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 (ψ) decreased from –0.2 to –2.1 MPa during drought in both species. The P_N decreased with ψ 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 (φ_{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 φ_{PS2} and quantum yield of non-photochemical excitation quenching (Y_n). Total soluble protein content on an area basis increased with ψ 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_{Nsat} = 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; φ_{PS2} = relative quantum yield of photosystem 2; ψ = morning leaf water potential; ψ_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 (φ_{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-photochemical energy dissipation measured as q_N, *i.e*. alternative mechanisms of excess electron dissipation, such as the violaxanthin cycle (Björkman and Demmig-Adams 1994), which reduces φ_{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 C_3 plants has been considered as an alternative sink for light-induced electron flow during periods of restricted CO_2 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 C_3 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 φ_{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_P 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 φ_{PS2} associated to an increase in the quantum yield of non-photochemical 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 φ_{PS2} and Y_n could indicate photoinhibition in species subjected to drought (Laisk *et al.* 1997). The Stern-Volmer coefficient of non-photochemical 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-photochemical 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 CO_2 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 ψ decreased from –0.3 to –2.0 MPa, relative stomatal limitation increasing by 63 %, while in *J. gossypifolia*, L_s 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 C_3 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 C_3 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-cm^3 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 1 500 μmol m^{-2} s^{-1}, temperature from 22±2 to 36±2 ºC, and relative humidity from 70±3 to 42±4 %.

Microclimatic parameters were measured every hour. PPFD was measured with a quantum sensor model *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 (ψ) 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 (ψ_s) was measured in the sap expressed from frozen and thawed leaves previously used for the determination of ψ, using a *Wescor 5000* osmometer (*Wescor*, Logan, Utah, USA); values were not corrected for apoplasmic 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* used in conjunction with a *PLC(B)* assimilation chamber (*PP Systems*, Hitchin, UK). Measurements were made at a $[CO_2]$ = 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 $A = b + de^{Kci}$, 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 $L_s = 100\ (P_{N0} - P_N)/P_{N0})$, where P_{N0} is the photosynthetic rate at $C_i = C_a$ (Farquhar and Sharkey 1982). The relative mesophyll limitation was calculated as $L_m = 100\ (P_{Nc} - P_{Ns})/P_{Nc})$ where P_{Nc} is P_N of control leaves at C_i = 800 μmol mol^{-1}, and P_{Ns} 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_v/F_m was measured *in situ* at the minimum dawn PPFD. Irradiance dependence curves of linear electron transport rate (J), φ_{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 φ_{PS2} at steady state photosynthesis is defined as $\varphi_{PS2} = (F'_m - F_s)/F'_m$ according to Genty *et al.* (1989), where F_s and F'_m are fluorescence at steady state photosynthesis and maximum fluorescence in the light, respectively. The quantum yield of non-photochemical quenching (Y_n) was calculated as $Y_n = (F_s/F'_m) - (F_s/F_m)$ (Laisk *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 $J = \varphi_{PS2}$ 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 ºC) 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 ºC 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} phenylmethylsulphonyl 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 ($4 \le n \le 6$) ± SE.

Results

Measurements under natural conditions: The species studied showed a high value of ψ during the rainy season, drought causing a considerable decrease of ψ in both species; ψ_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_{Nsat} and CE declined with drought (Fig. 1, Table 1). The CO_2 compensation concentration (Γ) increased with drought (Table 1). L_s increased by 17 % in *I. carnea*, while in *J. gossypifolia* it decreased by 27 % as ψ declined from –0.2 to –2.1 MPa. L_m increased with drought to 65 (*I. carnea*) and 51 % (*J. gossypifolia*) (Table 1).

Table 1. Changes in soil water content (SWC), xylem water potential (ψ) and osmotic potential (ψ_s), net photosynthetic rate (P_N), leaf conductance (g_s), intercellular CO_2 concentration (C_i), CO_2-saturated photosynthetic rate (P_{Nsat}), carboxylation efficiency (CE), CO_2 compensation concentration (Γ), relative stomatal (L_s) and mesophyll (L_m) limitations, total soluble protein (TSP), ribulose-1,5-bisphoshate 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.

