Photoinhibition and recovery of photosynthesis in *Coffea arabica* and *C. canephora*

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Abstract

Photosynthetic parameters were determined in disks from leaves of *C. arabica* cv. Red Catuaí and *C. canephora* cv. Kouillou grown in the field. Kouillou showed a relatively higher irradiance requirement for saturating photosynthesis, lower chlorophyll (Chl) content, and higher Chl *a/b* ratio than Catuaí. Photoinhibition of photosynthesis under bright irradiance was manifested by decreases in maximum photochemical efficiency (evaluated by the variable to maximum fluorescence ratio, F_v/F_m), as a consequence of an increased initial and a quenched maximum fluorescence. Restoration of F_v/F_m following photoinhibition in low irradiance was faster in Kouillou than in Catuaí. Chloramphenicol both accelerated photoinhibition (mainly in Kouillou) and blocked its recovery for at least 190 min in either cultivar. Photosynthetic oxygen evolution under photoinhibitory conditions was decreased by chloramphenicol; in control leaf disks this decrease was only observed in *C. arabica*, but with a rapid recovery within 90 min of low irradiance exposure. In both coffee cultivars, the depressed photochemical efficiency of photosystem 2 was not accompanied by a concomitant lowering in oxygen evolution during reversal from photoinhibition.

Additional key words: chloramphenicol; chlorophyll; fluorescence induction; irradiance; oxygen evolution; photochemical efficiency.

Introduction

Among more than 70 species of *Coffea*, only *C. arabica* and *C. canephora* are of economic importance, accounting for almost all the world's coffee production. These species were originally classified as shade obligatory; for this reason the early plantations were shaded by planting overstorey trees to simulate the conditions of its

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natural habitat. Gradually shade-adapted commercial coffee cultivars were grown in the open, in spite of their retaining some low-irradiance saturation characteristics (Kumar and Tieszen 1980, Fahl *et al.* 1994). Crop yields are yet great under high irradiance, although net photosynthetic rate is higher under moderate irradiance (Nutman 1937, Kumar and Tieszen 1980). For single leaves, the saturating irradiance shifts from about 300 to about 600 μmol(photon) m^{-2} s^{-1} in sun-grown arabica coffee (Kumar and Tieszen 1980, Fahl *et al.* 1994), with no depression in photosynthesis up to 1200-1300 μmol(photon) m^{-2} s^{-1} (Tió 1962, Kumar and Tieszen 1980). Because irradiances up to 2200 μmol(photon) m^{-2} s^{-1} in the field are common, it is assumed that they are high enough to oversaturate the photosynthetic apparatus of coffee leaves, and induce severe photoinhibition (Nunes *et al.* 1993).

Photoinhibition of photosynthesis has been characterized by a decrease in quantum efficiency of photosystem 2 (PS2), and often by a significant decline in rates of radiant energy-saturated photosynthesis, although lower PS2 efficiency does not always limit overall photosynthetic rates (Critchley and Russell 1994). When photosynthesis is radiant energy-saturated, photons are taken up by the chloroplasts at a faster rate than they can be dissipated *via* photosynthetic electron transport; the resulting excess energy must be dissipated to halt photodamage to PS2 (Bilger and Björkman 1994, Osmond 1994). The slowly reversible restoration of PS2 photochemistry after such damage can only occur *via* degradation and re-synthesis of the D1 protein and reassembly of the PS2 complex (Greer *et al.* 1993, Aro *et al.* 1994, Tyystjärvi and Aro 1996).

Very little information is available on photosynthesis of coffee plant at radiant energy oversaturation, as is usually found in the open, with photoinhibition. In this work an attempt was made to evaluate the significance of photoinhibition on the photosynthetic capacity of coffee tree. Two commercial species with different radiant energy requirements for saturating photosynthesis were used. In addition, the relative importance of the capacity of the PS2 repair cycle after photoinhibition of photosynthesis was studied.

Materials and methods

Leaves were obtained from two commercial coffee plantations, approximately six years old, one of *C. arabica* cv. Red Catuaí and the other of *C. canephora* cv. Kouillou. Young expanded leaves (the third pair from the apex) were detached from lateral branches oriented east-west and taken to the laboratory in plastic bags. They were placed in open desiccators containing, to the height of the base-plate, water, or a solution of 1 mol m^{-3} chloramphenicol (CAP), a compound blocking the synthesis of chloroplast-genome encoded D1 protein (Aro *et al.* 1994). The chemical was obtained from *Sigma Chemicals Co.*, St. Louis, MO, U.S.A. To hold the leaf upright and allow the uptake of water or solution, the petioles were inserted through the holes of the porcelain base-plate of the desiccator. The inhibitor was absorbed for 3 h at 20 °C in the darkness, in an open desiccator. Subsequently, disks from the middle of leaf blades were withdrawn for analysis.

