Photosynthesis and photoinhibition
in two xerophytic shrubs during drought

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Abstract
Seasonal changes in water relations, net photosynthetic rate ($P_N$), and fluorescence of chlorophyll (Chl) $a$ of two perennial C$_3$ deciduous shrubs, Ipomoea carnea and Jatropha gossypifolia, growing in a thorn scrub in Venezuela were studied in order to establish the possible occurrence of photoinhibition during dry season and determine whether changes in photochemical activity of photosystem 2 (PS2) may explain variations of $P_N$ in these species. Leaf water potential ($\psi$) decreased from –0.2 to –2.1 MPa during drought in both species. The $P_N$ decreased with $\psi$ in I. carnea and J. gossypifolia by 64 and 74 %, respectively. Carboxylation efficiency (CE) decreased by more than 50 and 70 % in I. carnea and J. gossypifolia, respectively. In I. carnea, relative stomatal limitation ($L_s$) increased by 17 % and mesophyll limitation ($L_m$) by 65 % during drought, while in J. gossypifolia $L_s$ decreased by 27 % and $L_m$ increased by 51 %. Drought caused a reduction in quantum yield of PS2 ($\phi_{PS2}$) in both species. Drought affected the capacity of energy dissipation of leaves, judging from the changes in the photochemical ($q_P$) and non-photochemical quenching (NPQ) coefficients. Photoinhibition during drought in I. carnea and J. gossypifolia was evidenced in the field by a drop in the maximum quantum yield of PS2 ($F_v/F_m$) below 0.8 and also by non-coordinated changes in $\phi_{PS2}$ and quantum yield of non-photochemical excitation quenching ($Y_n$). Total soluble protein content on an area basis increased with $\psi$ but the ribulose-1,5-bisphosphate carboxylase/oxygenase content remained unchanged. A reduction of total Chl content with drought was observed. Hence in the species studied photoinhibition occurred, which imposed an important limitation on carbon assimilation during drought.

Additional key words: fluorescence; Ipomoea carnea; Jatropha gossypifolia; net photosynthetic rate; quantum yield; photosystem 2; stomatal conductance; water stress; xerophytes.

Introduction
Drought limits plant production in many parts of the world. In many species, reductions in stomatal conductance ($g_s$) with increased water stress may limit diffusion of CO$_2$ to chloroplasts and consequently net photosynthetic rate ($P_N$) (Cornic 1994, 2000, Lawlor 2002). Water stress may also inhibit some metabolic processes, such as RuBP production, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activity, and ATP production (Giménez et al. 1992, Tezara and Lawlor 1995, Tezara et al. 1999, Lawlor and Cornic 2002) and/or photosystem 2 (PS2) activity and electron transport (Tezara et al. 2003).

During water deficit, restricted CO$_2$ availability due to stomatal closure may lead to increased susceptibility to

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Abbreviations: $C_i$ = intercellular CO$_2$ concentration; CE = carboxylation efficiency; Chl = chlorophyll; $F_v/F_m$ = maximum quantum yield of photosystem 2; $g_s$ = stomatal conductance; $J$ = total electron-transport rate in leaves; $L_s$ = relative stomatal limitation; $L_m$ = relative mesophyll limitation; NPQ = non-photochemical quenching coefficient; $PS$ = photosystem; $q_P$ = photochemical quenching coefficient of chlorophyll $a$ fluorescence; $P_N$ = net photosynthetic rate; $P_{N_{sat}}$ = CO$_2$-saturated $P_N$; PPFD = photosynthetic photon flux density; RuBPCO = ribulose-1,5-bisphosphate carboxylase/oxygenase; TSP = total soluble protein; $Y_n$ = quantum yield of non-photochemical quenching; $\phi_{PS2}$ = relative quantum yield of photosystem 2; $\psi$ = morning leaf water potential; $\psi_s$ = osmotic potential.

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photodamage (Powles 1984) but some studies have shown that such damage does not occur during water deficit under natural conditions (Epron et al. 1992), which suggests that the mechanisms of protection against an excess of absorbed excitation energy are efficient.

