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Water relations and photosynthetic capacity of two species of *Calotropis* in a tropical semi-arid ecosystem

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• *Background and Aims Calotropis procera* and *Calotropis gigantea*, originally from warm parts of Africa and Asia, are now pan-tropical and in ecological terms considered an indicator of overgrazed, disturbed lands; they grow successfully in dry areas. Variations in water relations, morphology and photosynthesis of the two species growing in the same habitat were studied to assess possible mechanisms of tolerance to drought and how these relate to their ecophysiological success. Also the hypothesis that their photosynthetic rate (*A*) under drought would be affected by stomatal and non-stomatal limitations was tested.
• *Methods* Water relations, gas exchange, water use efficiency (WUE), fluorescence parameters, pubescence and specific leaf area (SLA) of *Calotropis procera* and *C. gigantea* plants growing in the field were evaluated during the wet (WS) and dry (DS) seasons.
• *Results* The xylem water potential (ψ) was similar in both species during the WS and DS; drought caused a 28 % decrease of ψ. In *C. procera*, *A*, stomatal conductance (g_s) and carboxylation efficiency (CE) were higher in the WS with half the values of those during the DS, this species being more affected by drought than *C. gigantea*. A high $\delta^{13}C$ of *C. gigantea* ($-26 \cdot 2$ ‰) in the WS indicated a higher integrated WUE, in agreement with its lower g_s. Leaves of *C. gigantea* were more pubescent than *C. procera*. Relative stomatal and non-stomatal limitation of *A* increased with drought in both species; no changes in maximum quantum yield of photosystem II (PSII; $F_\sqrt{}/F_m$) were observed. The decrease in the relative quantum yield of PSII (ϕ_{PSII}) and in the photochemical quenching coefficient (q_P) was more pronounced in *C. procera* than in *C. gigantea*.
• *Conclusions* The photosynthetic capacity of *C. procera* was higher than that of *C. gigantea*. During the DS, *A* was regulated by stomatal and non-stomatal factors in a coordinated manner and drought did not cause chronic photoinhibition. A higher density of trichomes and leaf angle in *C. gigantea* may contribute to the maintenance of *A* and confer more efficient protection of photochemical activity in the DS. Ecophysiological traits such as high photosynthetic rate throughout the year even during the DS, and high WUE, highly pubescent leaves and low SLA observed in both species contribute to the establishment and growth of *Calotropis* in dry conditions.

Key words: Photosynthesis, *Calotropis procera*, *Calotropis gigantea*, fluorescence, non-stomatal limitation, stomatal limitation, drought.

INTRODUCTION

Global climatic change, with increased ambient CO_2 concentration and temperature of the planet, is predicted to alter precipitation patterns significantly, causing an increase in aridity in the semi-arid areas of the world (Lawlor, 2001; IPCC, 2007). High temperatures and dry conditions may be important factors in changing the success and regeneration of plant species (Wei *et al.*, 2009). Understanding how plants respond to drought can play a major role in the protection of natural vegetation in these areas. In most terrestrial ecosystems, water availability is the main environmental factor limiting photosynthesis, growth and productivity (Schulze *et al.*, 1987; Wullschleger *et al.*, 2002) even in plants well adapted to arid conditions, and influences the distribution and the abundance of many species of plants (Schulze *et al.*, 1987).

Seasonal loss of leaves, smaller photosynthetic leaf area and increased importance of photosynthetic stems, together with

high trichome density, succulence, development of a deep root system, low osmotic potential and high water use efficiency (WUE), are, among others, the main adaptations of genera and of species within genera to dry zones (Solbrig and Orians, 1977). For example, within a genus, *Eucalyptus cloeziana* maintains its water status under drought due to its deeper root system and a greater capacity to extract water from soil than *Eucalyptus argophloia* (Ngugi *et al.*, 2003). Also, photoprotective mechanisms involve physiological leaf adjustments in biochemistry, photochemistry, leaf morphology and anatomy (Havaux and Niyogi, 1999). Leaf pubescence has been reported to be an adaptation to arid environments by reducing the radiant energy absorbed by leaves, affecting their energy balance (Ehleringer, 1983), reducing transpiration whilst maintaining a favourable leaf temperature and so helping to keep a favourable water balance (Savé *et al.*, 2000; Galmés *et al.*, 2007). Variations between species within a genus have been shown in photosynthetic parameters

of *Mosla* and *Salix* (Liu *et al.*, 2003; Ge *et al.*, 2004); transpiration rate (*E*) in shrubs of *Caragana* (Ma *et al.*, 2004) and morphology and photosynthesis in two varieties of *Digitalis minor* growing in the Balearic Islands (Galmés *et al.*, 2007).

To analyse differences between genera or species which may be responsible for their ecological behaviour in relation to drought it is necessary to consider a number of potential mechanisms. Drought causes reductions in stomatal conductance (g_s) which may limit the photosynthetic rate (*A*) due to a direct effect on CO_2 availability to chloroplasts by limiting diffusion through stomata (Cornic, 2000) and/or reduction of the mesophyll conductance, g_m (Flexas *et al.*, 2008; Hassiotou *et al.*, 2009), or diminishing metabolic processes (Tezara *et al.*, 1999, 2008; Lawlor, 2002; Nunes *et al.*, 2008; Lawlor and Tezara, 2009) and/or photosystem II (PSII) activity and electron transport (Tezara *et al.*, 2003). To identify differences between species, an increase in the relative stomatal limitation (L_s) may be calculated from the response curve of *A* to the intercellular CO_2 concentration (C_i) to determine whether *A* is reduced only because of decreased g_s or if there as an increase in non-stomatal limitation (L_m; Farquhar and Sharkey, 1982). Jacob and Lawlor (1991) defined L_m as the proportional reduction in C_i-saturated *A* ($A_{CO2-sat}$) of plants subjected to stress relative to unstressed plants. Changes in L_m due to water deficit may reflect changes in photosynthetic processes such as Rubisco activity, ribulose bisphosphate (RuBP) production, ATP supply, electron transport rate (*J*) and light capture efficiency (Lawlor and Cornic, 2002; Tezara *et al.*, 2005; Lawlor and Tezara, 2009). Therefore, L_m is also affected by parameters such as chlorophyll *a* fluorescence and carboxylation efficiency (CE), among others. Measurement of some of these plant characteristics using such techniques may be used to identify the physiological and other factors responsible for the ecological differences between species.

