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UNIVERSIDAD DE GUADALAJARA

CENTRO UNIVERSITARIO DE CIENCIAS BIOLÓGICAS Y AGROPECUARIAS

DIVICIÓN DE CIENCIAS BIOLÓGICAS Y AMBIENTALES

EFFECTS OF SHADE, DROUGHT AND DAUGHTER CLADODES ON THE CO_2 UPTAKE BY CLADODES OF *Opuntia ficus-indica.*

TRABAJO DE TITULACIÓN EN LA MODALIDAD DE:
INVESTIGACIÓN Y ESTUDIOS DE POSGRADO
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QUE PARA OBTENER EL TÍTULO DE:
LICENCIADO EN BIOLOGÍA

PRESENTA:
ISAAC CASTILLO CRUZ

LAS AGUJAS, ZAPOPAN, JALISCO NOVIEMBRE 2007

Este trabajo esta dedicado:

A mis queridos padres Trinidad Castillo Beltrán y Cesaría Cruz Hernández, de quienes tengo la dicha de recibir amor y apoyo, por la oportunidad que me dieron y el esfuerzo tan grande que hicieron para sacarme a delante en mi formación ante la sociedad.

A mis hermanos David, Salomón, Jacob, Moisés, Josué, Abraham y ha mi hermana Sara Montserrat, que sin ellos a mi lado no podría haber tenido una vida mejor "llena de diversión".

A mis tíos Felipa y Libos por el apoyo que me brindaron y abrirme las puertas de su casa al llegar ha esta ciudad.

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Dr. Francisco Martín Huerta Martínez
Presidente del Comité de Titulación
Carrera de Licenciado en Biología
Centro Universitario de Ciencias Biológicas y Agropecuarias
P r e s e n t e.

Por este conducto me permito **poner a su consideración mi anteproyecto** de titulación modalidad: **Investigación y Estudios de Posgrado.**

Titulado: **Effects of shade, drought and daughter cladodes on the CO_2 uptake by cladodes of Opuntia ficus-indica** el cual se anexa para que sea turnado al Comité de Titulación de esta carrera para su revisión y, en su caso, aprobación.

Así mismo, señalo como director de titulación a: Dr. Eulogio Pimienta Barrios.

Sin otro particular y en espera de su resolución, quedo de usted con un cordial saludo.

Atentamente
Las Agujas, Zapopan, Jal., 17 de Septiembre del 2007

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ISAAC CASTILLO CRUZ
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Por medio de la presente comunicamos a usted que ha sido designado como **SINODAL**, para el trabajo de titulación: **"Effects of shade, drought and daughter cladodes on the CO_2 uptake by cladodes of *Opuntia ficus-indica*"**, elaborado por el alumno: **Isaac Castillo Cruz** con la modalidad: **Investigación y Estudios de Posgrado** opción **Artículo Científico Publicado.**.

Recuerde que como sinodal le corresponde a usted evaluar y en su caso aprobar el presente proyecto, para lo cual le suplicamos no exceder de 8 días hábiles.

Sin más por el momento, aprovechamos para enviarle un cordial saludo.

Alejandro Muñoz U.

A T E N T A M E N T E
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C. ISAAC CASTILLO CRUZ

PRESENTE:

Manifestamos a usted que con esta fecha ha sido aceptado su trabajo de titulación intitulado: **"Effects of shade, drought and daughter cladodes on the CO_2 uptake by cladodes of *Opuntia ficus-indica*"**, mediante la modalidad **Investigación y estudios de posgrado** opción: **Articulo publicado**.
Así mismo le notificamos que ha sido **aceptado como director** del trabajo de titulación el **Dr. Eulogio Pimienta Barrios.**

ATENTAMENTE
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FORMA F

Dr. Fco. Martín Huerta Martínez.
Presidente del Comité de Titulación.
Licenciatura en Biología.
CUCBA.
Presente

Nos permitimos informar a usted que habiendo revisado el trabajo de titulación, modalidad **Investigación y estudios de Posgrado**, opción **Articulo Cientifico Publicado** Con el título: **"Effects of shade, drought and daughter cladodes on the CO_2 uptake by cladodes of Opuntia ficus-indica"** que realizó el pasante **Isaac Castillo Cruz** con número de código **B00007803** consideramos que ha quedado debidamente concluido, por lo que ponemos a su consideración el escrito final para autorizar su impresión.