The photoinhibition of photosynthesis was evaluated in leaf disks (1000 mm^2) floating adaxial side up on water or CAP solution, under a photosynthetic photon flux (PPF) of 1.6 mmol m^{-2} s^{-1} at 22 °C. The high irradiance was provided by two 1000 W halogen tubes and was filtered through a running water layer (0.10 m thick). The recovery from photoinhibition was followed for 190 min at 22 °C under a PPF of 40 μmol m^{-2} s^{-1}, with the disks in the same medium as used during the photoinhibitory treatment. Photoinhibition and subsequent recovery were determined by measuring the fast fluorescence emission kinetics and photosynthetic oxygen evolution. Using a portable fluorometer (*PEA, Hansatech*, Norfolk, U.K.), the initial (F_0) and maximum (F_m) fluorescence were gauged at room temperature in leaf disks previously adapted to darkness for 20 min, and the variable to maximum fluorescence ratio (F_v/F_m) was then determined. The maximum photosynthetic capacity (P_{Nmax}) was measured by oxygen evolution, and determined under saturating irradiance and CO_2 at 35 °C using a gas phase oxygen electrode (*LD2 Leaf Chamber, Hansatech*, Norfolk, U.K.). Measurements of fluorescence emission and P_{Nmax} were made in the same disk, but different disks were used along the time. Actinic irradiation was provided by a 100 W halogen-tungsten bulb in a *LS2 Hansatech* light housing, giving approximately 1.2 mmol(photon) m^{-2} s^{-1} at the leaf disk surface. The CO_2 concentration in the chamber (30 mmol mol^{-1}) was generated by 0.2 cm^3 of carbonate/bicarbonate buffer solution (1 kmol m^{-3}), according to Walker (1987). Such conditions were also employed for determining the irradiance for saturating photosynthesis from the response curve of P_{Nmax} to irradiance, according to Delieu and Walker (1981). Irradiance at the disk height was adjusted from 14 to 1070 μmol m^{-2} s^{-1} by using different combinations of neutral density filters.

Chlorophylls (Chl) were extracted by grinding leaves in 80 % (v/v) acetone/water, and then determined spectrophotometrically according to Lichtenthaler (1987).

Results and discussion

Kouillou showed a relatively higher radiant energy requirement for saturating photosynthesis than Catuaí (Table 1). In addition, the former cv. exhibited a lower Chl content and higher Chl *a/b* ratio (Table 1), characterizing a better sun-adapted species (Anderson *et al.* 1988) when compared with Catuaí. These differences were probably related with the extent of photoinhibition, as evaluated by the F_v/F_m ratio. From the slope of the curve of F_v/F_m *versus* the duration of photoinhibition, the estimated rate constant of the decrease of PS2 photochemical efficiency (evaluated by F_v/F_m) was 14 % higher in Catuaí than in Kouillou (Fig. 1). The decrease in F_v/F_m was linear along the photoinhibitory treatment, either in the control or in CAP-treated disks. The results with control disks were similar to those observed in *Monstera deliciosa* by Demmig and Björkman (1987) and in bean by Green *et al.* (1986), but contrasted with values from the microalga *Ulva rotundata* given by Franklin *et al.* (1992), who reported the occurrence of two first-order processes, characterized by an initial fast and a later slow fall in F_v. The decline in F_v/F_m with the duration of photoinhibition may represent a decrease in the number of PS2 reaction centres

involved in linear electron transport, with a simultaneous rise in the number of photochemically inactive centres (Krause and Weis 1991, Critchley and Russell 1994).

Table 1. Values of irradiation for saturating photosynthesis (I_s), total chlorophyll (Chl), and Chl *a*/*b* ratio of two coffee cultivars grown in the field. Means of six replicates ± SE.