They have been applied to analyse the response of two species of *Calotropis* (Apocynaceae), known as milkweed, to seasonally dry conditions. *Calotropis* is a wasteland weed of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries (Singh *et al.*, 1996). *Calotropis procera* (Aiton) WT Aiton is native to Asia, Arabia and tropical Africa, and *C. gigantea* (L.) WT Aiton is native to southeast Asia (Malaysia, the Philippines, Sri Lanka, Thailand, India and China), and both were introduced into South America and the islands of the Caribbean (Lebrun, 1998). *Calotropis procera* grows up to an altitude of 1200 m (Sayed and Mohamed, 2000), while *C. gigantea* reaches 900 m (Usmani and Kushwaha, 2010). The genus *Calotropis* consists of common weeds which occur in arid ecosystems but have become naturalized in warm climates, where they grow commonly in disturbed areas. In Venezuela, *C. procera* shows a wider geographical distribution than *C. gigantea*. Both species are commonly found growing in hot and dry zones, generally next to the sea or in open and sunny places (Steyermark, 1994). Both are successful invaders, particularly in old fields. The two species of *Calotropis* studied here are perennial evergreen shrubs or small trees reaching 3–5 m tall, with large silver-green leaves, opposite, sub-sessile, clusters of waxy purple-tipped flowers, and inflated pale

green seed pods (Kleinschmidt and Johnson, 1977; Nicholson, 1991). These species differ in characteristics, particularly in leaf pubescence which may be important, as mentioned above: *C. procera* is less pubescent (Colombo *et al.*, 2007).

Most research regarding *Calotropis* has been concentrated on its medicinal properties (Lewis and Elvin-Lewis, 1977; Oudhia and Dixit, 1994; Oudhia, 1999*a*, *b*) as it is an important source of pharmaceutical compounds (Longanga *et al.*, 2000; Ahmed *et al.*, 2005; Usmani and Kushwaha, 2010). Also, *Calotropis* yields a durable fibre useful for ropes, carpets, fishing nets and sewing thread (Pérez-Arbeláez, 1978; Tuntawiroon and Samootsakorn, 1984). The effect of water deficit on the growth of *C. procera* in greenhouse-grown and cultivated plants has been evaluated (Boutraa, 2010), but there are few ecophysiological data on natural populations of species of this genus (Colombo *et al.*, 2007; Boutraa, 2010). Both species show high *A* throughout the year even during the dry season (DS), suggesting the occurrence of particular strategies of drought resistance (Khan and Beena, 2002; Colombo *et al.*, 2007; Khan *et al.*, 2007; Boutraa, 2010).

In order to gain insight into the possible mechanisms of tolerance to drought and assess differences in physiological responses to environmental variables of these two species of *Calotropis*, the seasonal changes in gas exchange, water relations and chlorophyll *a* fluorescence parameters of plants growing in their natural habitat were measured. The climate in the study area is typical of a tropical semi-arid ecosystem, characterized by high radiation, high temperatures, annual rainfall of ≤ 800 mm, with evaporation exceeding precipitation and so limiting water availability for vegetation growth during seasonal drought (Wilson, 1989). The following four hypotheses were tested: (1) that differences in ecophysiological traits related to maintenance of water balance and photosynthetic capacity contribute to the greater success of *C. procera* than *C. gigantea* in seasonally dry areas; (2) that the higher density of trichomes in *C. gigantea* provides a photoprotective mechanism that improves its photosynthetic performance compared with *C. procera*; (3) that *C. procera* is more widely adapted to short-term changes in temperature than *C. gigantea*; and (4) that limitation of photosynthesis to drought in xerophytic, successfully adapted species is caused by stomatal closure and not by metabolic limitation.

MATERIALS AND METHODS

Plant material and field site climate

The study was carried out in a thorn scrub on the coast of Vargas State in Venezuela at 10°34′ N, 67°09′ W and 38 m. The area has been defined as extreme, semi-desert, dry coastal xerophytic scrub vegetation (Huber and Alarcon, 1988). The species studied, *Calotropis procera* and *C. gigantea*, are important evergreen components of these ecosystem, although *C. procera* is more abundant than *C. gigantea* (a proportion of approx. 3:1 *C. procera*:*C. gigantea* plants in the 1000 m[2] study area). Ten plants of each of the two *Calotropis* species were sampled from May 2004 to April 2005, three times during the DS and three during the wet season (WS). The years of the study were characterized by a

precipitation pattern with a DS from December to April with 70 mm rainfall and a WS (May–November) with 617 mm. The mean monthly air temperatures (T_a) ranged from 26 to 34 °C (Colombo *et al.*, 2007).