Photoinhibition is a slowly reversible decline of maximum quantum yield of photosynthesis \( (F_{v}/F_{m}) \) associated with loss of PS2 activity (Powles 1984, Long et al. 1994). Adverse environmental conditions, such as high temperature and water stress that strongly limit photosynthetic carbon metabolism can intensify photoinhibition (Long et al. 1994). Photoinhibition is characterized by parallel decreases in \( P_{N} \) and quantum yield of photosystem 2 \( (\varphi_{PS2}) \) and is accompanied by a decline in \( F_{v}/F_{m} \) and an increase in minimal chlorophyll (Chl) fluorescence, \( F_{0} \) (Osmond and Grace 1995). After prolonged exposure to excess photons, the rate of photon-saturated \( P_{N} \) decreases (Long et al. 1994). During the dry season in tropical environments, high irradiance, high temperature, and water deficit can cause photoinhibition, determining a reduction in photosynthetic capacity of the plant (Powles 1984).

The major process involved in protection against photodamage is probably the increase in non-photothermal energy dissipation measured as \( q_{v} \), i.e. alternative mechanisms of excess electron dissipation, such as the violaxanthin cycle (Björkman and Demmig-Adams 1994), which reduces \( \varphi_{PS2} \) in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Weis and Berry 1987, Krause and Weis 1991). Furthermore, photorespiration in C3 plants has been considered as an alternative sink for light-induced electron flow during periods of restricted CO2 availability in the chloroplasts and high irradiance (Stuhlfauth et al. 1990, Lawlor and Cornic 2002). The photoprotective function of photorespiration has been well established in tobacco (Kozaki and Takeda 1996). In C3 plants subjected to different degrees of drought, more than 90% of the total energy absorbed by leaves is dissipated by the sum of thermal dissipation, photorespiration, and photosynthesis (Flexas and Medrano 2002).

Both \( \varphi_{PS2} \) and photon-saturated \( P_{N} \) decreased with increasing water deficit in Lycium nodosum, a spiny shrub sympatric to Ipomea carnea and Jatropha gossypifolia (Tezara et al. 2003). This suggests that either light-harvesting or electron transport were affected by water deficit (Tezara et al. 2003). The \( q_{v} \) at steady-state photosynthesis was not affected by water deficit in sunflower (Scheuermann et al. 1991) and wheat (Biehler and Fock 1993, 1996).

The measurement of Chl \( a \) fluorescence is a useful tool for quantification of the effect of stress on photosynthesis (Schreiber and Bilger 1987, Krause and Weis 1991, Schreiber et al. 1994). The \( F_{v}/F_{m} \) is one of the fluorescence parameters most widely used to estimate the degree of photoinhibition (Ball et al. 1994, Osmond and Grace 1995, Solhaug and Haugen 1998). A decrease in \( \varphi_{PS2} \) associated with an increase in the quantum yield of non-photothermal quenching \( (Y_{n}) \), i.e. \( \varphi_{PS2} + Y_{n} = 0.8 \), suggests an efficient control of the lifetime of excitation that minimizes the formation of triplet-state Chl, the production of singlet oxygen and radicals, and the occurrence of photoinhibition. Changes in the degree of coordination between changes in \( \varphi_{PS2} \) and \( Y_{n} \) could indicate photoinhibition in species subjected to drought (Laisk et al. 1997). The Stern-Volmer coefficient of non-photothermal quenching (NPQ), frequently used as an indicator of the excess radiant energy dissipation by heat in the PS2 antenna complex in the light-adapted state (Björkman and Demmig-Adams 1994), has the advantage of being an indicator of non-photothermal quenching without a measurement of \( F_{0} \) or minimum fluorescence at steady state photosynthesis, \( F_{0} \) (Buschmann 1999).

In a previous study, the effects of drought on \( P_{N} \) and \( g_{s} \) were examined in relation to different pathways of CO2 fixation in species from a semiarid ecosystem (Herrera et al. 1994, Tezara et al. 1998). In I. carnea and J. gossypifolia, stomatal closure was responsible for a 90% decline in \( P_{N} \) as \( g_{s} \) decreased from −0.3 to −2.0 MPa, relative stomatal limitation increasing by 63%, while in J. gossypifolia, \( L_{c} \) remained nearly constant (Tezara et al. 1998). However, the quantification of the relative contribution of different photon energy dissipation processes to total dissipation under different drought conditions was not assessed in either that study or in other ones (Flexas and Medrano 2002). For this reason, we measured water relations, gas exchange, and parameters of Chl \( a \) fluorescence in two xerophytic C3 shrubs, I. carnea and J. gossypifolia, in order to establish the possible occurrence of photoinhibition during drought and relate changes in \( P_{N} \) to PS2 activity. The main objectives of this research were to determine whether changes in photochemical activity of PS2 and mesophyll limitations explain the reductions in \( P_{N} \) in these xerophytic plants growing in the field and under greenhouse conditions and to establish the relative importance of stomatal and metabolic regulation of \( P_{N} \) in relation to progressive water deficit.

