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# Photosynthetic performance of two coffee species under drought

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## Abstract

*Coffea arabica* cv. Red Catuaí and *C. canephora* cv. Kouillou were grown in pots beneath a plastic shelter. When they were 14 months old, irrigation was withheld until the leaf pre-dawn water potential was about -1.5 and -2.7 MPa (designated mild and severe water stress, respectively). Under mild stress, net photosynthetic rate ($P_N$) decreased mainly as a consequence of stomatal limitations in Kouillou, whereas such decreases were dominated by non-stomatal limitations in Catuaí. Under severe drought, further decreases in $P_N$ and apparent quantum yield were not associated to any changes in stomatal conductance in either cultivar. Decreases were much more pronounced in Catuaí than in Kouillou, the latter maintained carbon gain at the expense of water conservation. In both cultivars the initial chlorophyll (Chl) fluorescence slightly increased with no changes in the quantum efficiency of photosystem 2. In response to rapidly imposed drought, the Chl content did not change while saccharide content increased and starch content decreased. Photoinhibition and recovery of photosynthesis, as evaluated by the ratio of variable to maximum fluorescence and by the photosynthetic $O_2$ evolution, were unaffected by mild drought stress. Photoinhibition was enhanced under severe water deficit, especially in Catuaí. In this cultivar the $O_2$ evolution did not resume upon reversal from photoinhibition, in contrast to the complete recovery in Kouillou.

*Additional key words*: chlorophyll; *Coffea arabica; Coffea canephora*; fluorescence kinetics; gas exchange; photoinhibition; saccharides; stomatal density and conductance; transpiration; water stress.

## Introduction

Soil water deficit is an environmental factor which largely decreases the productivity of coffee. Stomatal closure is often considered a primary indicator of water stress.

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Kumar and Tieszen (1980) report that stomatal limitation is the major factor restraining photosynthesis in coffee under drought. This agrees with results of Meinzer *et al.* (1990), who reported a close association between $P_N$ and stomatal conductance ($g_s$) in several coffee genotypes under different soil water contents. Nonetheless, factors other than $g_s$, such as mesophyll conductance and carboxylation efficiency, seem to control $P_N$ more directly when water availability is decreased (Rena *et al.* 1994). However, the understanding of most of these relationships is fragmentary and inconsistent in coffee, and can be merely circumstantial, due at least in part to methodological artefacts and lack of standardization of the measurements.

In this study, the relationships between leaf water status and some photosynthetic parameters were evaluated in one cultivar of *C. arabica* and another of *C. canephora*. The effects of high irradiance when associated with water deficit were also investigated.

## Materials and methods

The experiments were carried out with 14-month-old coffee plants (*C. arabica* L. cv. Red Catuaí and *C. canephora* Pierre cv. Kouillou), which showed potential differences in drought sensitivity (Da Matta *et al.* 1993, Maestri *et al.* 1995). They were grown in 6 000 $cm^3$ pots with an unlimited nutrient supply beneath a plastic shelter, and exposed to a maximum photosynthetic photon flux (PPF) of about 0.7 mmol $m^{-2}$ $s^{-1}$.

Water deficit was imposed by withholding watering. Pot soil surfaces were covered with grass mulch and a plastic sheet to minimize evaporation, thus allowing a slower establishment of the water stress. Photosynthetic parameters were estimated in fully expanded leaves of the third or fourth leaf pair from the apex on lateral branches. The measurements were made when leaves attained pre-dawn water potentials, $\Psi_{pd}$, of $-1.5\pm0.17$ (mild) and $-2.7\pm0.34$ MPa (severe, recoverable stress), which were achieved 4 and 6 d, and 6 and 9 d after suspension of irrigation, in cvs. Kouillou and Catuaí, respectively.

A portable fluorometer (*PEA, Hansatech*, Norfolk, U.K.) was used to measure fast fluorescence emission. The initial ($F_0$) and maximum ($F_m$) fluorescence were measured in the morning in attached leaves previously adapted to darkness for 30 min at room temperature, and the ratio of variable to maximum fluorescence ($F_v/F_m$) was then estimated.