Microclimatic parameters

The photosynthetic photon flux (PPF) was measured with a quantum sensor and meter (LI-185; LI-COR Inc., Lincoln, NE, USA). Air (T_a) and leaf temperature (T_L) were measured with YSI 405 and 409 B thermistors, respectively, connected to a telethermometer (Yellow Springs, OH, USA), and relative humidity was measured with a hair strand hygrometer (Abbeon model AB167B, Santa Barbara, CA, USA).

Physiological measurements

All physiological measurements described below were carried out on the fourth fully expanded intact leaf.

Water relations. Xylem water potential (ψ) was measured on leaf discs taken at 0630 h ($n = 4$) with a Wescor 5000 osmometer (Wescor, Inc., Logan, UT, USA). Leaf water content (LWC) was determined in leaf discs from ten leaves taken at 0700 h as LWC = [fresh weight (f. wt) – leaf dry weight (d. wt)]/leaf d. wt.

Soil samples ($n = 4$) were collected near the plants studied at a depth of 30 cm. The samples were placed in metal containers, weighed, dried at 100 °C for 72 h, re-weighed and the soil water content (SWC) calculated as: (soil f. wt – soil d. wt)/soil d. wt. Also, soil texture was determined by Bouyoucos analysis as described by Anderson and Ingram (1993).

Specific leaf area and pubescence. Specific leaf area (SLA = disc area/disc mass) was measured on discs ($n = 10$) from the same leaves used for the gas exchange determinations, dried to constant weight at 70 °C.

Leaf trichomes of each species were carefully removed with a brush, weighed and the pubescence was expressed as a proportion of the total leaf weight ($n = 15$). The PPF absorbed by the leaf was measured in leaves ($n = 20$) of each species with the 1800-12 integrating sphere (LI-COR). Also, leaf angle relative to the horizontal was determined using a protractor.

Leaf gas exchange. Gas exchange in attached leaves was measured with a portable infrared gas analyser [IRGA; CIRAS 2 with a PLC (B) assimilation chamber; PP Systems plc, Hitchin, UK]. Measurements were done at a CO_2 concentration (C_a) of 370 µmol mol^{-1}, unless otherwise stated. Instantaneous A was measured at 1000 h when the daily maximum A occurred, at a PPF = 1200 µmol m^{-2} s^{-1} and a T_L of 32 ± 0·5 °C. The integrated daily net photosynthetic rate (A_D) was calculated as the integration of the area below the curves of six daily courses of A. The A/C_i curves were generated during the WS ($n = 4$) and DS ($n = 6$) by reducing C_i as calculated by the program in the IRGA after Farquhar *et al.* (1980) from approx. 220 µmol mol^{-1}, when A at $C_a =$ 370 µmol mol^{-1} was initially measured, to zero and then progressively increasing C_i to 1400 µmol mol^{-1}. CO_2 was provided by a cylinder filled with pure gas inserted into the IRGA. The A/C_i curves were fitted to the empirical equation

$A = b + de^{KC_i}$, where $b = A_{CO2\text{-sat}}$ and $(b + d) = y$-intercept (Tezara *et al.*, 1998). The CE was calculated from the initial slope of the curve. The L_s was calculated as $L_s = 100 \times (A_o - A)/A_o$, where A_o is A at $C_i = C_a$ (Farquhar and Sharkey, 1982). The L_m was calculated as $L_m = 100 \times (A_C - A_D)/A_C$, where A_C is the A of WS leaves at $C_i = 700$ µmol mol^{-1} and A_D is the rate of droughted leaves (DS) at the same C_i (Jacob and Lawlor, 1991). Response curves of A vs. PPF and A vs. T_L were generated between 0900 and 1100 h at ambient C_a, using a leaf microclimate control system connected to an LCA-4 IRGA (Analytical Development Co., Hoddesdon, UK). The PPF was varied using neutral filters (Balzers, Handelsbank, Zürich, Switzerland); the light source was either the sun or a 50 W dichroic lamp (Phillips, Caracas, Venezuela). No gas exchange measurements were done on leaves without trichomes due to a strong wilting (loss of turgor) and stomatal closure half an hour after pubescence was removed.

Carbon isotope ratio ($\delta^{13}C$). Leaf samples were ground and then analysed for $\delta^{13}C$ ($n = 4$) with an isotope ratio mass spectrometer at the Ecology and Evolution Department of Biological Sciences (University of Illinois at Chicago).

Chlorophyll a fluorescence. Chlorophyll fluorescence was measured on attached dark-adapted leaves ($n = 6$) with a Mini PAM fluorometer (Walz, Effeltrich, Germany) using the protocol described by Genty *et al.* (1989). The F_v/F_m was measured *in situ* at the minimum dawn PPF. The ϕ_{PSII} at steady state A was calculated as $\phi_{PSII} = (F'_m - F_s)/F'_m$, where F_s and F'_m are fluorescence at steady-state A and maximum fluorescence in the light, respectively. The photochemical quenching coefficient q_P and non-photochemical quenching (NPQ) were calculated from measurements of fluorescence. The electron transport rate of PSII was estimated by the method of Krall and Edwards (1992) as $J = \phi_{PSII} \times$ PPF $\times a \times 0·5$, where a is the fraction of incident PPF absorbed by the leaf.

Statistical analysis

Results are presented as means ± s.e. Significance at $P < 0·05$ was assessed by one- and two-way analysis of variance (ANOVA) using the statistical package Statistica. Curves were fitted using the Sigmaplot package.