Sin otro particular quedamos de usted con un cordial saludo.

Atentamente
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Supl. Cecilia Neri Luna	Cecilia Neri Luna	9 OCTUBRE 2007

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WATER USE EFFICIENCY

Effects of shade, drought and daughter cladodes on the CO_2 uptake by cladodes of *Opuntia ficus-indica*

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Keywords
Crassulacean acid metabolism; drought; net CO_2 uptake; *Opuntia ficus-indica*; relative water content

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Abstract

The effects of shade on the physiology of opuntias have received little attention, notwithstanding that shade regularly occurs in both wild stands and cultivated populations. This research evaluates the effects of shade on the physiology of cladodes of *Opuntia ficus-indica*, with and without daughter cladodes, as they are exposed to progressive drought. The stress caused by shade, drought and daughter cladodes reduced photosynthesis by mother cladodes and was associated with decreases in relative water content, parenchyma thickness and chlorophyll content. Shade exacerbated the physiological drought of mother cladodes imposed by daughter cladodes and by reduced soil water content.

Introduction

How much global photosynthesis occurs under light limitation is unclear (Osmond *et al.*, 1999). Moreover, the effects of shade for plants in arid environments have been neglected because it is widely accepted that light is an abundant resource there (Smith *et al.*, 1997), where water scarcity is considered to be the key environmental factor controlling plant functioning and productivity (Chesson *et al.*, 2004). In fact semiarid ecosystems in México are characterised by long-dry spring season, with high irradiance and little or no precipitation (Pimienta-Barrios *et al.*, 2002). Nevertheless, shade can affect plant functioning in arid ecosystems (Nobel, 1982, 1988). For instance, wild mature opuntias can develop closed crowns with successive layers of opaque thick cladodes oriented semivertically (López *et al.*, 1977), which lowers the efficiency of light penetration compared with typical C_3 plants (Nobel & Bobich, 2002). As a consequence, shade effects are common for cladodes in the inner part of the crown or on the opposite side from the sun's main trajectory (Pimienta-Barrios, 1990). Also, opuntias cultivated for young cladodes for human consumption (nopalitos) are commonly grown under plastic-covered tunnels, which greatly reduce light availability (Pimienta-Barrios, 1993). Net CO_2 uptake for opuntias can increase linearly with the photosynthetic photon flux (PPF) (Nobel, 1982, 1988). The utilisation of crassulacean acid metabolism (CAM) is more expensive energetically than is C_3 photosynthesis because of the greater quantum costs of CAM (Lüttge, 2004). In this regard, the interactive effects of drought, daughter cladodes and reduced light availability on the physiology of opuntias have not been studied. Here the combined effects of low light and daughter cladodes for *O. ficus-indica* exposed to a gradual reduction in soil water content during the spring dry period and an increase at the beginning of the wet period are considered with respect to net CO_2 uptake. Previous studies have found that plant performance during drought is more critical in the shade than in full sunlight (Valladares & Pearcy, 2002). Therefore, the photosynthetic response of *O. ficus-indica* to the physiological drought caused by its daughter cladodes is hypothesised to be exacerbated by low light because all factors tend to affect the activity of photosynthetic enzymes.

Materials and methods

Study sites, plant material and experimental design

Measurements were made during the spring and early summer of 2005 at an experimental field of the Departamento de Ecología, Universidad de Guadalajara. The site is in central Jalisco, México, at 20°5′N, 103°32′W, and 1420 m above sea level. The climate is temperate subtropical. The average annual rainfall is 1104 mm, and the average annual temperature is 19.5°C (climatic data provided by the Fifth Military Base of the Mexican Air Force at 20°45′N, 103°27′W, and 1625 m above sea level).

