

UNIVERSIDAD DE GUADALAJARA
CENTRO UNIVERSITARIO DE CIENCIAS BIOLÓGICAS
Y AGROPECUARIAS

POSGRADO EN CIENCIAS BIOLÓGICAS



EFFECTO DE UN FACTOR ONTOGENICO EN LA RESPUESTA
FISIOLÓGICA DE CLADODIOS MADUROS DE *Opuntia ficus-*
***indica* EN CONDICIONES DE HUMEDAD Y SEQUÍA**

Por

JULIA ZAÑUDO-HERNÁNDEZ

Tesis presentada como requisito parcial para obtener el grado de

DOCTOR EN CIENCIAS (AREA ECOLOGIA)

LAS AGUJAS, ZAPOPAN, JALISCO
JULIO DE 2005



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INDICE DE CONTENIDO

	Página
EFFECTS OF AN ONTOGENIC FACTOR ON THE PHYSIOLOGICAL RESPONSE OF MOTHER CLADODES OF <i>Opuntia ficus-indica</i> UNDER DRY AND WET CONDITIONS	vii
ABSTRACT	vii
EFFECTOS DE UN FACTOR ONTOGENICO EN LA RESPUESTA FISIOLÓGICA DE CLADODIOS MADUROS DE <i>Opuntia ficus-indica</i> EN CONDICIONES DE HUMEDAD Y SEQUÍA	ix
RESUMEN	ix
1. INTRODUCCIÓN	1
2. YOUNG DAUGHTER CLADODES AFFECT CO₂ UPTAKE BY MOTHER CLADODES OF <i>Opuntia ficus-indica</i>	8
2.1 Abstract	9
2.2 Introduction	10
2.3 Materials and methods	11
2.3.1 Site description, plant material, and experimental design	11
2.3.2 Gas exchange measurements	12
2.3.3 Chlorophyll content	13
2.3.4 Soil water potential, soil water content, and cladode relative water content	13
2.3.5 Statistical analysis	14
2.4 Results	14
2.5 Discussion	25
2.6 Literature cited	31
3. EFFECTS OF YOUNG CLADODES ON THE GAS EXCHANGE OF BASAL CLADODES OF <i>Opuntia ficus-indica</i> (CACTACEAE) UNDER WET AND DRY CONDITIONS	38
3.1 Abstract	39
3.2 Introduction	40
3.3 Materials and methods	41
3.3.1 Site description	41
3.3.2 Climatic and microclimatic data	41
3.3.3 Experimental design	42
3.3.4 Soil water potential and cladode relative water content	43
3.3.5 Gas exchange measurements	43
3.3.6 Statistical analysis	44
3.4 Results	44
3.4.1 Daughter cladodes growth	44
3.4.2 Climatic and microclimatic conditions	44
3.4.3 Mother cladode relative water content	47
3.4.4 Daily patterns of net CO ₂ uptake	49
3.4.5 Total daily net CO ₂ uptake	53
3.4.6 Intercellular net CO ₂ concentrations	55
3.4.7 Net CO ₂ uptake in CAM phases	57
3.5 Discussion	60
3.6 Literature cited	65
4. CONCLUSIONES	71
5. APÉNDICE	72