RESULTS

Microclimatic conditions

No differences were observed in microclimatic parameters between seasons. The PPF between 0700 and 1600 h ranged from 100 to 1500 µmol m^{-2} s^{-1}, T_a from 25 ± 0·5 to 32·0 ± 0·2 °C and relative humidity from 85 ± 2 to 65 ± 1 %. The daily course of T_L was the same for both species and did not change between seasons; from 1000 to 1500 h T_L was maintained at 31 ± 0·5 °C; leaf–air water vapour deficit pressure (Δw) ranged between 0·5 and 1·6 kPa for both species.

Water status and gas exchange

The soil at the site was sandy, consisting of 71 % sand, 14 % clay and 16 % lime. Table 1 shows the seasonal changes in

TABLE 1. *Seasonal changes in soil water content (SWC; n = 24), leaf water content (LWC; n = 10), specific leaf area (SLA; n = 10), pubescence (weight of trichomes; n = 15), fraction of incident PPF absorbed by the leaf (*a*; n = 20) and leaf angle (n = 50) of plants of* Calotropis procera *and* C. gigantea *growing in the field*

Parameter	*C. procera*		*C. gigantea*	
	WS	DS	WS	DS
SWC (% dry matter)	8·3 (0·4)[c]	1·4 (0·2)[a]	4·3 (0·1)[b]	1·3 (0·2)[a]
LWC (% dry matter)	86 (4)[b]	74 (3)[a]	86 (3)[b]	78 (3)[a]
SLA (cm^2 g^{-1})	127 (5)[a]	126 (6)[a]	203 (9)[c]	167 (2)[b]
mg trichomes g^{-1}	1·3 (0·1)[a]	1·8 (0·4)[a]	3·3 (0·2)[b]	4·4 (0·5)[b]
a	0·82 (0·01)[b]	0·79 (0·01)[b]	0·75 (0·00)[a]	0·74 (0·00)[a]
Leaf angle (°)	20 (1)[a]	23 (0·7)[a]	48 (1)[b]	51 (1·1)[b]

Results are presented as means (s.e.). Different letters indicate significant differences between seasons and species for each parameter at $P < 0.05$.

SWC and several leaf variables. In the DS, SWC decreased 83 % where *C. procera* grows, in contrast to a 69 % reduction where *C. gigantea* grows. Both species showed a similar LWC in the two seasons and, after several months of drought (from August 2004 to April 2005), LWC was significantly reduced by 14 and 9 % in *C. procera* and *C. gigantea*, respectively. The SLA was significantly lower in *C. procera* than in *C. gigantea*, decreasing 69 % with drought in *C. gigantea* and remaining unaffected in *C. procera*. Pubescence was unaffected by season in both species; in *C. gigantea*, trichome mass was approx. 2·5 times higher than in *C. procera*. The fraction of PPF absorbed by leaves of *C. procera* was significantly higher than by those of *C. gigantea*, while the leaf angle was smaller in the former.

The δ^{13}C was higher in *C. gigantea* ($-26·2 \pm 0·4$ ‰) than in *C. procera* ($-27·4 \pm 0·06$ ‰) during the WS.

No seasonal differences in ψ between the species were observed; a decrease of $-0·4$ MPa on average occurred with drought (Fig. 1). Differences in gas exchange were observed between species and seasons (Fig. 1). Leaves of *C. procera* showed values of A and g_s approx. 28 and 66 % higher than *C. gigantea* during the WS, this difference disappearing during the DS. During the DS, A and g_s decreased 48 and 49 %, respectively in *C. procera*, while in *C. gigantea* A was reduced by 34 % and g_s was unchanged. In *C. gigantea*, E was significantly lower than in *C. procera* and during the DS a reduction of 48 % was observed in both species (Fig. 1). The WUE was higher in *C. gigantea* and remained unaffected in both species during the DS.

The C_i/C_a ratio decreased little in *C. procera*, while no change was observed in *C. gigantea* (Table 2). The integrated daily net photosynthetic rate (A_D) followed the same trend observed in A, i.e. higher in *C. procera* during the WS than in *C. gigantea*; A_D decreased by 39 and 31 % in *C. procera* and *C. gigantea*, respectively, during the DS (Table 2).

Through the analysis of A/C_i curves it was found that both $A_{CO2-sat}$ and CE declined with drought in *C. procera*, whereas CE was unaffected in *C. gigantea* (Fig. 2; Table 2). During drought, the CO_2 compensation point (Γ) increased approx. 2 and 2·4 times in *C. procera* and *C. gigantea*, respectively. The L_s increased 33 % in both species, whereas L_m increased with drought by 45 and 58 %, in *C. procera* and *C. gigantea*, respectively.

During the WS, A ($A_{PPF-sat}$) was saturated at a PPF of 1500 µmol m^{-2} s^{-1} in both species and was higher in *C. procera* (Table 3). Significant differences were observed between the species in ϕ_{CO2} and the light compensation point (LCP), while no differences were observed in the dark respiration rate (R_D).

The optimum temperature for A, when 90 % of maximum A occurs, showed a wider range (25–40 °C) in *C. procera* than in *C. gigantea* (33–38 °C). A higher value of A, measured at 35 °C T_L, was observed in *C. procera* than in *C. gigantea* (Fig. 3).

Chlorophyll a *fluorescence*

Seasonal changes in fluorescence parameters were observed (Fig. 4). The F_v/F_m for both species was 0·84 and was not affected by drought. During the DS, decreases in ϕ_{PSII} of 42 and 32 % at high PPF were observed in *C. procera* and *C. gigantea*, respectively; consequently, J was reduced by 32 and 28 % in *C. procera* and *C. gigantea*, respectively. The q_P followed the same trend as ϕ_{PSII}, decreasing 30 % with drought, while NPQ increased 60 % in *C. procera* and approx. 100 % in *C. gigantea* in the DS, although NPQ was lower in *C. gigantea*. The J was higher in *C. procera* due to higher ϕ_{PSII} and q_P.