The photosynthetic photon flux (PPF) (wavelengths of 400–700 nm) was measured weekly from 13 April to 15 July 2005 on a horizontal plane; it was recorded hourly from sunrise to sunset with an LI-250 quantum sensor (LI-COR, Lincoln, NE, USA) and then integrated to obtain the total daily PPF. On the dates of gas exchange measurement, air temperature was determined hourly with a mercury thermometer and air relative humidity with a digital humidity gauge (RadioShack, Los Angeles, CA, USA). The length of daughter cladodes was measured weekly from 18 April to 20 July 2005, using a ruler with the cladode base as a reference.

Sixty mature 12-month-old cladodes (flattened stem segments) of *O. ficus-indica* (L.) Miller (Cactaceae) averaging 35 cm long, 19 cm wide and 1.8 cm thick were harvested on 3 February 2005 from a cultivated plantation at Nextipac, Jalisco, México. These cladodes were planted on 18 February 2005 in plastic pots (19 L) filled with a vermiculite/sand mixture (1:1, v/v). One cladode was planted per pot. To promote development of new cladodes, the planted cladodes were watered with 3 L per pot on two dates: 7 and 11 April 2005. Afterward, watering was suspended, allowing the soil around the mother cladode to dry at a rate determined by the conditions prevailing at the study site in the spring. No fertilisers or nutritive solutions were applied to the experimental plants.

Daughter cladodes were allowed to develop freely on the mature cladodes; on 17 April 2005, daughter cladodes were selectively removed, leaving only peripheral cladodes that developed around the apex of the mature cladodes. Two groups were formed (each composed of 30 mature cladodes): (a) in one group at least four daughter cladodes were allowed to develop and (b) in the other group all the daughter cladodes were eliminated once they began to sprout (zero daughter cladodes). Half of the plants in each group were exposed to full sunlight (FS) and half shaded (Sh). The Sh plants were covered with a plastic net (open at the sides) placed 3.5 m above ground level and 2.30 m above the mature cladodes, reducing the incident light by 45%. Each treatment was thus replicated 15 times, with a completely randomised design.

Chlorophyll determination

Ten samples for determination of chlorophyll content were removed from the centre of mother cladodes using a cork borer 1 cm in diameter for each treatment on the dates for gas exchange measurement. Chlorophyll was extracted by homogenising frozen material in cold acetone (80%, v/v). The homogenates were centrifuged for 10 min at 12 000 g at −4°C; the insoluble material was re-extracted and re-centrifuged. Chlorophyll content (μg cm^{-2}) was calculated from spectrophotometric measurements at 645 and 663 nm (Bruinsma, 1961).

Soil water content and cladode relative water content

Soil water content from the fine root proliferation zone, located from 3 to 12 cm from the base of mother cladodes (a depth of 10–15 cm) was determined every 3 to 6 days from 7 April to 27 July 2005 for 10 soil samples of 50 g each that were dried at 105°C to constant mass (generally within 72 h); data are expressed as percentage water content: [(fresh mass − dry mass)/fresh mass] × 100 (Torres, 1984). (Soil of similar water content was returned to the experimental pots.)

The relative water content (RWC) was determined for five segments (1.1 × 3.0 cm) of mother cladodes from 10:00 to 11:00 h on the dates of gas exchange measurement. The segments were immediately weighed to determine their fresh mass and then oven dried at 80°C to constant mass. Saturated mass was obtained after exposing stem sections (obtained with a cork borer 11 mm in diameter) and equilibrated with water-saturated filter paper at 30°C for 1 h. The RWC (%) was calculated as (fresh mass − dry mass)/(saturated mass − dry mass) (Koide *et al.*, 2000).

Gas exchange measurements

The rates of instantaneous net CO_2 uptake were measured for six mother cladodes every 2 h over 24 h periods on 22–23 April, 26–27 May, 15–16 June and 15–16 July 2005 with a portable LI-6200 photosynthesis system (LI-COR). A 0.25-L leaf chamber was modified by replacing the distal half-cylinder with a narrow opening (2 × 4 cm) lined with a closed-pore foam gasket that was firmly pressed against an approximately southwest-facing surface of the cladodes.