Variable	*I. carnea*		*J. gossypifolia*	
	rainy	dry	rainy	dry
SWC [% DM]	10.9±1.2 b	2.6±0.2 a	10.9±1.2 b	2.6±0.2 a
ψ [MPa]	-0.23±0.03 b	-2.10±0.60 a	-0.23±0.03 b	-2.10±0.58 a
ψ_s [MPa]	-1.09±0.04 a	-0.99±0.03 b	-1.03±0.03 b	-1.25±0.02a
P_N [μmol m^{-2} s^{-1}]	14.6±3.0 b	5.3±0.6 a	14.9±2.1 b	3.9±0.3 a
g_s [mmol m^{-2} s^{-1}]	440±6 b	78±8 a	280±10 b	141±41 a
C_i [μmol mol^{-1}]	308±14 a	315±2 a	296±16 a	323±4 b
P_{Nsat} [μmol m^{-2} s^{-1}]	32.2±2.3 b	11.4±2.4 a	37.8±5.4 b	18.4±1.8 a
CE [mol m^{-2} s^{-1}]	0.075±0.010 b	0.034±0.001 a	0.114±0.020 b	0.033±0.009 a
Γ [μmol mol^{-1}]	45±2 a	95±4 b	58±3 a	130±5 b
L_s [%]	35.0±0.5 a	42.0±0.3 b	35.0±2.2 b	26.0±0.8 a
L_m [%]	0 a	65±4 b	0 a	51±2 b
TSP [g m^{-2}]	4.9±1.3 a	17.2±3.6 b	4.2±0.7 a	17.9±3.6 b
RuBPCO [g m^{-2}]	2.30±0.40 a	2.00±0.03 a	1.40±0.10 a	1.70±0.10 a
TSP of RuBPCO [%]	47 b	12 a	33 b	10 a
Chl [g m^{-2}]	0.199±0.012 b	0.112±0.012 a	0.209±0.080 b	0.114±0.017 a
F_v/F_m	0.820±0.003 b	0.780±0.008 a	0.810±0.005 b	0.710±0.01 a

At PPFD of 0–300 μmol 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. 2*A,B*). In *J. gossypifolia*, φ_{PS2} was slightly lower in droughted plants (Fig. 2*C,D*). The q_P followed the same trend as φ_{PS2}, decreasing during the dry season, but q_P did not change in *I. carnea* (Fig. 2*E,F*). NPQ showed a strong increase with drought in both species (Fig. 2*G,H*).

An inverse linear relationship was found between φ_{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. gossypifiolia,* 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 ψ are shown in Fig. 4*A,B*. After 28 d under the water stress treatment, a decrease in ψ to –1.5 MPa was observed; the ψ_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. 4*C–F*).

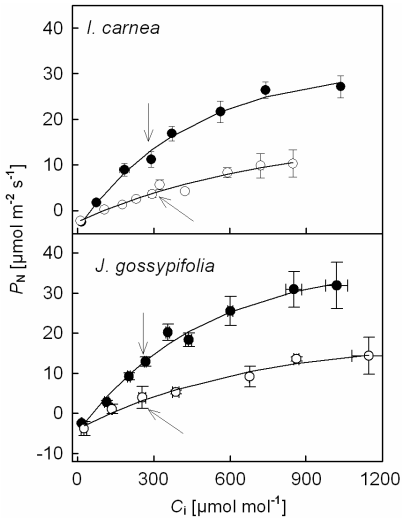

Fig. 1. Responses of net photosynthetic rate (P_N) to intercellular CO_2 concentration (C_i) in leaves of plants of *I. carnea* and *J. gossypifolia* during the rainy (*filled circles*) and dry season (*empty circles*). Means ± SE (n = 4); standard errors are shown when greater than the symbol. *Arrows* indicate the mean value of operational C_i at 350 μmol mol^{-1} of CO_2 (C_a).

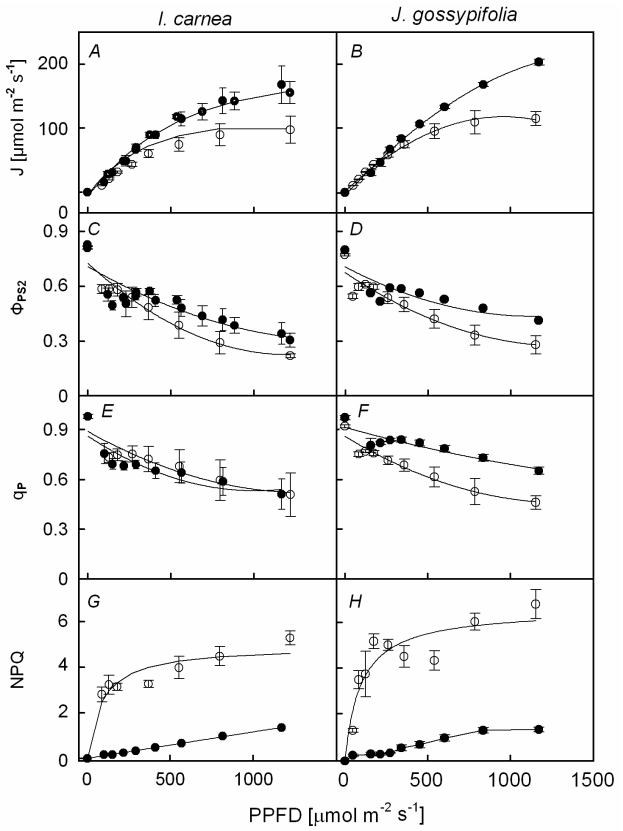

Fig. 2. Seasonal changes in the rate of total linear electron transport, J (*A, B*), the relative quantum yield of photosystem 2, φ_{PS2} (*C, D*), the photochemical quenching coefficient, q_P (*E, F*), and the non-photochemical quenching coefficient, NPQ (*G, H*) of fluorescence as a function of PPFD 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.