DISCUSSION

Calotropis species are drought (Colombo *et al.*, 2007) and salt tolerant (Khan *et al.*, 2007); however, the mechanism behind their successful distribution across arid regions is not well understood. Analysis of the responses of two closely related species, *C. procera* and *C. gigantea*, which have invaded seasonally dry desert areas of Venezuela, in a continent from which they did not originate, was aimed at increasing understanding of their mechanisms of adaptation to dry conditions and how these, and differences between the two species, may affect their ecology. Most studies on *Calotropis* focus on the pharmacological and medical aspects, and studies on the ecophysiology of *Calotropis* are needed to improve the basis for management of this plant species. In addition, analysis of the changes to leaf physiology caused by water deficit and effects of temperature enhances understanding of the way in which drought under natural conditions affects plants of semi-desert areas. Basic physiological responses of desert plants are not well understood, and such information may be compared with responses of crop plants which have been
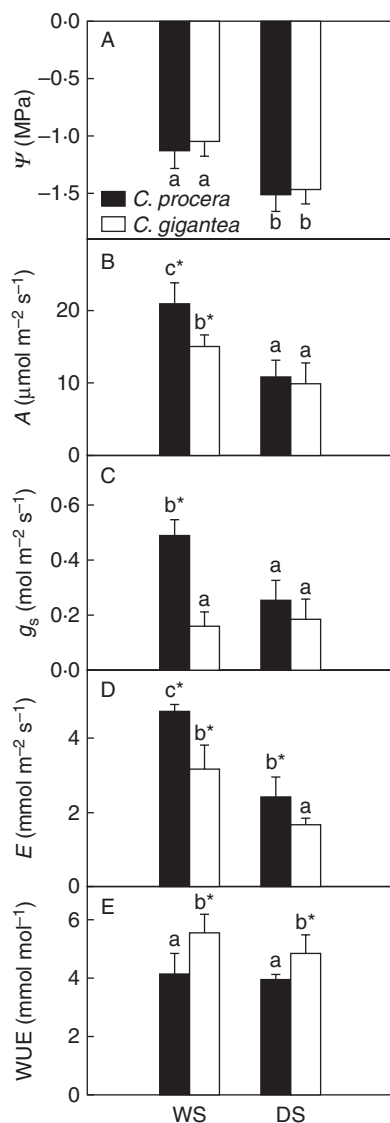
FIG. 1. Seasonal changes in (A) xylem water potential, (B) photosynthetic rate, (C) stomatal conductance, (D) transpiration rate and (E) water use efficiency of leaves of *Calotropis procera* and *Calotropis gigantea*, as indicated, during the wet (WS) and dry seasons (DS). Values are means ± s.e. ($n = 36$) of measurements made three times during the DS and three times during the WS. Gas exchange was measured between 1000 and 1100 h under a PPF of $1200 \pm 70 \ \mu mol \ m^{-2} \ s^{-1}$, $32 \pm 0.5 \ °C$ leaf temperature, and ambient CO_2 concentration of $370 \ \mu mol \ mol^{-1}$. Different letters indicate significant differences between seasons, and asterisks indicate significant differences between species for each parameter at $P < 0.05$.

somewhat better studied (Boutraa, 2010). Also the potential pharmacological value of *Calotropis* species, and the need for management to decrease their impact on invaded ecosystems, warrant these and further studies.

Responses of C. procera *and* C. gigantea *during the wet season*

Values of ψ of *C. procera* and *C. gigantea* during the WS were the same, lower than in other xerophytic species (Tezara *et al.*, 2005) but similar to those reported for some evergreen trees and drought-avoiding shrubs (Tezara *et al.*,
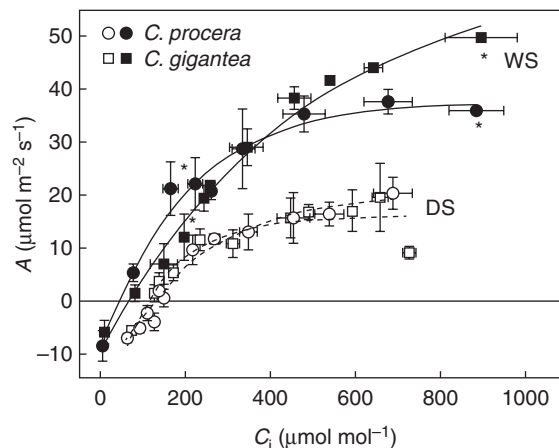

FIG. 2. Response of photosynthetic rate to intercellular CO_2 concentration in leaves of *Calotropis procera* and *Calotropis gigantea*, as indicated, growing in the field during the wet (WS, filled symbols, $n = 4$) and dry season (DS, open symbols, $n = 6$). Values are means ± s.e.; standard errors are shown when larger than the symbols. Asterisks indicate significant differences at $P < 0.05$ of $A_{CO2\text{-sat}}$ and carboxylation efficiency between species in the WS.