Statistical analysis

Data were analysed using a hierarchical analysis of variance (Zar, 1999); means were separated by a least significant difference test (Little & Hills, 1975). Data are presented as means ± SE (n = number of measurements).

Results

Microclimatic conditions and cladode growth

No rainfall occurred from February to April 2005. Substantial rainfall occurred during the third week of May and in late June; both increased the soil water content to 30% (Fig. 1A and Fig. 1B). The soil water content was further increased by considerable rainfall in July, although the daughter cladodes of *O. ficus-indica* showed

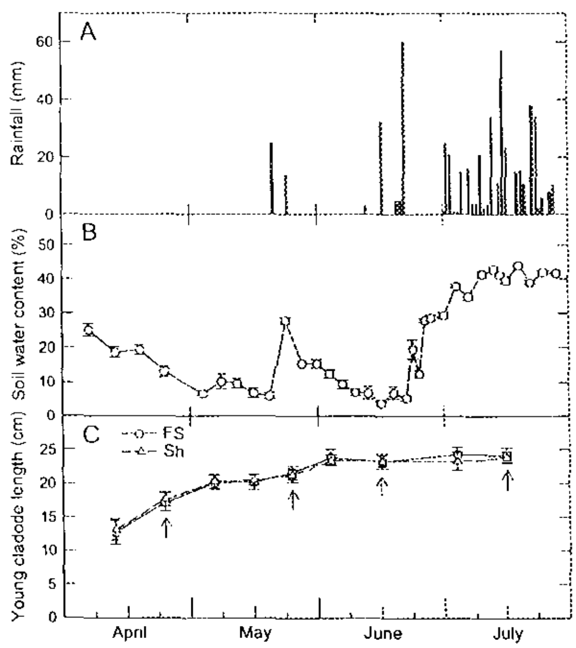

Figure 1 Rainfall (A), soil water content (B) and elongation of developing daughter cladodes of *Opuntia ficus-indica* (C) in the late spring and early summer 2005. Arrows indicate dates of gas exchange measurement. Data are means ± SE (n = 10). FS indicates full sunlight and Sh 45% shading

no further elongation then (Fig. 1C). The elongation rate of daughter cladodes was similar in full sunlight (FS) and when shaded by 45% (Sh). For the four dates of measurement of net CO_2 uptake (Fig. 1C), the total daily PPF ranged from 44 mol m^{-2} day^{-1} on 22–23 April to 47 mol m^{-2} day^{-1} on 15–16 July 2005 in FS; in Sh, the total daily PPF ranged from 22 mol m^{-2} day^{-1} on 26 May to 26 mol m^{-2} day^{-1} on 15 July 2005. Average day/night air temperatures (all ±2°C) were similar on 22–23 April (25/19°C), 26–27 May (25/20°C), 15–16 June (25/20°C) and 15–16 July (24/20°C) in FS and Sh. The average day/night relative humidities (all ±4%) were 27/56% on 22–23 April, 31/48% on 26–27 May, 40/69% on 15–16 June and 55/93% on 15–16 July 2005 in both FS and Sh.

Gas exchange measurements

Most of the total daily CO_2 assimilation and the highest instantaneous rates of net CO_2 uptake occurred at night for mother cladodes with and without daughter cladodes and were higher without daughter cladodes in both FS and Sh (average daily CO_2 assimilation of 505 mmol m^{-2} day^{-1} without daughter cladodes and 286 mmol m^{-2} day^{-1} with them, $P < 0.05$; Fig. 2). The daily CO_2 assimilation was higher under FS than Sh (463 mmol m^{-2} day^{-1} vs 329 mmol m^{-2} day^{-1}, $P < 0.05$; Fig. 2). Assimilation of CO_2 in the late afternoon (phase IV of CAM; Osmond, 1978) and early morning (phase II) was observed for mother cladodes without daughter cladodes on all measurement dates. For mother cladodes with daughter cladodes, CO_2 uptake in phase IV was observed in May in FS only and in July in both FS and Sh (Fig. 2F and Fig. 2H), and CO_2 assimilation in phase II was observed on all four dates except July in Sh. With the exception of FS in May, daughter cladodes tended to decrease daytime CO_2 uptake by mother cladodes in phase IV as soil water content decreased. The interruption of drought by rains in July allowed mother cladodes with daughter cladodes to recover daytime CO_2 uptake in phase IV (Fig. 2H).