INDICE DE TABLAS Y FIGURAS

	Página
2. YOUNG DAUGHTER CLADODES AFFECT CO₂ UPTAKE BY MOTHER CLADODES OF <i>Opuntia ficus-indica</i>	
Fig.1. Elongation of developing daughter cladodes of <i>Opuntia ficus-indica</i> (A), rainfall (bars) (B), soil water content (C), and soil water potential (D). Arrows indicate dates of measurement of gas exchange.	16
Fig. 2. Net CO ₂ uptake rates over 24-h periods for mother mature cladodes of <i>O. ficus-indica</i> .	20
Table 1. Relationships between number of daughter cladodes and relative water content and chlorophyll content for <i>Opuntia ficus-indica</i> on dates of gas exchange measurement.	18
Table 2. Total net CO ₂ uptake during CAM phases, highest rates of CO ₂ uptake, total daily net CO ₂ uptake and mean night-time intercellular CO ₂ mole fraction for mother cladodes in response to number of daughter cladodes on different dates.	22
Table 3. Stomatal conductance during CAM phases for mother cladodes in response to number of daughter cladodes on different dates.	24
3. EFFECTS OF YOUNG CLADODES ON THE GAS EXCHANGE OF BASAL CLADODES OF <i>Opuntia ficus-indica</i> (CACTACEAE) UNDER WET AND DRY CONDITIONS	
Fig. 1. Elongation of developing daughter cladodes of <i>Opuntia ficus-indica</i> under wet and dry conditions (A), daily rainfall (B), and soil water potential (C).	46
Fig. 2. Relative water content for mother cladodes of <i>O. ficus-indica</i> with variable number of daughter cladodes under wet and dry conditions on the dates of gas exchange measurement.	48
Fig. 3. Net CO ₂ uptake rates over 24-h periods under wet conditions for mother cladodes of <i>O. ficus-indica</i> .	50
Fig. 4. Net CO ₂ uptake rates over 24-h periods under dry conditions for mother cladodes of <i>O. ficus-indica</i> .	52
Fig. 5. Total daily net CO ₂ uptake for mother cladodes of <i>O. ficus-indica</i> with a variable number of daughter cladodes under wet (A) and dry (B) conditions.	54
Fig. 6. Mean night-time intercellular CO ₂ mole fraction for mother cladodes of <i>O. ficus-indica</i> with a variable number of daughter cladodes under wet (A) and dry (B) conditions	56
Table 1. Net CO ₂ uptake during CAM phases under wet and dry conditions by mother cladodes in responses to number of daughter cladodes on different dates	59

**EFFECTS OF AN ONTOGENIC FACTOR ON THE PHYSIOLOGICAL
RESPONSE OF MOTHER CLADOES OF *Opuntia ficus-indica* UNDER DRY
AND WET CONDITIONS**

ABSTRACT

Drought affects C₃ and C₄ crops as well as cultivated CAM plants in the semiarid lands of central Mexico. Damage by drought to cultivated CAM species of *Opuntia* is commonly observed when mother cladodes are planted during the dry spring season, these species are resistant to drought, but their responses can be affected by development of new organs, such as young developing daughter cladodes that behave like C₃ plants, with daytime stomatal opening and water loss. In contrast, wild populations of *Opuntia* are less affected by drought, because they do not develop daughter cladodes under extreme drought conditions. The main objective of this work is to evaluate the effects of the number of daughter cladodes on gas exchange parameters of mother cladodes of *Opuntia ficus-indica* exposed to varying soil water contents growing in spring under dry and wet conditions. Rates of net CO₂ uptake, stomatal conductance, intercellular CO₂ concentration, chlorophyll content, and relative water content were measured. Daily net CO₂ uptake by mother cladodes of *O. ficus-indica* was significantly reduced as the number of daughter cladodes increased under dry conditions but was less affected under wet conditions. This was accompanied by decreased mother cladode relative water content, suggesting movement of water from mother to daughter cladodes. Stress caused by daughter cladodes and by dry soil affected the

expression of CAM phases in mother cladodes, curtailing CO₂ uptake in the late afternoon (phase IV) and reducing carbon gain during the night (phase I) and early morning (phase II), the reduction being most marked under dry conditions. Daughter cladodes significantly hasten the effects of drought on mother cladodes by competition for the water supply and thus decrease daily carbon gain by mother cladodes mainly by inhibiting phase IV of CAM.

Under wet conditions the relative water content of mother cladodes was only slightly affected by daughter cladodes; nevertheless, daughter cladodes affected the recovery of phases I, II and IV after substantial rainfall interrupted drought, and affected also the net CO₂ uptake in phases II and IV; this is one of the first times that an ontogenic factor has been shown to affect net CO₂ uptake under well watered conditions and dry conditions. The increase in the number of daughter cladodes did not increase the photosynthetic capacity of mother cladodes under wet or dry conditions as has been shown on C₃ and C₄ plants.

