2} s^{-1}) between –0.2
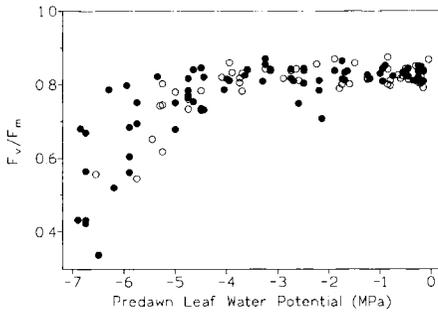
Fig 1. Relationships between predawn leaf water potential and maximal photochemical efficiency of PS II in the dark (F_v/F_m) for 8-year-old seedlings of *Q ilex* (closed circles) and *Q pubescens* (open circles).

and −1.7 MPa. In this species, at an irradiance level of 1 000 μmol m^{-2} s^{-1}, $\Delta F/F_m'$ was lowered only by 12% between −0.2 and −1.7 MPa. However, for the same irradiance, decrease of $\Delta F/F_m'$ rose above 75% between −1.7 and −5.1 MPa. Observed values were not significantly different for *Q pubescens* (15 and 72%). For the lowest values of leaf water potential (*Q ilex*: −5.1 MPa; *Q pubescens*: −4.5 MPa), the irradiances related to a lowering of 50% of the PS II photochemical yield were, respectively, 310 and 270 μmol m^{-2} s^{-1} for *Q ilex* and *Q pubescens*.

Diurnal course of photochemical efficiency of PS II of 30 min dark-adapted leaves

Diurnal course of F_v/F_m was also analysed during a typical sunny summer day, incident global radiation reaching 850 Wm^{-2}, minimal and maximal air temperature being 20 and 29 °C, respectively, relative humidity covering the range of 58 to 83%. The courses of this ratio displayed the same shape for *Q ilex* and *Q pubescens* (fig 3). A diurnal decline was observed in relation to

the level of drought: very stressed seedlings showed a sharper decline. *Q ilex* exhibited the beginning of a recovery at the end of the afternoon (around 1700 hours TST) in both control and stressed (−6.4 MPa) treatment; the complete recovery was observed around 1900 hours. *Q pubescens* showed only 70% of recovery in stressed (−6.1 MPa) plants at the same time. The delay between solar noon and F_v/F_m minimum seems to be water-stress dependent.

In addition, changes in F_0 and F_m were analysed (fig 4). Whatever the level of drought, F_0 did not show any clear diurnal time-course. In contrast, a diurnal decline was observed on F_m in relation to the value of predawn leaf water potential: F_m decreased to 60% in the afternoon between −0.5 and −6.4 MPa for *Q ilex*, and up to 80% between −0.5 and −6.1 MPa for *Q pubescens*.
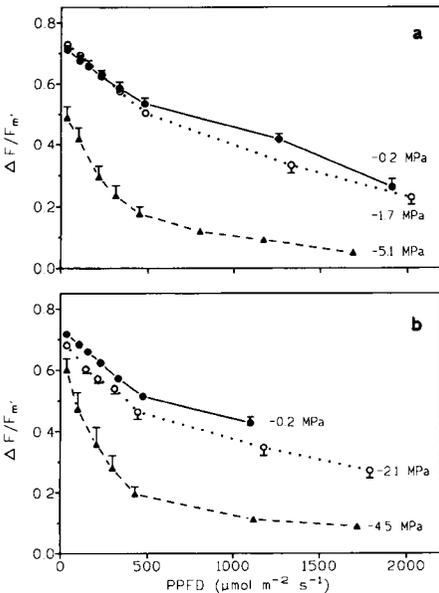
DISCUSSION AND CONCLUSION

The effects of leaf water potential on photochemical efficiency of PS II in two Mediterranean oak species were analysed. Chlorophyll fluorescence was used in understanding the mechanisms by which drought alters photosynthetic capacity. Due to a quasi-planophile distribution function of leaves of our young trees, considering only the adaxial surfaces was justified. Any other type of leaf distribution would require measurements on both sides (Araus and Hogan, 1994).

The responses of maximal photochemical efficiency of PS II measured predawn and photochemical efficiency of 30 min dark-adapted leaves need to be closely examined. Indeed, according to Demmig and Winter (1988), Krause and Weis (1991) and as observed by Epron et al (1992), a slow component of nonphotochemical quenching q_N ('photoinhibitory quenching') may exist and is not readily reversible in the dark. In this

case, only the amount of q_N which relaxed slowly was determined.