Changes in relative water content

During the dry period (April to June), the RWC of mother cladodes without daughter cladodes decreased 6% in FS and 12% in Sh (Fig. 3A), whereas for mother cladodes with daughter cladodes, the decreases were 31% in FS and 24% in Sh ($P < 0.05$; Fig. 3B). The RWC of mother cladodes without daughter cladodes continued to decrease from June to the middle of July (Fig. 3A); however, the RWC of mother cladodes with daughter cladodes increased in July for both FS and Sh (15% and 3%,

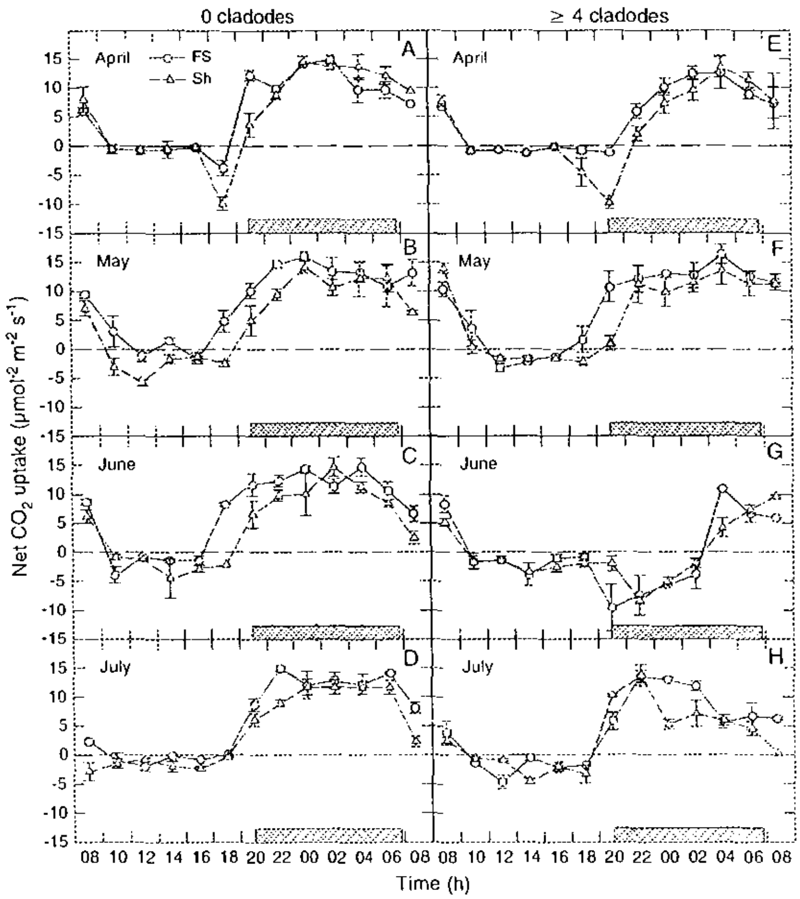

Figure 2 Net CO_2 uptake rates over 24-h periods for mother cladodes of *Opuntia ficus-indica* without (A–D) and with four or more daughter cladodes (E–H) in full sunlight (FS) and shade (Sh) on 22–23 April, 26–27 May, 15–16 June and 15–16 July 2005. Data are means ± SE (n = 6 plants).

respectively; Fig. 3B). Chlorenchyma thickness of mother cladodes increased in FS from 3.6 mm in April to 5.8 mm in July without daughter cladodes and to 4.7 mm with daughter cladodes ($P < 0.05$; Fig. 4A), while little change occurred in Sh (Fig. 4B). For mother cladodes without daughter cladodes in both FS and Sh, little net change occurred in parenchyma thickness from mid-April to the end of July, averaging 17.1 mm (Fig. 4C and Fig. 4D). However, the presence of daughter cladodes led to a 72% decrease in parenchyma thickness by the end of June for mother cladodes in FS (Fig. 4C) and a 45% decrease for Sh ones (Fig. 4D), followed by a recovery in thickness in July, especially under FS.