Stomatal conductance to water vapour ($g_s$), internal $CO_2$ concentration ($C_i$), transpiration rate ($E$), and net $CO_2$ assimilation rate ($P_N$) were measured under artificial, saturating PPF (0.85-0.90 mmol $m^{-2}$ $s^{-1}$) at an ambient $CO_2$ concentration of about 355 µmol $mol^{-1}$, with a portable open-system infrared gas analyser and a Parkinson leaf chamber (*LCA-2, Analytical Development Co.*, Kings Lynn, U.K.). Measurements were made in the morning when the plants had already been exposed for about 1 h to artificial, moderate PPF (0.30-0.35 mmol $m^{-2}$ $s^{-1}$). During these measurements, the air relative humidity was about 80 %, and the leaf temperature ranged from 22 to 24 °C.

To determine the $CO_2$ concentration sufficient to completely overcome stomatally imposed diffusive resistance in water-stressed plants, four $CO_2$ concentrations were tested: 32 mmol mol$^{-1}$, generated by 0.2 cm$^3$ of 1 kmol mol$^{-1}$ carbonate/bicarbonate buffer solution (as quantified by gas chromatography), and 50, 100, and 150 mmol mol$^{-1}$, obtained from commercial gas mixtures which besides $CO_2$ contained 210 mmol($O_2$) mol$^{-1}$ plus $N_2$ as a balancing gas (*White Martins*, Brazil). Photosynthetic $O_2$ evolution ($P_{Nmax}$) decreased by about 30 % in 32 and 150 mmol($CO_2$) mol$^{-1}$; in the first case, this was probably caused by non-saturation of the photosynthetic machinery, and in the second case probably by leaf-cell acidification (Chaves 1991, Ögren and Evans 1993). Subsequently, $P_{Nmax}$ was measured in 100 mmol($CO_2$) mol$^{-1}$.

Oxygen evolution was measured in leaf disks (1000 mm$^2$) under saturating irradiance at 35 °C using a gas-phase $O_2$ electrode in a leaf chamber (*LD2, Hansatech*, Norfolk, U.K.). Radiant energy was supplied from a 100 W halogen-tungsten bulb mounted in a light source (*LS2, Hansatech*) giving approximately 1.2 mmol(photon) m$^{-2}$ s$^{-1}$ at the surface of the leaf disk. These conditions were also used for determining the apparent quantum yield of the photosynthetic $O_2$ evolution ($\Phi$), which was estimated from the linear portion of the initial slope of the response curve of $P_{Nmax}$ to irradiance, according to Delieu and Walker (1981). The PPF at the disk surface was adjusted from 14 to 1070 µmol m$^{-2}$ s$^{-1}$ by using different combinations of neutral density filters.

Photoinhibition of photosynthesis was evaluated using 1000 mm$^2$ leaf disks floating adaxial side up on water, under a PPF of 1.5 mmol m$^{-2}$ s$^{-1}$, for 2 h at 22 °C. High irradiance was provided by two 1000 W halogen tubes, and the radiation was filtered through a running water layer (0.10 m thick). The recovery from photoinhibition was assessed during 3 h at 20 °C, under a PPF of 50 µmol m$^{-2}$ s$^{-1}$ by measuring fast fluorescence emission and $P_{Nmax}$.

Chlorophylls were extracted by grinding leaves in 80 % (v/v) acetone/water, and then determined spectrophotometrically according to Lichtenthaler (1987). Starch and total saccharides' contents were quantified by the method of McCready *et al.* (1950).

Abaxial epidermal impressions from the middle of the leaves were obtained with *Krylon* (*Bordon*, Ohio, U.S.A.); the impressions were evaluated microscopically to determine the stomatal density (SD).

## Results and discussion

The photosynthetic performance of Kouillou was clearly superior to that of Catuaí, either in unstressed or water-stressed plants (Table 1). Kouillou had a higher SD, which might account for the doubling in $g_s$ and in $P_N$ compared to Catuaí, under full hydration. Both Kouillou and Catuaí had a similar $P_{Nmax}$, but $\Phi$ was 21 % higher in the former.