Observed values of F_v/F_m for well-watered plants are within the range of 0.75–0.85 given by Björkman and Demmig (1987). Our data also confirm the analysis of Genty et al (1987), Kaiser (1987), Cornic et al (1989), Epron et al (1992) and Havaux (1992), who showed the stability of the PS II when plants are faced with water stress. Epron et al (1993) observed similar responses of F_v/F_m to declining leaf water potential on three temperate oak species: Q petraea, Q rubra and Q cerris. The observed decreases began at stress intensities for which net assimilation rates were almost nil.

The predawn leaf water potential where the decline of F_v/F_m begins must be considered. Rambal and Debussche (1995)

compiled data of leaf water potential on Mediterranean evergreen mature oak species in six stands of Q ilex, three stands of Q coccifera, and various locations of Q dumosa, Q turbinella and Q agrifolia in California and Arizona. Regardless of the study
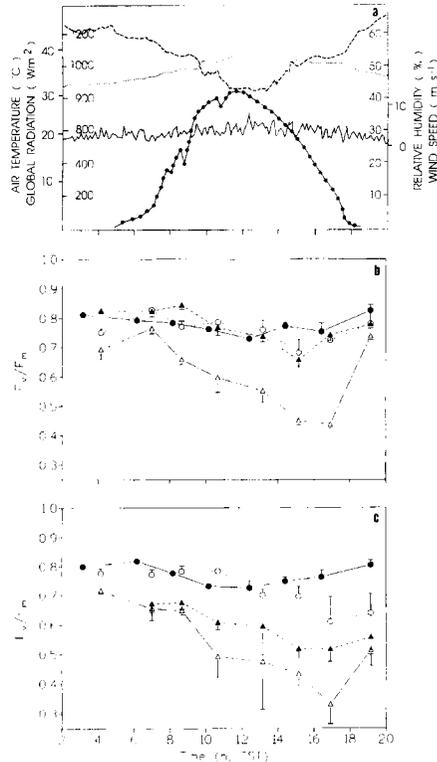

Fig 3. Daily course of: (**a**) climatic parameters recorded on 10 August 1993: (–•–) global radiation; (•••) air temperature; (– – –) relative humidity; (—) wind speed. (**b**) PS II photochemical efficiency of dark-adapted leaves of 5-year-old seedlings of Q ilex. Predawn leaf water potentials (closed circles) –0.5 MPa; (open circles) –1.0 MPa; (closed triangles) –2.0 MPa and (open triangles) –6.4 MPa. (**c**) PS II photochemical efficiency of dark-adapted leaves of 5-year-old seedlings of Q pubescens. Predawn leaf water potentials: (closed circles) –0.5 MPa; (open circles) –4.7 MPa; (closed triangles) –5.7 MPa; (open triangles) –6.1 MPa. Vertical lines indicate ± SE (n = 24 control seedlings, n = 6 water-stressed seedlings).

Fig 2. Relationships between PS II photochemical yield ($\Delta F/F_m$) and PPFD for different predawn leaf water potentials of 8-year-old seedlings of Q ilex (**a**) and Q pubescens (**b**). Vertical lines indicate ± SE (n = 3).

site and the species, predawn and minimum leaf water potentials were always greater than −3.8 and −4.4 MPa, the values reached by Q ilex in a northeast Spain location and by Q coccifera near Montpellier, France. The mean values were −3.3 MPa ($n = 10$, SD = 0.3 MPa) and −4.1 MPa ($n = 12$, SD = 0.2 MPa) for predawn and minimum leaf water potentials, respectively. Consequently, a decline of F_v/F_m would probably not be observed on mature oak in the field. This decline especially concerns the drought response at the seedling stage. Such a decline was also observed by Rambal et al (1994) on seedlings of Q suber grown in ambient and in 2 x CO_2. In a study on the comparative physiology and biogeography of Q gambelii, a broad-leaved deciduous

Fig 4. Daily course of initial fluorescence F_0 (symbols only) and maximal fluorescence F_m (symbols with lines) of dark-adapted leaves of 5-year-old seedlings of Q ilex (**a**) and Q pubescens (**b**). For each species, predawn leaf water potentials are the same as in figure 3. Vertical lines indicate ± SE ($n = 24$ control seedlings, $n = 6$ water-stressed seedlings).

oak, and Q turbinella, an evergreen oak, Neilson and Wullstein (1985) observed that seedlings of either species reached lethal drought stress at ca −7.0 MPa leaf water potential. This value is consistent with the results of Oechel (1988) on Q dumosa, a Mediterranean evergreen oak of California. Q dumosa seedlings tolerated a water potential of −6.2 MPa but survival decreased to 67% at a plant water potential of −8.7 MPa.