Changes in chlorophyll content

From mid-June until the end of July, the chlorophyll content steadily increased for mother cladodes without daughter cladodes in FS and Sh (Fig. 5A). For mother cladodes with daughter cladodes, chlorophyll content tended to decrease as soil water content decreased but increased from mid-June to mid-July (Fig. 5B).

Discussion

Shade can ameliorate drought effects by reducing leaf and air temperatures, the vapour pressure deficit, and photoinhibition (Quero *et al.*, 2006). The physiological stress caused by the combined effects of shade, lowered soil water content and daughter cladodes indeed reduced the photosynthetic ability of mother cladodes of *O. ficus-indica*, similar to effects on *Heteromeles arbutifolia* (Valladares & Pearcy, 2002). In southern Texas, *Opuntia lindheimerii* does not occur beneath the canopies of mature *Prosopis* but can form solid stands where the plants experience full sunlight after land clearing. Additionally, plants of

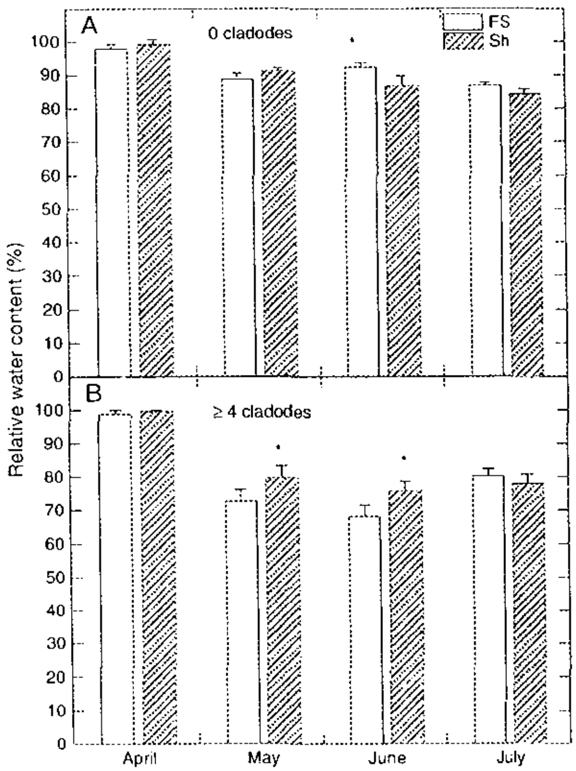

Figure 3 Relative water content of mother cladodes without (A) and with daughter cladodes (B) in full sunlight (FS; open bars) and shaded by 45% (Sh, hatched bars). Data are means ± SE (n = 10 plants).

O. lindheimerii tend to die in a non-irrigated lawn as a nearby *Prosopis* overtopped it and shaded it (P. Felker, personal communication). The lower soil water content from April to June coincided with a lower RWC in mother cladodes and a greatly reduced thickness of the water storage parenchyma. Daughter cladodes also hindered the photosynthetic recovery in July following substantial rainfall. In any case, succulence allowed the mother cladodes, especially those without daughter cladodes, to have substantial carbon gain during the dry period, which is enhanced by morphological adaptations such as thick cuticles and low stomatal frequency (Nobel & Bobich, 2002). Previous work showed that young cladodes exhibit C_3 photosynthesis with daytime stomatal opening (Osmond, 1978), leading to water import from the mother cladodes (Wang *et al.*, 1997). The development of daughter cladodes under dry conditions increases water loss to the environment, thereby reducing the ecological advantages of succulence in such plants (Pimienta-Barrios *et al.*, 2005).