Under moderate water stress, $g_s$ decreased by 45 % in Kouillou and by 75 % in Catuaí; such reductions were matched by a decline of 58 % in $E$ in Catuaí, with no significant decrease in Kouillou (Table 1). Thus, $g_s$ in coffee was more sensitive to early drought than was $E$, as had already been reviewed by Nunes (1976). The $E$ in Kouillou was lowered by about 45 % only when water deficit was severe, but to a lesser magnitude than in Catuaí (Table 1). As water deficit is often accompanied by elevated temperatures, the higher $E$ shown by Kouillou ensuing from the higher $g_s$ probably increases evaporative cooling and lowers its leaf temperature; this could be an ecological adaptation of Kouillou, reflecting its origin from African equatorial lowlands, in contrast to highlands' origin of *C. arabica*.

Table 1. Stomatal density (SD) [$\mathrm{mm}^{-2}$], transpiration rate ($E$) [mol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$], stomatal conductance to water vapour ($g_s$) [mmol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$], internal $CO_2$ concentration ($C_i$) [µmol $\mathrm{mol}^{-1}$], net carbon assimilation ($P_N$) [µmol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$], photosynthetic oxygen evolution as measured under saturating irradiance and $CO_2$ ($P_{\mathrm{Nmax}}$) [µmol $\mathrm{m}^{-2}\,\mathrm{s}^{-1}$], apparent quantum yield ($\Phi$) [mmol($O_2$) $\mathrm{mol}^{-1}$(photon)], initial ($F_0$) and maximum ($F_m$) fluorescence, ratio of variable to maximum fluorescence ($F_v/F_m$), and contents of chlorophylls (Chl) [g $\mathrm{m}^{-2}$], total saccharides (Sacch), and starch [g $\mathrm{kg}^{-1}$(DM)] measured in coffee leaves under full irrigation, mild, and severe water stress (pre-dawn water potentials, $\Psi_{\mathrm{pd}}$ = -0.2, -1.5, and -2.7 MPa, respectively). Means ± SE of six replicates.

| | Cv. Catuai | | | Cv. Kouillou | | |
|---|---|---|---|---|---|---|
| $\Psi_{\mathrm{pd}}$ | -0.2 | -1.5 | -2.7 | -0.2 | -1.5 | -2.7 |
| SD | 214 ± 31 | | | 373 ± 38 | | |
| $E$ | 1.2 ± 0.3 | 0.5 ± 0.1 | 0.4 ± 0.1 | 1.8 ± 0.2 | 1.4 ± 0.3 | 1.0 ± 0.1 |
| $g_s$ | 40 ± 10 | 10 ± 0 | 10 ± 0 | 90 ± 10 | 50 ± 10 | 40 ± 10 |
| $C_i$ | 265 ± 15 | 329 ± 11 | 349 ± 16 | 264 ± 15 | 276 ± 18 | 303 ± 11 |
| $P_N$ | 3.0 ± 0.6 | 0.9 ± 0.2 | -0.3 ± 0.2 | 5.7 ± 0.7 | 2.8 ± 0.8 | 1.5 ± 0.4 |
| $P_{\mathrm{Nmax}}$ | 27.8 ± 5.6 | 12.4 ± 1.8 | 5.1 ± 1.3 | 29.8 ± 3.5 | 25.5 ± 2.9 | 17.8 ± 2.2 |
| $\Phi$ | 77 ± 5 | 37 ± 5 | 23 ± 3 | 93 ± 7 | 71 ± 6 | 54 ± 6 |
| $F_0$ | 644 ± 14 | 656 ± 23 | 718 ± 46 | 592 ± 31 | 616 ± 44 | 661 ± 22 |
| $F_m$ | 3613 ± 114 | 3743 ± 187 | 3939 ± 247 | 3389 ± 227 | 3493 ± 210 | 3655 ± 295 |
| $F_v/F_m$ | 0.82 ± 0.01 | 0.82 ± 0.01 | 0.82 ± 0.01 | 0.83 ± 0.01 | 0.82 ± 0.00 | 0.82 ± 0.01 |
| Sacch | 29.7 ± 2.5 | 32.5 ± 1.0 | 37.9 ± 2.6 | 28.8 ± 1.7 | 37.0 ± 3.3 | 42.3 ± 2.8 |
| Starch | 24.7 ± 2.2 | 21.6 ± 2.2 | 15.3 ± 2.4 | 26.2 ± 3.1 | 18.4 ± 1.7 | 11.1 ± 0.8 |
| Chl | 0.59 ± 0.04 | 0.60 ± 0.05 | 0.57 ± 0.05 | 0.53 ± 0.04 | 0.50 ± 0.05 | 0.51 ± 0.06 |