Materials and methods

The study site was a thorn scrub near the city of Coro in Venezuela (11°25’N–69°36’W) at ca. 20 m a.s.l. Two C3 deciduous shrubs, I. carnea Jacq. and J. gossypifolia L., were studied during the rainy and dry seasons (1999–2000) under natural conditions.

Greenhouse experiments: Plants collected in the field were grown in 15 000-cm3 pots filled with commercial garden fertile soil in the greenhouse in Caracas (ca. 1 000 m a.s.l.). Daily watering for one month ensured the production of abundant foliage; plants were fertilized.
weekly with a commercial fertilizer (N : P : K = 15 : 15 : 15). Ten plans were daily watered (control plants) and ten subjected to water deficit, which was induced by withholding irrigation during 28 d. Plants were grown under natural irradiance. Photosynthetic photon flux density (PPFD) between 08:00 and 13:00 h ranged from 200 to 1500 µmol m$^{-2}$ s$^{-1}$, temperature from 22±2 to 36±2 °C, and relative humidity from 70±3 to 42±4 %.

**Microclimatic parameters** were measured every hour. PPFD was measured with a quantum sensor model LI-190-S connected to a meter model LI-185 (LI-COR, Lincoln, NE, USA). Air temperature was measured with YSI 400 thermistors connected to a telethermometer (Yellow Springs Instruments, Ohio, USA), and relative humidity with a hair strand hygrometer (Abbeon model AB167B, Santa Barbara, CA, USA).

**Water status**: Xylem water potential ($\psi$) was measured between 06:00 and 06:30 h on four youngest fully expanded leaves using a pressure chamber (PMS, Corvallis, Oregon, USA). Osmotic potential ($\psi_o$) was measured in the sap expressed from frozen and thawed leaves previously used for the determination of $\psi$, using a Wescor 5000 osmometer (Wescor, Logan, Utah, USA); values were not corrected for apoplastic water content. Soil water content in the field was determined in four samples taken at a 30-cm depth, placed in metal containers, weighed, dried at 100 °C for 72 h, and re-weighed.

**Gas exchange** was measured with a portable IRGA model CIRAS 1 in conjunction with a PLC(B) assimilation chamber (PP Systems, Hitchin, UK). Measurements were made at an [CO$_2$] = 350 µmol mol$^{-1}$ and a PPFD = 1 200±20 µmol m$^{-2}$ s$^{-1}$. Instantaneous $P_N$ was measured at 10:00–11:00 h, but the daily maximum $P_N$ was determined in a previous study (Tezara et al. 1998).

**Response curves of $P_N$ vs. intercellular [CO$_2$] ($C_i$)**: Under natural conditions the $P_N$-$C_i$ curves were done by increasing $C_i$ from 0 to 1 200 µmol mol$^{-1}$. The CO$_2$ was provided by a cylinder filled with pure gas inserted into the IRGA. The $P_N$-$C_i$ curves were fitted to the empirical equation $A = b + de^{C_i}$, where $b$ = CO$_2$-saturated $P_N$ and (b + d) = y-intercept (Tezara et al. 1998). Carboxylation efficiency (CE) was calculated from the initial slope of the curve. Measurement conditions were 1.7 kPa leaf-air water vapour concentration gradient, 32±2 °C leaf temperature, and 1 200±20 µmol m$^{-2}$ s$^{-1}$ PPFD. The relative stomatal limitation of the photosynthetic rate was calculated as $L_s = 100 \frac{(P_{N0} - P_N)}{P_{N0}}$, where $P_{N0}$ is the photosynthetic rate at $C_i = C_a$ (Farquhar and Sharkey 1982). The relative mesophyll limitation was calculated as $L_m = 100 \frac{(P_{Nc} - P_N)}{P_{Nc}}$ where $P_{Nc}$ is $P_N$ of control leaves at $C_i = 800$ µmol mol$^{-1}$, and $P_{Nc}$ the rate of stressed leaves at the same $C_i$ (Jacob and Lawlor 1991). Thus, $L_m$ is a measure of the capacity of the mesophyll to fix CO$_2$ at $C_i = 800$ µmol mol$^{-1}$ and it is zero in control leaves.