Cultivar	I_s [μmol(photon) m^{-2} s^{-1}]	Chl (*a*+*b*) [g m^{-2}]	Chl *a*/*b*
Catuaí	652 ± 46	0.538 ± 0.042	1.72 ± 0.08
Kouillou	895 ± 90	0.412 ± 0.036	2.19 ± 0.12

Photoinhibition was clearly enhanced by CAP, in both Kouillou and Catuaí, with a greater sensitivity in the former (Fig. 1). In Catuaí, the rate constant of the decrease in F_v/F_m of CAP-treated leaf disks was only 17 % higher than that in the control disks (Fig. 1*A*), compared with 55 % in Kouillou (Fig. 1*B*). Thus, Catuaí appeared to have a lower D1 repair cycle capacity as a defence mechanism to protect against photoinhibition than Kouillou, as shown by its lower sensitivity of photoinhibition to CAP. In effect, as hypothesized by Öquist *et al.* (1992) and Tyystjärvi *et al.* (1992), plants with higher radiant energy requirements for saturating photosynthesis rely more on the D1 synthesis and the PS2 repair cycle to counteract photoinhibition than do plants with lesser radiant energy requirements. Hence, photoinhibition exacerbated by CAP could be an indication of a fast and tightly coordinated irradiance-induced turnover of D1 protein in the absence of the inhibitor (Leitsch *et al.* 1994, Schnettger *et al.* 1994), especially in plants with high radiant energy saturation characteristics.

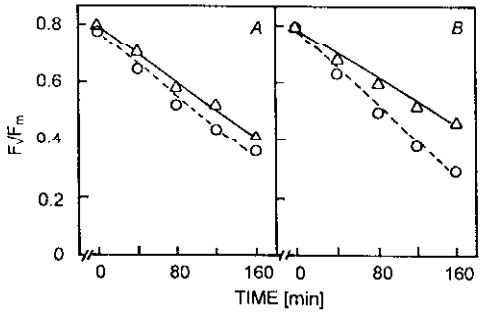

Fig. 1. Decrease of F_v/F_m, the variable to maximum fluorescence ratio [relative], of Catuaí (*A*) and Kouillou (*B*) coffee leaf disks, floating on water (*solid line*) and chloramphenicol solution (*dotted line*), as induced by a photoinhibitory treatment for up to 160 min. Photoinibition was carried out at 22 °C, under 1.6 mmol(photon) m^{-2} s^{-1}. Each symbol is the mean of six replicates; the standard error did not exceed 5 % of the mean value.

Fig. 2*A* shows an increase of about 19 and 16 % in F_0 in leaf disks of Catuaí and Kouillou, respectively, after a 2 h photoinhibitory treatment. These increases were nearly sustained during the recovery phase (especially in Catuaí) and might indicate an impaired efficiency of trapping energy in the PS2 reaction centres or a partial disconnection of the antennae from the centres (Somersalo and Krause 1990). The increase in F_0 paralleled a 63 % decrease in F_m (Fig. 2*B*); these changes together halved the photochemical efficiency of PS2 in both cultivars after high irradiance

(Fig. 3*C*). At the end of the leaf recovery phase from radiation stress, F_v/F_m reached values around 82 and 93 % when compared to control disks of Catuaí and Kouillou, respectively (Fig. 2*C*). In the former cv., the recovery of F_v/F_m was due exclusively to gains in F_m, as F_0 did not change, whereas in Kouillou F_0 decreased to similar values of that of non-photoinhibited disks at the end of the recovery phase.

Restoration of the F_v/F_m ratio after photoinhibition is biphasic, with a fast initial phase (20-60 min), and a second slow one in which the resynthesis of D1 protein is required for the PS2 cycle to function (Krause and Weis 1991, Schnettger *et al.* 1992, Leitsch *et al.* 1994, Rintamäki *et al.* 1994). In this work, however, a separation into fast and slow phases in the recovery of F_v/F_m was not observed. This was better demonstrated with CAP-treated leaf disks; the inhibitor completely blocked, for at least 190 min, the recovery of F_0, F_m, and F_v/F_m after photoinhibition (Fig. 2*D,E,F*). However, a relatively long time of darkness (20 min) was required for the fluorescence signals to relax; it is believed that the fast recovery phase usually found after photoinhibition is associated to protective energy dissipation, the so-called down-regulation of PS2, which chiefly relaxes in the darkness (Aro *et al.* 1994). Anyhow, the present results are consistent with those observed using lincomycin in pea (Aro *et al.* 1994) and CAP in the microalga *Ulva rotundata* (Franklin *et al.* 1992), but differ markedly from results obtained with streptomycin in spinach (Leitsch *et al.* 1994), and with CAP in bean (Greer *et al.* 1986), kiwifruit (Greer *et al.* 1993), and wheat (Hurry and Huner 1992). In these divergent cases, there was a substantial recovery of F_v/F_m but to a lesser extent than in control plants, probably as a consequence of the inactivation, degradation or even export of the inhibitor used (Greer *et al.* 1993).