Decreases in $g_s$ were associated with a decline of 51 and 70 % in $P_N$ in Kouillou and in Catuaí, respectively. In the former, a 24 % drop in $\Phi$ paralleled a 15 % non-significant reduction in $P_{\mathrm{Nmax}}$, suggesting that the decrease in $P_N$ was mainly determined by stomatal limitation. Thus, the photosynthetic machinery of Kouillou was relatively more resistant to early stages of leaf desiccation; it was able to function at high rates provided there was a sufficient supply of $CO_2$ to the chloroplasts, as shown by Grieu *et al.* (1995) with *Trifolium repens*. In Catuaí, a decrease around 55 % in $P_{\mathrm{Nmax}}$ and $\Phi$ probably resulted from non-stomatal limitations, as the measurements were made under a saturating $CO_2$ concentration. This indicates that the photosynthetic apparatus of Catuaí has a lower tolerance to leaf dehydration than has that of Kouillou.

Under severe drought, the decrease in $P_N$ was not associated with further decreases in $g_s$ in either cultivar (Table 1). Notwithstanding, $C_i$ increased by 15 and 32 % in Kouillou and Catuaí, respectively, probably as a result of increased photorespiration and/or some damage to the photosynthetic machinery. Even if a patchy stomatal closure occurred that could induce an overestimation of $C_i$ (Parkhurst 1994), the saturating $CO_2$ concentration used was unable to halt further decreases in $P_{Nmax}$ and $\Phi$ (which were much more pronounced in Catuaí). Therefore, the major limitation to photosynthesis under severe drought must be ascribed to direct effects of water stress on the photosynthetic reactions at the chloroplast level.

There were differences in the water loss rate of Kouillou and Catuaí; in the former cultivar, $\Psi_{pd}$ attained values of about -2.7 MPa after withholding irrigation for 6 d. In Catuaí this value was attained after 9 d, probably as a result of lower SD and $g_s$ when compared to Kouillou. However, the lower $g_s$ did not account for a larger instantaneous water use efficiency ($P_N/E$) in Catuaí since its $P_N$ was low, as can be inferred from Table 1. In Kouillou, $P_N$ was maintained at the expense of water conservation. Kouillou has a greater potential for osmotic adjustment (Da Matta *et al.* 1993), a higher capacity to accumulate compatible solutes (Maestri *et al.* 1995), and a deeper root system (Ramos and Lima 1980) than has Catuaí. These features and a larger $P_N/E$ in Kouillou may be an advantage for productivity, at least under rapidly imposed drought.

Initial fluorescence slightly increased (by about 12 %) in severely droughted coffee plants (Table 1), indicating a small decrease in the non-photochemical dissipation of radiant energy by the antennae or by photosystem 2 (PS2) reaction centres (Havaux 1994). Changes in $F_0$ paralleled a non-significant rise in $F_m$ that contributed to maintain $F_v/F_m$ ratio unchanged (Table 1). These results corroborate several reports concerning the high tolerance of PS2 photochemical efficiency to water deficit (*e.g.*, Havaux 1992, Benveniste-Levkovitz *et al.* 1993, Cornic 1994, Eastman and Camm 1995), and therefore they did not match the marked differences in $P_{Nmax}$ and $\Phi$ shown in Catuaí and Kouillou under drought (Table 1).

The Chl content did not change under water stress in either cultivar (Table 1), as also noted in four *C. arabica* genotypes subjected to soil water stress (Almeida and Maestri 1996), despite Chl being highly sensitive to drought (Castrillo and Trujillo 1994, Tuba *et al.* 1996). Similar results have been reported rarely (*e.g.*, Kulshreshtha *et al.* 1987). Curiously, when the cv. Kouillou was subjected to water stress under 80 $\mu$mol(photon) $m^{-2}$ $s^{-1}$ (7 d without irrigation, $\Psi_{pd}$ = -2.3 MPa), the Chl content increased by 37 %, especially that of Chl *b*. Coffee leaves maintain a high relative water content under dehydrating conditions (Josis *et al.* 1983, Da Matta *et al.* 1993), about 93 % for a water potential of -2.3 MPa, as found in our laboratory. This may explain the above results, because a decreased water content is required for Chl degradation (Haspel-Horvatovič and Holúbková 1981).