**Chl a fluorescence** of PS2 was measured on attached dark-adapted leaves ($n = 5$) with a mini-PAM fluorometer (Walz, Effeltrich, Germany) using the protocol described by Genty et al. (1989). $F_i/F_m$ was measured in situ at the minimum dawn PPFD. Irradiance dependence curves of linear electron transport rate ($J$), $\Phi_{PS2}$, and NPQ were done in leaves dark-adapted for at least 2 h by automatically raising the “actinic light” in eight consecutive steps at 2-min intervals. The $\Phi_{PS2}$ at steady state photosynthesis is defined as $\Phi_{PS2} = (F_m - F_i)/F_m$ according to Genty et al. (1989), where $F_i$ and $F_m$ are fluorescence at steady state photosynthesis and maximum fluorescence in the light, respectively. The quantum yield of non-photocatalytic quenching ($Y_q$) was calculated as $Y_q = (F_i/F_m) - (F_i/F_m)$ (Laik et al. 1997). Whole chain electron transport rate in the leaves ($J$) was estimated by the method of Krall and Edwards (1992) from the equation $J = \Phi_{PS2} PPFD a 0.5$, where $a$ is the fraction of incident PPFD absorbed by the leaf. Leaf absorptivity was measured ($n = 20$) using an integrating sphere model 1800-12 (LI-COR, Lincoln, NE, USA). The value of $a$, 0.80±0.01, did not change throughout the seasons.

**Biochemical determinations**: Total soluble protein content (TSP), ribulose-1,5-bisphosphate carboxylase/oxygenase (RubPco) content, and Chl content were determined in samples taken after gas exchange measurements by freeze clamping (~20 °C) the leaf section previously enclosed in the assimilation chamber (4 cm$^2$). Leaf samples were stored in liquid N$_2$ before determinations. RubPco was extracted at 0–4 °C in 1 cm$^3$ buffer (100 mol m$^{-3}$ bicine, pH 8.0, 20 mol m$^{-3}$ MgCl$_2$, 50 mol m$^{-3}$ mercaptoethanol), 10 mm$^{-3}$ of 40 mol m$^{-3}$ phenylmethysulphonyl fluoride, and 10 mg acid-washed sand. Leaf Chl content was determined after Bruinsma (1963) in acetone extracts. An aliquot of the crude extract was used to determine TSP by Coomasie blue binding (Bradford 1976) with bovine serum albumin as standard. The amount of RubPco was measured by 15 % SDS-PAGE of the native protein identified and quantified by comparison with standard RubPco protein (Lawlor et al. 1989).

**Statistics**: The statistical analyses were done using the Statistica 4.0 and Sigmaplot softwares. All linear single regressions, correlations, and one-way ANOVA were tested for significance at $p<0.05$. Results are presented as means ($\pm$SE).
Results

Measurements under natural conditions: The species studied showed a high value of \( \psi \) during the rainy season, drought causing a considerable decrease of \( \psi \) in both species; \( \psi_s \) was also affected by drought (Table 1). \( P_N \) and \( g_s \) were significantly higher during the rainy season than in the dry season (Table 1). With drought, \( P_N \) decreased by 64 and 74 % as \( g_s \) declined by 82 and 50 % in \( I. \) carnea and \( J. \) gossypifolia, respectively. \( C_i \) was higher in the dry season in both species.