1998). In contrast, very low values of ψ (-5 MPa) have been reported for shrubs from a dry-deciduous ecosystem (Smith and Nobel, 1986) and herbs in a tropical thorn scrub during the DS (Tezara *et al.*, 1998). There were differences between the photosynthetic performances of the two species: A, g_s and A_D were greater (larger photosynthetic capacity) in *C. procera* than in *C. gigantea*, but these interspecific photosynthetic differences disappeared in the DS. Also, in *C. procera*, both ϕ_{CO2} and the PPF-saturated photosynthetic rate ($A_{PPF\text{-sat}}$) were higher than in *C. gigantea*, which suggests that the former has either a larger light-harvesting capacity or a greater electron transport rate. Also, the CE was larger in *C. procera* than in *C. gigantea*, indicating that it has a higher activity and/or amount of Rubisco. Differences in light absorption (*a*) due to the variation in leaf pubescence may explain photochemical differences between the two species: leaves of *C. gigantea* with a greater trichome density and a higher angle are less prone to overheating and have a smaller NPQ. Changes in NPQ associated with differences in pubescence have been reported in species of *Caragana* (Ma *et al.*, 2004), *Encelia* (Ehleringer and Björkman, 1978) and varieties of *D. minor* (Galmés *et al.*, 2007).

The larger $\delta^{13}C$ of *C. gigantea* than *C. procera* in the WS indicated a better WUE (long term) in this species, related to its lower g_s. The $\delta^{13}C$ for both species in this study agree with data of *C. procera* ($-27.9 ‰$) in southern Sinai, supporting the conclusion that the photosynthetic metabolism is C_3 (Sayed and Mohamed, 2000).

In the WS, *C. procera* showed a broad temperature optimum (T_{opt}; short-term responses) for A and higher values compared with *C. gigantea*, allowing a greater carbon gain over a wider range of environmental temperatures. The leaf temperature for both species was around $31 \pm 0.5 \ °C$ from 1000 to 1500 h, where A was 98 % of maximum for *C. procera*; in contrast *C. gigantea* showed 75 % of maximum A during the same time interval, contributing to the lower A_D of *C. gigantea*

TABLE 2. *Seasonal changes in intercellular CO_2/ambient CO_2 concentration ratio (C_i/C_a ratio; n = 36), integrated daily net photosynthetic rate (A_D; n =18). CO_2-saturated photosynthetic rate ($A_{CO2-sat}$), carboxylation efficiency (CE), CO_2 compensation concentration (Γ), relative stomatal and mesophyll limitations (L_s and L_m; n = 4 for the WS and n = 6 for the DS for A/C_i parameters), in leaves of* Calotropis procera *and* C. gigantea *growing in the field*

	C. procera		C. gigantea	
Parameter	WS	DS	WS	DS
C_i/C_a ratio	0·63 (0·03)[a]	0·51 (0·02)[b]	0·61 (0·003)[a]	0·58 (0·02)[a]
A_D (mmol m^{-2} d^{-1})	271[c]	167[a]	234[b]	162[a]
$A_{CO2-sat}$ (μmol m^{-2} s^{-1})	38·9 (1·9)[b]	26·7 (3·9)[a]	53·7 (5·6)[c]	24 (5·7)[a]
CE (mol m^{-2} s^{-1})	0·29 (0·05)[b]	0·16 (0·02)[a]	0·18 (0·01)[a]	0·16 (0·02)[a]
Γ (μmol mol^{-1})	62·7 (12)[a]	117·6 (7)[b]	51·3 (8)[a]	125·3 (7)[b]
L_s (%)	31·6 (5)[a]	42·1 (3)[bc]	35·1 (1)[ab]	46·7 (2)[c]
L_m (%)	0	45 (8)[a]	0	58 (9)[a]

Results are presented as means (s.e.). Different letters indicate significant differences between seasons and species for each parameter at $P < 0.05$.

TABLE 3. *Photosynthetic photon flux-saturated photosynthetic rate ($A_{PPF-sat}$), apparent quantum yield of CO_2 fixation (ϕ_{CO_2}), light compensation point (LCP) and dark respiration (R_D) in leaves of plants of* Calotropis procera *and* C. gigantea *growing in the field during the WS*

Parameter	C. procera	C. gigantea
$A_{PPF-sat}$ (μmol m^{-2} s^{-1})	18·4 (2)[b]	10·8 (0·5)[a]
ϕ_{CO2} (μmol μmol photon^{-1})	0·017 (0·000)[b]	0·014 (0·002)[a]
LCP (μmol m^{-2} s^{-1})	140 (28)[a]	216 (20)[b]
R_D (μmol m^{-2} s^{-1})	2·2 (0·3)[a]	2·6 (0·3)[a]

Results are presented as means (s.e.) ($n = 4$). Different letters represent significant differences between species for each parameters at $P < 0.05$.
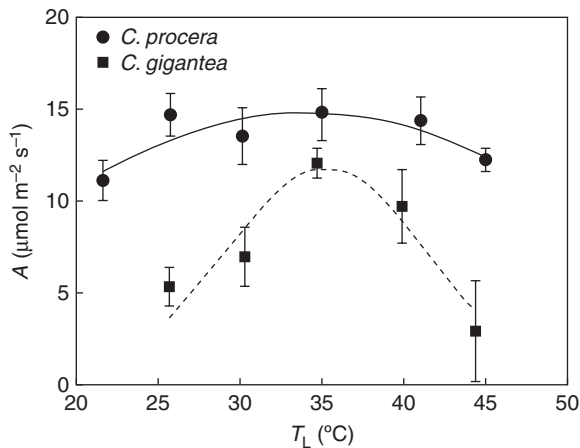

FIG. 3. Response of photosynthetic rate to leaf temperature of *Calotropis procera* and *Calotropis gigantea*, as indicated, growing in the field during the wet season, WS. Values are means ± s.e. ($n = 4$); standard errors are shown when larger than the symbols. Measurements were done under a PPF of 1200 ± 70 μmol m^{-2} s^{-1} and ambient CO_2 concentration of 370 μmol mol^{-1}.