Metabolic impairment may cause a large decline in daily carbon gain by mother cladodes with daughter cladodes at the end of the dry spring by affecting photophosphorylation, presumably leading to an inadequate supply of ATP (Lawlor, 2002). The reduction of ATP may be particularly critical for CAM plants such as opuntias because CAM metabolism is energetically more expensive than is C_3 photosynthesis (Lüttge, 2004). Interestingly, the rainfall that interrupted drought just before the May measurement augmented CO_2 uptake in full sunlight, suggesting that the drought endured by mother cladodes early in the spring did not impair their photosynthetic biochemistry and photochemistry (Cornic, 2000; Souza *et al.*, 2004). Actually, the rapid recovery of photosynthetic activity observed for mother cladodes of *O. ficus-indica*, particularly those with daughter cladodes, is commonly observed for perennial plants in arid and semiarid environments (Hanscom & Ting, 1978; Holmgren *et al.*, 2006; Potts *et al.*, 2006). The sink strength apparently contributed to the marked increase in carbon assimilation ability from April to May because at this time daughter cladodes are actively growing. Indeed, when sink activity is high during rapid growth, photosynthesis rates can also be high (Paul & Driscoll, 1997).

Drought and high irradiances generally reduce chlorophyll concentrations, while shade tends to increase them (Raveh *et al.*, 1998; Duan *et al.*, 2005; Ivanov *et al.*, 2006; Mohsenzadeh *et al.*, 2006). Unexpectedly, mother cladodes without daughter cladodes that were exposed to a progressive reduction in soil water content showed a gradual increase in chlorophyll content; nevertheless, chlorophyll content decreased when the mother cladodes had developing daughter cladodes, suggesting that the physiological drought caused by daughter cladodes was important, which may be related to the reduction in carbon gain during the dry period.

The amount of light received by CAM plants during the daytime determines the degree of dark fixation of CO_2 at night (phase I; Nobel & Hartsock, 1984; Keller & Lüttge, 2006). Here, CO_2 uptake in phase I was not different between full sunlight and shading by 45% for mother cladodes with and without daughter cladodes during the dry period. Nevertheless, CO_2 uptake at night was affected, particularly in April and May, if mother cladodes developed daughter cladodes, which also lessen the photosynthetic recovery when plants received substantial rainfall in July, especially in the shade.

The water-storage parenchyma greatly decreased in thickness as drought progressed, particularly in full sunlight for mother cladodes with daughter cladodes. However, the chlorenchyma thickness of mother cladodes showed little changes during the dry and the wet periods, suggesting water movement from the parenchyma to the chlorenchyma (Nobel, 2006). Therefore, the severe