In \( I. \) carnea and \( J. \) gossypifolia, both \( P_{\text{Nsat}} \) and CE declined with drought (Table 1). The CO\(_2\) compensation concentration (\( \Gamma \)), relative stomatal (\( L_s \)) and mesophyll (\( L_m \)) limitations, total soluble protein (TSP), ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content, percentage of TSP in RuBPCO, total chlorophyll (Chl) content, and maximum quantum yield of photosystem 2 (\( F_v/F_m \)) in plants of \( I. \) carnea and \( J. \) gossypifolia growing in the field. Means ± SE (\( n = 4 \), for the last five items \( n = 6 \)). Different letters indicate statistically significant differences at \( p<0.05 \) between seasons for each parameter and species.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( I. ) carnea</th>
<th>( J. ) gossypifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rainy</td>
<td>dry</td>
</tr>
<tr>
<td>SWC [% DM]</td>
<td>10.9±1.2 b</td>
<td>2.6±0.2 a</td>
</tr>
<tr>
<td>( \psi ) [MPa]</td>
<td>-0.23±0.03 b</td>
<td>-2.10±0.60 a</td>
</tr>
<tr>
<td>( \psi_s ) [MPa]</td>
<td>-1.09±0.04 a</td>
<td>-0.99±0.03 b</td>
</tr>
<tr>
<td>( P_N ) [mumol m(^{-2}) s(^{-1})]</td>
<td>14.6±3.0 b</td>
<td>5.3±0.6 a</td>
</tr>
<tr>
<td>( g_s ) [mumol m(^{-2}) s(^{-1})]</td>
<td>440±6 b</td>
<td>78±8 a</td>
</tr>
<tr>
<td>( C_i ) [mumol mol(^{-1})]</td>
<td>308±14 a</td>
<td>315±2 a</td>
</tr>
<tr>
<td>( P_{\text{Nsat}} ) [mumol m(^{-2}) s(^{-1})]</td>
<td>32.2±2.3 b</td>
<td>11.4±2.4 a</td>
</tr>
<tr>
<td>CE [mumol m(^{-2}) s(^{-1})]</td>
<td>0.075±0.010 b</td>
<td>0.034±0.001 a</td>
</tr>
<tr>
<td>( \Gamma ) [mumol mol(^{-1})]</td>
<td>45±2 a</td>
<td>95±4 b</td>
</tr>
<tr>
<td>( L_s ) [%]</td>
<td>35.0±0.5 a</td>
<td>42.0±0.3 b</td>
</tr>
<tr>
<td>( L_m ) [%]</td>
<td>0 a</td>
<td>65±4 b</td>
</tr>
<tr>
<td>TSP [g m(^{-2})]</td>
<td>4.9±1.3 a</td>
<td>17.2±3.6 b</td>
</tr>
<tr>
<td>RuBPCO [g m(^{-2})]</td>
<td>2.30±0.40 a</td>
<td>2.00±0.03 a</td>
</tr>
<tr>
<td>Chl [g m(^{-2})]</td>
<td>0.199±0.012 b</td>
<td>0.112±0.012 a</td>
</tr>
<tr>
<td>( F_v/F_m )</td>
<td>0.820±0.003 b</td>
<td>0.780±0.008 a</td>
</tr>
</tbody>
</table>

At PPFD of 0–300 mumol m\(^{-2}\) s\(^{-1}\) there was no effect of drought on \( J \), whereas at high PPFD a reduction with drought was observed in both species (Fig. 2A,B). In \( J. \) gossypifolia, \( \varphi_{PS2} \) was slightly lower in droughted plants (Fig. 2C,D). The \( \varphi_{PS2} \) followed the same trend as \( \varphi_{PS2} \), decreasing during the dry season, but \( \varphi_{PS2} \) did not change in \( I. \) carnea (Fig. 2E,F). NPQ showed a strong increase with drought in both species (Fig. 2G,H). An inverse linear relationship was found between \( \varphi_{PS2} \) and \( Y_n \) in leaves of \( I. \) carnea and \( J. \) gossypifolia (Fig. 3). During the rainy season, \( \varphi_{PS2} + Y_n \) = 0.8 for both species, while during drought, although linearity in the relationship was maintained, \( \varphi_{PS2} + Y_n \) < 0.8, indicating loss of coordination between these parameters.

Total soluble protein content (TSP) increased with drought in both species, RuBPCO content remaining unchanged; the proportion of TSP represented by RuBPCO decreased with drought 4 and 3 times in \( I. \) carnea and \( J. \) gossypifolia, respectively (Table 1). Both Chl content and maximum quantum yield of PS2 were reduced by drought (Table 1).