The average F_v/F_m was 0.83±0.01 in both species values decreasing after 7 d of drought but resuming control values after 28 d of drought (Fig. 5*A,B*); however, a significant decrease in φ_{PS2} at PPFD = 1 000 μmol m^{-2} s^{-1} was observed as water deficit increased (Fig. 5*C,D*); consequently, J measured at high PPFD was reduced by 70 and 77 % after 28 d of water deficit in *I. carnea* and *J. gossypifolia*, respectively (Fig. 5*E,F*). The q_P followed the same trend as φ_{PS2}, decreasing with time under stress (Fig. 5*G,H*), while NPQ increased with water deficit (Fig. 5*I,J*).

Discussion

The parameters characterizing plant water status (ψ, ψ_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 ψ than ψ_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 ψ and ψ_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 ψ 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 ψ, 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_{Nmax}, CE, and g_s. The decreased CE and P_{Nsat} suggest a loss of RuBPCO activity with decreasing ψ. 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_{Nmax} support the earlier conclusion (Tezara *et al.* 1999) that factors associated with decreased ψ 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 ψ declined in *I. carnea,* whereas it decreased by 27 % in *J. gossypi*

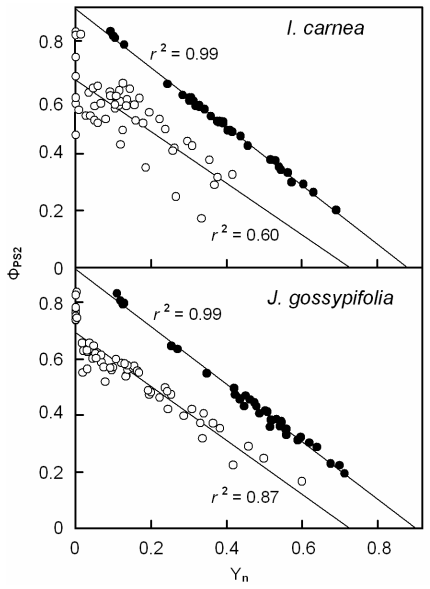

Fig. 3. Relationship between the quantum yield of photosystem 2, φ_{PS2} and non-photochemical excitation quenching, Y_n in plants of *I. carnea* and *J. gossypifolia* during the rainy (*filled circles*) and dry (*empty circles*) seasons. Values are individual datapoints.

folia. 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 ψ 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

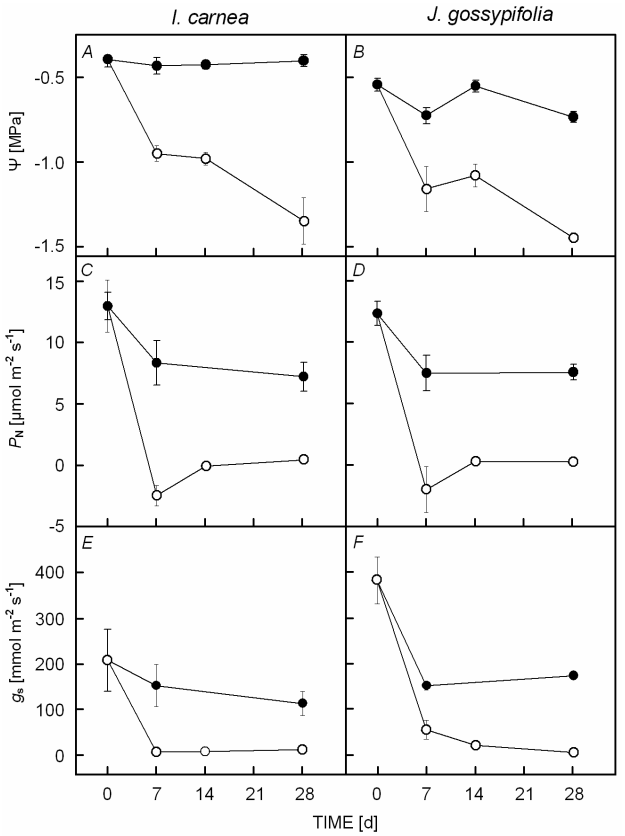

Fig. 4. Time-course of changes in plants of *Ipomoea carnea* (*A, C, E*) and *J. gossypifolia* (*B, D, F*) subjected to water deficit in greenhouse experiments in control plants (*filled symbols*) and droughted plants (*empty symbols*). (*A*, *B*) water potential, ψ (*C, D*), net photosynthetic rate, P_N, and (*E, F*) leaf conductance, g_s. Means ± SE (n = 5).