(14 % lower than *C. procera*). Differences between species within a genus with respect to the T_{opt} for A have been reported for the genus *Caragana* (Ma *et al.*, 2004). In contrast, species from the same genus (*Encelia farinosa* and

E. californica) showed a similar T_{opt} (Ehleringer and Björkman, 1978).

Responses of C. procera *and* C. gigantea *during the dry season*

Plants of *C. procera* as well as *C. gigantea* were able to withstand a severe reduction in SWC without a marked decrease in water status, as denoted by maintenance of rather high values of ψ compared with the wet, but drought did cause a slight reduction in ψ and LWC in both species. Maintenance of the water status in both species is probably due to their deep root systems, enabling water absorption from deep soil. Sharma (1968) showed that *C. procera* has a very deep, stout taproot reaching depths of $1.7-3.0$ m with few or no near-surface lateral roots in Indian sandy desert soils. We measured smaller SLA in *C. procera* than *C. gigantea*, but this seems to have little correlation with the responses, despite SLA being used as an index of scleromorphism; low values have been related to a high capacity to resist low water availability (Ogaya and Peñuelas, 2006).

Despite the xeromorphic characteristics and the relatively small decrease in water potential and LWC, water deficit did inhibit gas exchange of both species, but more so in *C. procera* than in *C. gigantea*. The photosynthetic capacity [instantaneous photosynthetic rate, $A_{PPF-sat}$, $A_{(at\ 35°C)}$, CE, A_D and ϕ_{CO2}] of *C. procera* was higher than that of *C. gigantea*. The reduction in both $A_{CO2-sat}$ and A_D may be attributed to stomatal closure as well as a reduction in mesophyll capacity, with similar responses from both species. Some of the ecophysiological traits observed in the two species of *Calotropis* (e.g. a positive and high photosynthesis rate throughout the year in the field and a high instantaneous WUE even during the DS, very pubescent leaves and a low SLA) may contribute to their successful growth in areas with lack of water. Similar results have been reported for *C. procera* growing in the Karachi desert (Khan and Beena, 2002). Values of A_D throughout the year were greater than observed in other xerophytic C_3 species (Tezara *et al.*, 1998) and less affected by drought, suggesting why this plant is successful in dry conditions and thus invasive. The positive effects of pubescence on decreasing the radiation load on the photosynthetic apparatus may contribute to photoprotection
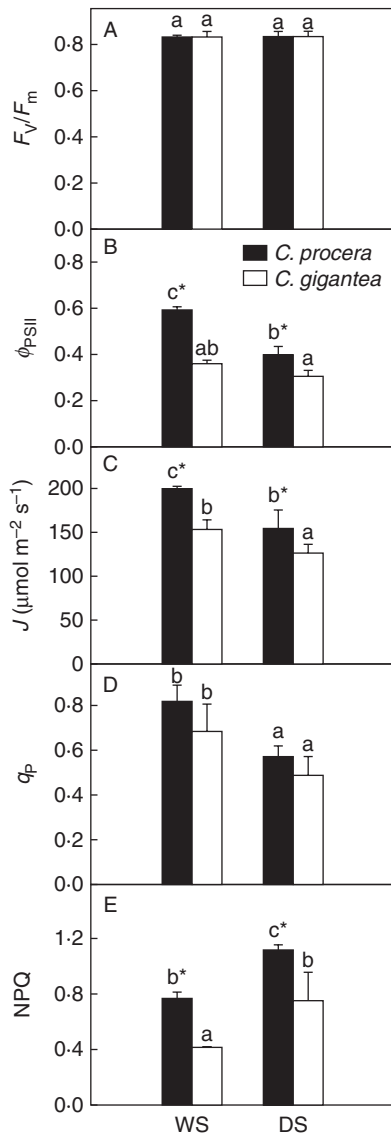
FIG. 4. Seasonal changes in (A) maximum quantum yield, (B) relative quantum yield of PSII, (C) total electron transport rate, (D) coefficient of photochemical quenching and (E) coefficient of non-photochemical quenching of fluorescence in plants of *Calotropis procera* and *Calotropis gigantea*, as indicated. Values are means ± s.e. ($n = 36$). Fluorescence parameters were measured three times during the dry season (DS) and three times during the wet season (WS) at 1000 h under a PPF of $1170 \pm 40 \, \mu mol \, m^{-2} \, s^{-1}$. Asterisks indicate significant differences at $P < 0.05$ of A_{max} and carboxylation efficiency between species in the WS.

long period to the DS. However, drought led to partial stomatal closure (g_s reduced by 58 %) in *C. procera* but not in *C. gigantea*, probably due to its greater pubescence and leaf angles, similar to the response of other plants of such habitats, e.g. *E. farinosa* (Ehleringer and Björkman, 1978) which decrease water loss by transpiration.

Water deficit markedly reduced $A_{CO2\text{-}sat}$, and CE in *C. procera*, while CE was unaffected in *C. gigantea*. The lower values of CE and $A_{CO2\text{-}sat}$ suggest loss of Rubisco amount and/or activity with decreasing ψ. The amount and activity of Rubisco and the availability of RuBP affect CE, and thus *A* (Lawlor and Tezara, 2009). Similar results were found for *Medicago truncatula* (Nunes *et al.*, 2008), *Ipomoea carnea* and *Jatropha gossypifolia* (Tezara *et al.*, 2005), and *Lycium nodosum* (Tezara *et al.*, 2003). In contrast, CE was unaffected by drought in *E. californica* and *E. farinosa* (Ehleringer and Björkman, 1978). The changes in $A_{CO2\text{-}sat}$ support the earlier conclusion that factors associated with decreased ψ which progressively reduced *A* increase non-stomatal limitations of *A* (Lawlor, 2002; Lawlor and Cornic, 2002; Lawlor and Tezara, 2009).