Greenhouse experiments: Changes due to water stress in \( \psi \) are shown in Fig. 4A,B. After 28 d under the water stress treatment, a decrease in \( \psi \) to –1.5 MPa was observed; the \( \psi_s \) values were –1.0 and –1.6 MPa in \( I. \) carnea and \( J. \) gossypifolia, respectively, without further changes after 28 d of water stress (data not shown). Control values were similar to those measured in Coro, whereas values measured in the greenhouse after 28 d of water deficit were higher than in the field. Control values of \( P_N \) and \( g_s \) were similar to those measured in plants growing in the field during the rainy season. After 28 d of treatment, \( P_N \) and \( g_s \) decreased by 98 and 94 % in both species (Fig. 4C–F).
PHOTOSYNTHESIS AND PHOTOINHIBITION IN TWO XEROPHYTIC SHRUBS DURING DROUGHT

**Discussion**

The parameters characterizing plant water status ($\psi$, $\psi_s$) in the field decreased with increasing water stress in the two xerophytic species studied, in agreement with our earlier report (Tezara et al. 1998). Lower values of $\psi$ than $\psi_s$ with drought may reflect development of negative turgor potential, which may be caused by an effect of dilution by apoplasmatic water.

Decreases in $P_N$ and $g_s$ with water deficit (lower $\psi$ and $\psi_s$) were observed in *I. carnea* and *J. gossypifolia*. Similar results with water deficit have been reported in these two species (Herrera et al. 1994, Tezara et al. 1998) and in the sympatric species *L. nodosum* (Tezara et al. 2003). Inhibition of $P_N$ by drought is one of the effects that water deficits can have on growth and metabolism of xerophytes. The decline in $P_N$ with decreasing $\psi$ was correlated with a reduction in $g_s$. This may indicate that under water deficit stomata were imposing a larger limitation on $P_N$. However, in our study values of $C_i$ associated with each $P_N$ value during drought increased with decreasing $\psi$, suggesting that $g_s$ was not the main cause of the reduction of $P_N$.

Drought significantly affected the shape of the $P_N$-$C_i$ response in both species. Water deficit markedly reduced $P_{N_{\text{max}}}$, CE, and $g_s$. The decreased CE and $P_{N_{\text{max}}}$ suggest a loss of RuBPCO activity with decreasing $\psi$. The amount and specific activity of RuBPCO and the availability of RuBP affect CE and thus $P_N$ (Tezara et al. 2003). The changes in $P_{N_{\text{max}}}$ support the earlier conclusion (Tezara et al. 1999) that factors associated with decreased $\psi$ progressively reduced photosynthetic capacity in sunflower. The mechanism was considered to be decreased ATP synthesis, shown by lower ATP content and the consequent reduction in RuBP synthesis and content (Tezara et al. 1999, Lawlor 2002, Lawlor and Cornic 2002).

In this study, $L_s$ increased by 16% as $\psi$ declined in *I. carnea*, whereas it decreased by 27% in *J. gossypifolia*. Fig. 2. Seasonal changes in the rate of total linear electron transport, $J$ (A, B), the relative quantum yield of photosystem 2, $\phi_{PS2}$ (C, D), the photochemical quenching coefficient, $q_P$ (E, F), and the non-photochemical quenching coefficient, NPQ (G, H) of fluorescence as a function of PPFD in plants of *I. carnea* and *J. gossypifolia* during the rainy (filled circles) and dry (empty circles) seasons. Means ± SE (n = 3); standard errors are shown when greater than the symbol.

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The $L_m$ increased to 65 and 51 % with water deficit in both species, suggesting that as stress increased, metabolic regulation of photosynthesis became more important than stomatal closure; similar results were found in *L. nodosum* (Tezara et al. 2003).

Conclusions concerning stomatal and metabolic limitations of $P_N$ based on $P_N$-$C_i$ curves may in some cases be misleading due to erroneous calculation of $C_i$ because of stomatal patchiness (Downton et al. 1988, Terashima et al. 1988). The validity of calculated $C_i$, particularly with respect to water deficits (see Lawlor and Cornic 2002) has been questioned, but we consider it valid. Patchiness occurs in heterobaric leaves, i.e. leaves in which the mesophyll continuity is interrupted by vascular bundles spanning the entire cross section, but not in homobaric leaves (Terashima et al. 1988) such as those of *I. carnea* and *J. gossypifolia*.