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 φ_{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 Demmig-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 φ_{PS2} and J due to water deficit was lower than the decrease in P_N, possibly due to higher photorespiration at low ψ, as suggested by Lawlor and Cornic (2002) and Tezara *et al.* (2003). This is supported by the observation that Γ was twice as high in droughted than watered plants.

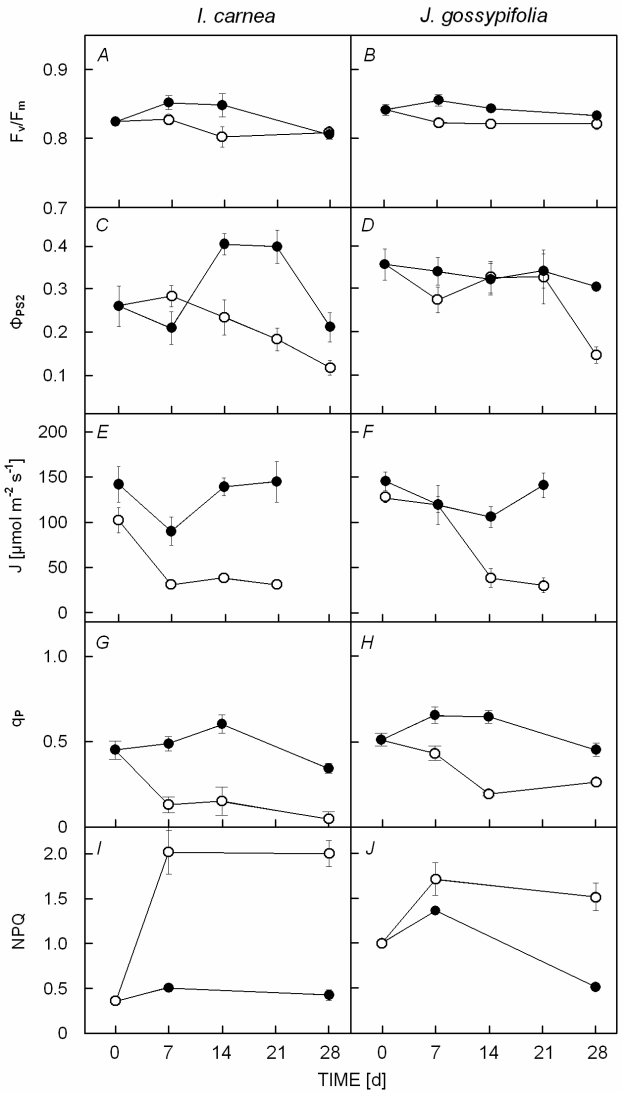

Fig. 5. Time-course of changes in plants of *I. carnea* (*A, C, E, G, I*) and *J. gossypifolia* (*B, D, F, H, J*) subjected to water deficit in greenhouses experiments in (*A, B*) maximum quantum yield of photosystem 2, F_v/F_m (control plants, *filled symbols*) and droughted plants (*empty symbols*), (*C, D*) relative quantum yield of photosystem 2, φ_{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 φ_{PS2}, J, q_P, and NQP were measured at a PPFD = 1 000±20 μmol m^{-2} s^{-1}. Means ± SE (n = 5).

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 φ_{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 ψ_w (see Lawlor and Cornic 2002).

Tezara *et al.* (2003) suggested that the reductions in φ_{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_i curves, P_{Nsat} 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 φ_{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 φ_{PS2} and Y_n (*i.e.* the increase in Y_n was smaller than the decrease in φ_{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 φ_{PS2}, although linearity was still maintained (Laisk *et al.* 1997). Decreases in φ_{PS2} compensated by proportional increases in Y_n have been reported in *Clusia hilariana* Schlecht. (Franco *et al.* 1999). In sunflower and tobacco under conditions of photoinhibition and thermoinhibition the complementary relationship between φ_{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 ψ 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_v/F_m was unaffected by drought.

TSP content decreased with decreasing ψ but the RuBPCO content did not change significantly and the

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 φ_{PS2} and Y_n (rainy season) was lost in leaves of these C_3 shrubs during drought, when much lower values of ψ, P_N, 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 (φ_{PS2}, J, q_P) and CE, which may partly explain the increase in L_m. However, future research concerning ATP and RUBP contents and RuBPCO activity are necessary in order to establish the consequences of metabolic impair 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.

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