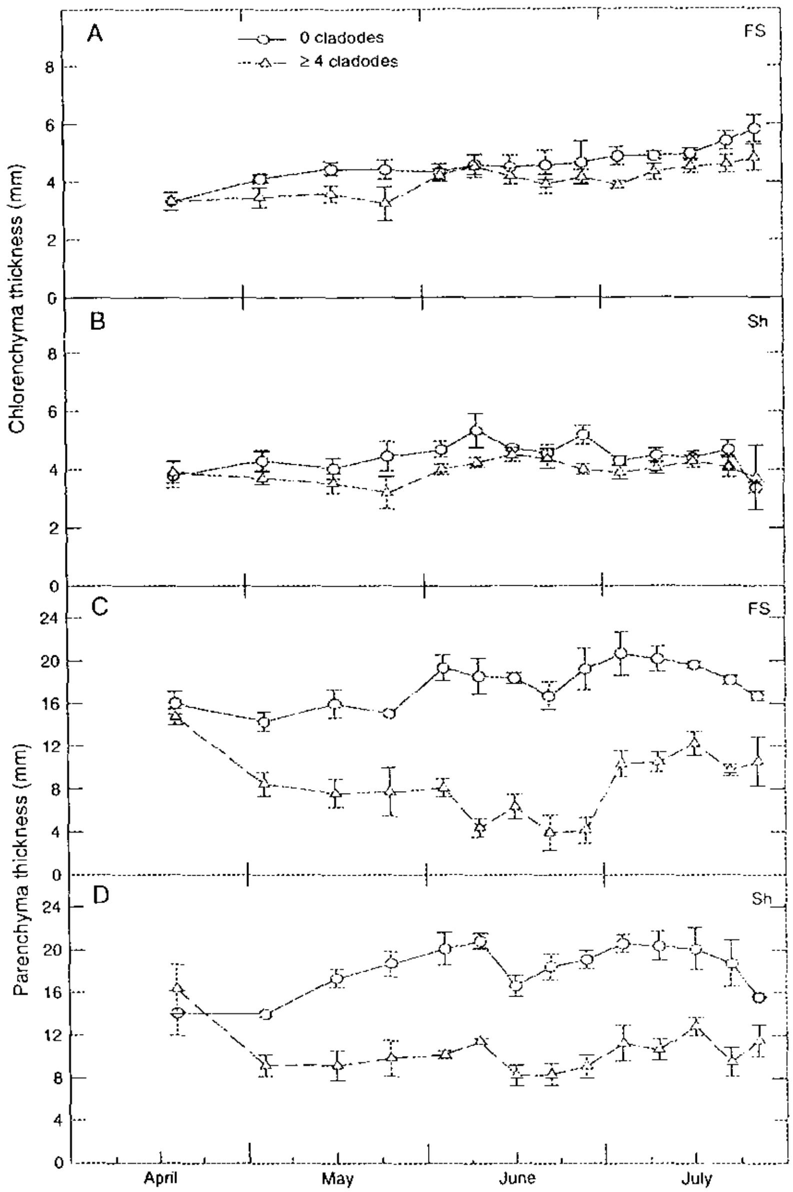

Figure 4 Chlorenchyma (A, B) and parenchyma (C, D) thicknesses in full sunlight (FS) and shaded by 45% (Sh) for mother cladodes without (○) and with (△) daughter cladodes. Data are means ± SE (*n* = 10 plants).

reduction of CO_2 uptake in June and the slow recovery in July suggests that the chlorenchyma experienced an increased osmotic pressure, causing osmotic stress, because the redistribution of water from parenchyma to the chlorenchyma as drought progresses generally is accompanied by solute uptake (Goldstein *et al.*, 1991).

Shading mother cladodes reduced daily net CO_2 uptake under both wet and dry conditions, and the effect was augmented when mother cladodes developed daughter cladodes that led to increased tissue dehydration. Therefore, soil water stress *per se* is not the main cause of the reduction in photosynthesis. The impairment of photosynthetic activity for mother cladodes in June was associated with the physiological drought caused by the combined effect of reduced soil water availability and reduction in the chlorophyll content.

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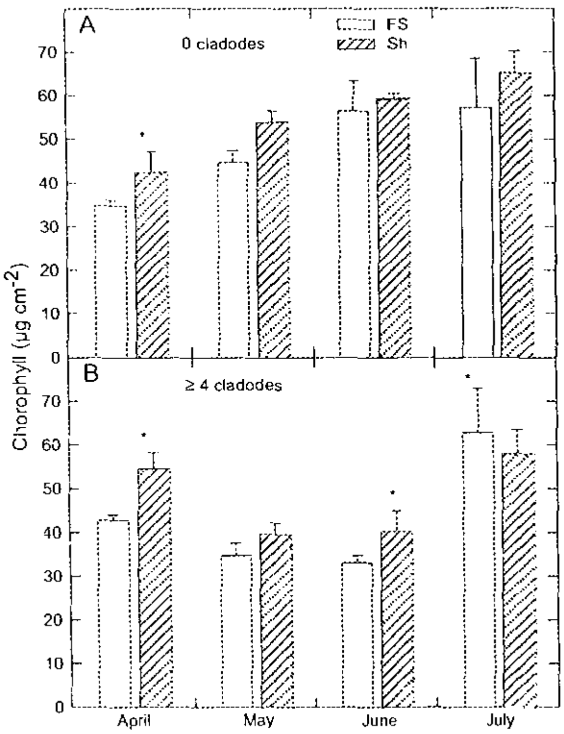

Figure 5 Chlorophyll content for mother cladodes without (A) and with daughter cladodes (B) in full sunlight (FS; open bars) and shaded by 45% (Sh, hatched bars). Data are means ± SE (*n* = 10 plants).

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