One of the most widely used fluorescence parameters, $F_v/F_m$, might estimate the degree of photoinhibition (Ball et al. 1994, Osmond and Grace 1995, Solhaug and Haugen 1998). Seasonal changes in fluorescence parameters, especially in $F_v/F_m$, reflect the degree of photoinhibition in the species of this study. Thus, the lowest values of $F_v/F_m$ were found during the dry season in *I. carnea* and *J. gossypifolia* under natural conditions, suggesting that water deficit during the dry season is a stress factor that suggests possible photoinhibition. However, in greenhouse experiments, $F_v/F_m$ did not change, perhaps because $\psi$ at the end of the treatment was higher than under natural conditions. In our plants growing under natural conditions, $F_v/F_m$ was affected by water deficit, in disagreement with other studies (Tezara et al. 1999, 2003, Lawlor and Cornic 2002), suggesting that harsher microclimatic conditions in the field had a marked effect on PS2 activity.

Under well-watered conditions (rainy season), $J$ was not photon saturated at maximum PPFD in *J. gossypifolia*. However, drought caused photon saturation of $J$ in both species and $J$ was 60 % higher during the rainy season than the dry season in both species. Lower saturated $J$ and $\phi_{PS2}$ during drought were observed, suggesting that the photochemical system was down-regulated by changes in leaf water status and that these leaves are sensitive to photoinhibition. Similarly, $J$ was reduced by approximately 40 % in maize leaves subjected to water deficit (Scheuermann et al. 1991).

Drought affected the energy dissipation in leaves of *I. carnea* and *J. gossypifolia*, judging from the changes in $q_P$ and NPQ. During the dry season, NPQ increased strongly, indicating that a greater proportion of the energy was thermally dissipated, thus accounting for the apparent down-regulation of PS2. In contrast, in plants of *L. nodosum*, changes in fluorescence parameters supporting the protective role of the non-photochemical quenching against photoinhibition were observed (Tezara et al. 2003). Alternative mechanisms of excess electron
dissipation, such as the violaxanthin cycle (Björkman and Demming-Adams 1994), related to non-photochemical quenching, increase markedly when turgor is lost whilst photochemical quenching is either unaffected or decreased (Lawlor 1995). In both species of this study, the reduction in $\phi_{PS2}$ and $J$ due to water deficit was lower than the decrease in $P_N$, possibly due to higher photorespiration at low $\psi_w$ as suggested by Lawlor and Cornic (2002) and Tezara et al. (2003). This is supported by the observation that $I$ was twice as high in droughted than watered plants.

The analysis of fluorescence components of these xerophytic species confirms that during the dry season and, more generally, when $P_N$ is inhibited, there is a fraction of PS2 centres that remain open and are able to perform charge transfer. Horton et al. (1994) suggested that a certain fraction of PS2 centres stays open when $P_N$ is very low and the $\phi_{PS2}$ is greatly reduced as, for instance, by stressful environment. Water deficiency decreased electron flux, $J$, through PS2 as expected from the decrease in $P_N$, but much less than the decrease in $P_N$, due to higher photorespiration at low $\psi_w$ (see Lawlor and Cornic 2002).

Tezara et al. (2003) suggested that the reductions in $\phi_{PS2}$, $J$, $q_P$, and CE may partly explain the increase in $L_m$ and the occurrence of co-limitation of photosynthesis in plants under drought; such reductions were found in both species of this study. The decrease in $J$ of $I. carnea$ and $J. gossypifolia$ may have contributed to the increase in $L_m$ through a reduction in ATP and/or RuBP content (Tezara et al. 1999, Lawlor and Cornic 2002) since in $P_N$-$C$ curve, $P_{Sat}$ equals the maximum rate of RuBP regeneration and the maximum $J$ (Farquhar et al. 1980). Increased $L_m$ under stress may also be caused by decreased activity of some Calvin cycle enzymes (for example reduction in RuBPCO activity and/or amount, which would be seen as a decrease in CE), and/or decreased mesophyll conductance to CO$_2$ (Flexas et al. 2002, Centritto et al. 2003).