The increase in L_s and L_m with declining ψ shows that stomatal (Farquhar and Sharkey, 1982) and mesophyll (Lawlor, 2002; Nunes *et al.*, 2008; Lawlor and Tezara, 2009) factors limit *A* under drought conditions. Also, the nearly constant value of C_i with an average $C_i/C_a = 0.58$ in both species and seasons suggests that there is a balance between the stomata limitation and the mesopohyll activities even under the longer term drought (Schulze and Hall, 1982; Lawlor, 2002). Many studies have reported that both stomatal and metabolic components are responsible for decreased *A*, not only in mesophytic crop plants under relatively rapid drought [e.g. *Helianthus annuus* (Tezara *et al.*, 1999; Lawlor, 2002)] but also of *Amaranthus palmeri* (Ehleringer, 1983), *E. farinosa* (Ehleringer and Cook, 1984), and *L. nodosum*, *I. carnea* and *J. gossypifolia* (Tezara *et al.*, 2003, 2005).

An increase in L_m probably reflects reductions in photochemical activity related to ATP supply which determines RuBP regeneration, thereby decreasing CE. It has also been suggested that increased L_m under stress is caused by decreased activity of some Calvin cycle enzymes, for example Rubisco, which would be seen as a decrease in CE, but evidence for this is weak (Lawlor and Tezara, 2009). Another factor that may contribute to increased L_m is shrinkage of tissue with decreasing RWC (Hassiotou *et al.*, 2009). This could be the case in *C. gigantea*, in which SLA was reduced by 20 %. A more compact mesophyll would decrease gaseous diffusion pathways, thus preventing ready access of CO_2 to the mesophyll cells. However, in mesophytes, increasing the ambient CO_2 does not overcome the limitation (which may be partly caused by shrinkage), although the relative magnitude of the mesophyll limitation might be smaller because of shrinkage. Maintenance of relatively constant C_i/C_a could also reflect long-term adaptation to conditions in metabolism and with the stomata.

Water deficit often does not affect F_v/F_m (Lawlor and Cornic, 2002; Tezara *et al.*, 2003; Nunes *et al.*, 2008) and did not do so in this study, so photoinhibition was not observed during the DS, suggesting that PSII activity is very drought resistant in *C. procera* and *C. gigantea*. However, F_v/F_m was

and so stress did not cause chronic photoinhibition. Also, it clearly reduces water loss by transpiration so can also help both species to avoid substantial loss of water and photosynthetic activity.

In both species of *Calotropis* photosynthesis is limited by both stomatal and non-stomatal factors. In this respect, the response is similar to that of crop plants where a relatively rapid decrease in water supply leads to dehydration of leaves with lower RWC and ψ and loss of turgor, which can result in stomatal closure (Lawlor, 2001; Lawlor and Tezara, 2009). Such mechanisms may not apply to plants of dry regions such as *C. procera* and *C. gigantea*, adapted over a

decreased and photoinhibition occurred in the shrubs *I. carnea* and *J. gossypifolia* under natural conditions (Tezara *et al.*, 2005) and in the herb *D. minor* (glabrous variety) (Galmés *et al.*, 2007). The high leaf trichome density in *Calotropis* species could be an efficient mechanism of photochemical photoprotection.

The lower ϕ_{PSII} of droughted plants of both species of *Calotropis* was accompanied by a lower q$_P$ and J, i.e. with a higher reduction state of the Q_A pool, compared with plants in the WS: this is related to the increase in L_m. This is similar to the decreased ϕ_{PSII}, indicating that J was affected by drought, in other xerophytic species (Tezara *et al.*, 2003, 2005). Drought increased NPQ in both species of *Calotropis*, suggesting that a greater proportion of the energy was thermally dissipated, thus accounting for the apparent downregulation of PSII and supporting the photoprotective role of NPQ.

We conclude that hypothesis 1 is substantiated, but the responses are complex. During the WS both species of *Calotropis* had a similar water status, but A, A_D and g_s were higher in *C. procera* than in *C. gigantea*, showing a higher photosynthetic capacity, possibly related to its greater leaf thickness. Also *C. procera* had a greater and broader short-term temperature optimum (substantiating hypothesis 3). The larger photosynthetic capacity of *C. procera* shown by these short-term measurements is an explanation of its wider and more frequent distribution than *C. gigantea*. Both species showed high (instantaneous) WUE compared with other xerophytic species in the north of Venezuela, indicating that they are well adapted to seasonally dry habitats. The presence of highly pubescent leaves together with a deep root system probably helps these two species to tolerate drought, particularly *C. gigantea* during drought when photosynthesis was less inhibited that that of *C. procera* (supporting hypothesis 2). In the DS, water potential and RWC decreased in both species, but not as severely as in other xerophytes due, probably, to the deep root system attested to by the literature, hairs (which favour *C. gigantea*, reducing the effect on stomata, etc.) and the large instantaneous WUE, so water deficit did not cause chronic photoinhibition and some photosynthesis was maintained during the DS. Photosynthesis was co-limited by stomatal and non-stomatal factors (not supporting hypothesis 4). Co-limitation would operate as a mechanism that optimizes water use and resource allocation when carbon acquisition is impaired. Tolerance to high irradiance allows the plants to maintain a positive carbon balance and growth in semi-arid coastal habitats characterized by large temporal and spatial variations in rainfall.

ACKNOWLEDGEMENTS

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