Increasing PPFD reduced the photochemical efficiency of PS II ($\Delta F/F_m'$) of both water-stressed and control leaves. The electron transport rate is strongly limited at the lowest leaf water potentials, probably due to stomatal closure and (or) direct inhibition of the dark reactions. Increased photorespiration has been suggested (Cornic and Briantais, 1991) as a possible mechanism of dissipating excess photochemical energy in water-stressed plants. In the present case, as shown by Epron et al (1993) on three deciduous oak species, the decrease in $\Delta F/F_m'$ indicates that photorespiration or cyclic electron transport around PS I may not have been sufficient to maintain high electron rates and that the diurnal transient decrease in F_v/F_m is at least partly due to an increase in thermal energy dissipation (Oquist and Huner, 1991).

Powles (1984) showed the susceptibility of PS II to photoinhibition. Furthermore, it is established that water deficits interact with light intensity to cause greater damage and larger reductions in growth than either factor on its own (Björkman and Powles, 1984). Ögren and Öquist (1985) showed that F_v/F_m is a good indicator of photoinhibitory damage when plants are subjected to a wide range of environmental stresses. A reversal of the quenching of F_v (expressed as a decrease in the ratio F_v/F_m) is correlated with the recovery from photoinhibition (Krause and Weis, 1991). The diurnal timecourses we observed in F_v/F_m reflect an alteration of the PS II activity.
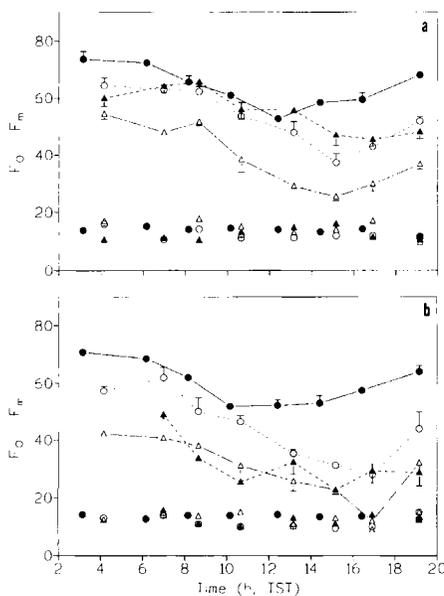
According to Krause (1988), a photoin-hibition-induced decline in photosynthetic rate at limiting light level results both from induction of photoprotective mechanisms (associated with the dissipation of excessive energy in the photosynthetic apparatus), as well as from photodamage to PS II. In examining changes in F_v/F_m , increases in F_0 are to be distinguished from decreases in F_m (Araus and Hogan, 1994). F_0 depends on the size of PS II chlorophyll antenna and on the functional integrity of PS II reaction centers (Krause and Weis, 1991). We did not observe any significant change in F_0; however, depending on the efficiencies in the trapping of excitation energy by the inhibited and by the photochemically active population of centers, changes in F_0 can be ambiguous (Giersch and Krause, 1991). Moreover, one can observe the recovery in Q ilex for the highest level of drought. Recovery is probably actual in Q pubescens as morning values were always above 0.7.

In contrast, a diurnal decrease in F_m was shown. This decrease cannot be produced by direct temperature effects on the photosynthetic apparatus. Indeed, Terzaghi et al (1989) observed the stability of F_m (and F_0) in a wide range of temperatures for 32 temperate and tropical species. The results of Dreyer et al (1992) for dehydrating leaves of Q ilex, Q pubescens, Q robur, Q petraea, Q rubra, Q cerris and five other temperate or tropical trees displayed similar features. An increase in nonphotochemical quenching may produce this decrease in F_m (Krause and Weis, 1991). This decline shows an increase in rate constant of thermal de-excitation and seems to assume the onset of photoprotective mechanisms (Butler, 1978; Demmig and Björkman, 1987; Osmond et al, 1987). Such mechanisms were observed by Epron et al (1992) on adult trees of Q petraea. Avoidance of permanent photodamages is probably an important component of water-stress tolerance in Q ilex and Q pubescens.

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