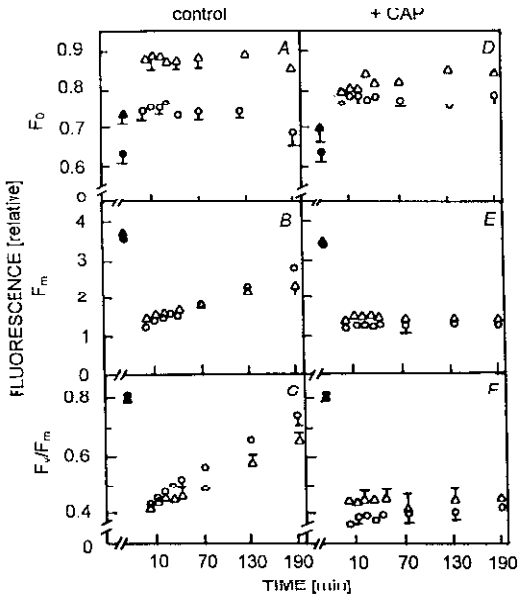

Fig. 2. Changes in initial (F_0) and maximum (F_m) fluorescence, and variable to maximum fluorescence ratio (F_v/F_m) of Catuaí (Δ) and Kouillou (○) coffee leaf disks floating on water (*A,B,C*) or choramphenicol solution (*D,E,F*) during recovery from 2 h photoinhibitory treatment, carried out under 40 μmol(photon) m^{-2} s^{-1} at 22 °C . Each symbol is the mean of six replicates and the bars indicate SE; when not shown, the SE was smaller than the symbols. Full symbols on the left of each figure indicate the values of the parameters measured before photoinhibitory treatment.

The low PS2 photochemical efficiency after photoinhibition and during its recovery did not limit P_{Nmax} in Kouillou, reducing it only slightly in Catuaí, but with a rapid recovery within 90 min from the end of exposure to high irradiance (Fig. 3*A*). These results are consistent with those of Anderson *et al.* (1993) who propose that less than 40 % of the functional PS2 centres in photoinhibited leaves can provide the same P_{Nmax} as completely functional centres in non-inhibited leaves. From this aspect, photoinhibition is not necessarily a destructive phenomenon, but may represent a strategy of acclimation of PS2, providing protection against high irradiances. Moreover, the present results are strictly valid for individual leaves; because many leaves are partly to heavily shaded in a canopy of coffee tree, any possible disadvantage from excessive irradiance may thus be minimized by taking into account the coffee tree as a whole.

Chloramphenicol strongly affected P_{Nmax} and fully inhibited its restoration after a recovery from photoinhibitory treatment (Fig. 3*B*), mainly in Catuaí, even when leaf disks were subjected to low irradiance, and with no apparent change in F_v/F_m. Streptomycin also promoted an inhibitory side-effect on photosynthesis under low irradiance before coffee leaf disks were exposed to bright irradiance, but to a slightly lesser degree than did CAP (results not shown). Schnettger *et al.* (1994) and Greer *et al.* (1993) also reported comparable results with CAP (at similar concentrations to that of this study) in leaves of spinach and kiwifruit, respectively. Such effects are consistent with a strong oxidizing power of CAP, acting possibly as PS1 electron acceptor and thus affecting the level of PS2 photoinhibition (Okada *et al.* 1991).

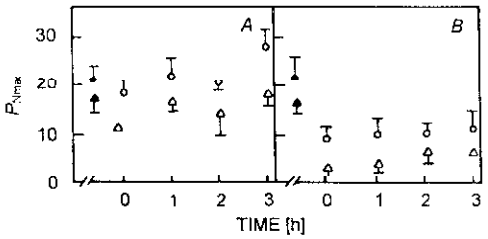

Fig. 3. Changes in photosynthetic capacity (P_{Nmax}, μmol m^{-2} s^{-1}), as detected by oxygen evolution, of Catuaí (Δ) and Kouillou (○) coffee leaf disks, floating on water (*A*) or chloramphenicol solution (*B*), during recovery from photoinhibition. See legend to Fig. 2 for details.

In summary, Kouillou apparently is a better sun-adapted species than Catuaí. This might rely upon the lesser sensitivity to photoinhibition and a relatively faster recovery of PS2 photochemistry after excess irradiance by Kouillou. This might be an indication of a greater capacity of Kouillou to repair photodamaged PS2 reaction centres; this assumption is strengthened by the restoration of F_0 (but not in Catuaí) at the end of recovery phase from photoinhibition and also by the effects of CAP. Because the decrease of the efficiency of PS2 did not limit P_{Nmax}, photoinhibition *per se* might not result in substantial depressions in the yield of coffee plant grown in the open. This can, at least partially, explain the successful cultivation of commercial cultivars under full sun, despite some shade adaptation attributes displayed by *Coffea* leaves.

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