A decreased starch content was closely associated with an increased total saccharide concentration under water stress, with Kouillou showing more pronounced changes (Table 1). It is likely that a rise in saccharide content contributes to the osmotical adjustment observed in coffee under water deficit (Da Matta *et al.* 1993; see also reviews by Chaves 1991, Pereira and Chaves 1993). These results do not support the assumption of Da Matta *et al.* (1993) that a lowering of specific leaf area in some droughted coffee genotypes was caused by an accumulation of leaf starch level. Indeed, there was no change in specific leaf area associated with water stress in the present study.

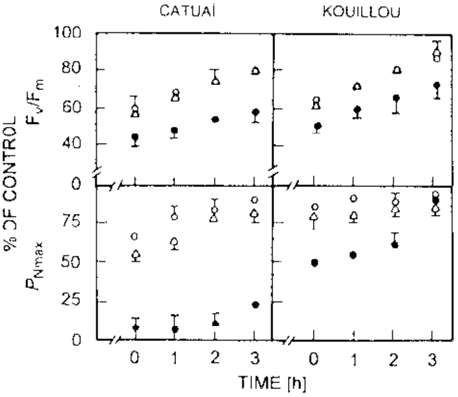

Fig. 1. Changes in the ratio of variable to maximum fluorescence ($F_v/F_m$) and photosynthetic oxygen evolution ($P_{Nmax}$) measured in leaves from coffee plants under full irrigation (○), mild (Δ), and severe (●) water stress, during recovery from photoinhibition, carried out under 50 μmol(photon) $m^{-2}$ $s^{-1}$ at 20 °C [previous high irradiance treatment was assessed under 1.5 mmol(photon) $m^{-2}$ $s^{-1}$ during 2 h at 22 °C]. Each symbol represents the mean of five replicates, and the bars indicate the SE; when not shown, the SE was smaller than the symbols. Values are presented as % of control (before the photoinhibitory treatment). $F_v/F_m$ was 0.82 ± 0.01 irrespective of the cultivar and watering regime. $P_{Nmax}$ was 25.9 ± 3.8, 11.4 ± 2.0, 6.4 ± 0.9 μmol($O_2$) $m^{-2}$ $s^{-1}$ for Catuaí leaves from irrigated, mildly, and severely droughted plants, and 30.3 ± 2.9, 23.6 ± 4.0, and 18.0 ± 2.7 μmol($O_2$) $m^{-2}$ $s^{-1}$ for Kouillou leaves, in that order.

Photoinhibition and recovery of photosynthesis, as evaluated by the $F_v/F_m$ ratio and by $P_{Nmax}$, were unaffected by moderate water stress in both cultivars (Fig. 1). The $F_v/F_m$ and $P_{Nmax}$ were not related, as also noted by Da Matta and Maestri (unpublished). As the fluorescence measurements showed that the efficiency of PS2 photochemistry was nearly restored during recovery from photoinhibition, it was assumed that no sustained photodamage occurred in the PS2 reaction centres. Photoinhibition was enhanced when the drought was severe; in the cv. Catuaí (Fig. 1) the $F_v/F_m$ decreased by 55 % after exposure to high irradiance, compared with 48 % in the cv. Kouillou. At the end of the recovery from excessive irradiance, $F_v/F_m$ reached values of about 58 and 73 % of those in non-photoinhibited leaf disks of Catuaí and Kouillou, respectively. Oxygen evolution did not recover in Catuaí on the reversal from photoinhibition, in contrast to the complete reversal in Kouillou. These results indicate that the photosynthetic apparatus of Kouillou is more tolerant of a water shortage associated with photoinhibitory irradiance. This might contribute to the larger crop yield of most *C. canephora* cvs. usually found under field conditions, as already suggested by the results of Table 1.

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