A reduction in $\phi_{PS2}$ co-ordinated with an increase in $Y_n$ suggests that photoinhibition does not occur in irrigated plants (Laisk et al. 1997). This could be explained because, under irrigation and saturating irradiance, more than 50% of absorbed radiation is thermally dissipated (Flexas and Medrano 2002). Drought caused a lack of complementarity between $\phi_{PS2}$ and $Y_n$ (i.e. the increase in $Y_n$ was smaller than the decrease in $\phi_{PS2}$), suggesting occurrence of photoinhibition. Similarly, this relationship was not always complementary in plants of sunflower and cotton leaves grown at higher temperatures or at lower irradiance, the increase in $Y_n$ being less than the decrease in $\phi_{PS2}$, although linearity was still maintained (Laisk et al. 1997). Decreases in $\phi_{PS2}$ compensated by proportional increases in $Y_n$ have been reported in Clusia hiliariana Schlecht. (Franco et al. 1999). In sunflower and tobacco under conditions of photoinhibition and thermo-inhibition the complementary relationship between $\phi_{PS2}$ and $Y_n$ was lost (Laisk et al. 1997).

Water deficits decreased $q_P$, showing that the reduction state of the acceptor $Q_A$ was increased as well as NPQ so a greater proportion of the energy was thermally dissipated at low $\psi_w$ in both species. Such effects have been frequently observed (see Lawlor and Cornic 2002). In droughted plants of $I. carnea$ and $J. gossypifolia$, photochemical activity decreased and photoinhibition occurred. However, in $L. nodosum$ (Tezara et al. 2003) and in sunflower (Tezara et al. 1999) there was no evidence of photoinhibition as $F_7/F_m$ was unaffected by drought.

TSP content decreased with decreasing $\psi_w$ but the RuBPCO content did not change significantly and the

Fig. 5. Time-course of changes in plants of $I. carnea$ ($A$, $C$, $E$, $G$, $I$) and $J. gossypifolia$ ($B$, $D$, $F$, $H$, $J$) subjected to water deficit in greenhouses experiments in ($A$, $B$) maximum quantum yield of photosystem 2, $F_7/F_m$ (control plants, filled symbols) and droughted plants (empty symbols), ($C$, $D$) relative quantum yield of photosystem 2, $\phi_{PS2}$, ($E$, $F$) photosynthetic electron transport rate, $J$, ($G$, $H$) coefficient of photochemical quenching, $q_P$, and ($I$, $J$) coefficient of non-photochemical quenching of fluorescence, NPQ. The parameters $\phi_{PS2}$, $J$, $q_P$, and NPQ were measured at a PPFD = 1 000±20 µmol m$^{-2}$ s$^{-1}$. Means ± SE ($n=5$).
RuBPCO/TSP ratio decreased. CE decreased probably due to a decrease in the specific activity of RuBPCO, since amounts of RuBPCO remained constant.

Our results suggest that drought causes photoinhibition and down-regulation of PS2 (decrease in photochemical activity) in leaves of *I. carnea* and *J. gossypifolia*. Photoinhibition during drought in *I. carnea* and *J. gossypifolia* was evidenced since maximum quantum yield of PS2 was lower than 0.8 ($F_v/F_m<0.8$). A coordinated change between $q_{PS2}$ and $Y_n$ (rainy season) was lost in leaves of these C3 shrubs during drought, when much lower values of $y$, $P_{n0}$, $F_v/F_m$, and total Chl, and an enormous increase in NPQ were found. These results indicated that photoinhibition could be a cause of the photosynthetic inhibition observed in these xerophytic shrubs during the dry season.

The analysis of stomatal and metabolic limitation suggested that metabolic regulation is more important than stomatal closure under drought in the species studied. This is supported by the reductions in photochemical activity ($\Phi_{PS2}$, $J$, $q_P$) and CE, which may partly explain the increase in $L_{max}$. However, future research concerning ATP and RUBP contents and RuBPCO activity are necessary in order to establish the consequences of metabolic impairment during drought. A significant reduction in $F_v/F_m$ suggests occurrence of photoinhibition. However, future studies of D1 protein and xanthophyll cycle are needed to confirm these results.

**References**


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