EFFECTOS DE UN FACTOR ONTOGÉNICO EN LA RESPUESTA FISIOLÓGICA DE CLADODIOS MADUROS DE *Opuntia ficus-indica* EN CONDICIONES DE HUMEDAD Y SEQUÍA

RESUMEN

La sequía afecta a los cultivos C₃ y C₄ también como a las plantas CAM cultivadas en las regiones semiáridas del Centro de México. El daño por sequía en las especies CAM cultivadas de *Opuntia* es comúnmente observada cuando los cladodios madres son plantados o sembrados durante la primavera, éstas especies son resistentes a la sequía, pero su respuesta puede ser afectada por el desarrollo de nuevos órganos, como es el desarrollo de cladodios hijos que se comportan como plantas C₃, con apertura diurna de estomas y pérdida de agua. En contraste, las especies silvestres de *Opuntia* son menos afectadas por la sequía, porque ellas no desarrollan cladodios hijos dentro de condiciones extremas de sequía. El objetivo de este trabajo fue evaluar el efecto del número de cladodios hijos en el intercambio de gases de los cladodios madre de *Opuntia ficus-indica* expuestos a diferente condición de sequía y humedad del suelo. Las variables que se midieron fueron: tasas instantáneas de asimilación de CO₂, conductancia estomática, concentración de CO₂ intercelular, contenido de clorofila y contenido relativo de agua. La asimilación neta diaria de CO₂ por los cladodios madre de *O. ficus-indica* se redujo significativamente conforme el número de cladodios hijos incrementó dentro de las condiciones de sequía pero fue menos afectada en condiciones de humedad. Esto fue acompañado por la disminución en

el contenido relativo de agua en los cladodios madre, sugiriendo un movimiento de agua de los cladodios madre a los hijos. El estrés causado por los cladodios hijos y por el suelo seco afectó la expresión de las fases CAM en los cladodios madre, acortando la asimilación de CO_2 al final de la tarde (fase IV) y reduciendo la ganancia de carbono durante la noche (fase I) y al amanecer (fase II), la reducción fue más marcada en condiciones de sequía. Demostrando que los cladodios hijos aceleran significativamente el efecto de la sequía en los cladodios madre por la competencia para suplir agua y esto disminuye la ganancia de carbono principalmente por inhibición de la fase IV CAM.

Dentro de condiciones de humedad el contenido relativo de agua de los cladodios madre fue ligeramente afectado por los cladodios hijos, sin embargo, estos afectaron la recuperación de la fase I, II y IV después de que la sequía fue interrumpida por la caída de la lluvia, y afectó también la asimilación de CO_2 en las fases II y IV; este es uno de los primeros trabajos que muestran el efecto de un factor ontogénico en la asimilación neta de CO_2 en condiciones de humedad y sequía. El incremento en el número de cladodios hijos no aumentó la capacidad fotosintética en los cladodios madre en condiciones de humedad y sequía como se ha mostrado en plantas C_3 y C_4 .

1. INTRODUCCIÓN

Las plantas que presentan el metabolismo ácido de las crasuláceas (plantas CAM) presentan una amplia distribución en ambientes contrastantes, ya que se encuentran en regiones áridas, y tropicales. Las plantas CAM se encuentran típicamente asociadas con plantas que habitan ambientes áridos y semiáridos, en los cuales el agua es un factor limitante para el desarrollo de la vegetación, debido a que la precipitación pluvial es escasa y errática, aunque también se localizan donde la precipitación pluvial es relativamente abundante, como ocurre en los habitats en donde crecen plantas epífitas tropicales (Cushman, 2001; Lüttge, 2004). Dentro de las plantas CAM cultivadas destacan la piña (*Ananas comosus*), los agave (*Agave*), las cactáceas (Cactaceae), y las orquídeas (Orchidaceae) (Lüttge, 2004). El metabolismo CAM esta correlacionado con diferentes características morfológicas que reducen la pérdida de agua, incluyendo cutículas gruesas, la relación baja superficie-volumen, células grandes con vacuolas prominentes que mejoran la capacidad de almacenamiento de agua (i.e. succulencia) y la baja frecuencia de estomas (Osmond, 1978; Keeley y Rundel, 2003; Mauseth, 2004; Lüttge, 2004).

La fijación inicial de CO_2 llevada a cabo por la enzima fosfoenol piruvato carboxilasa (PEP_C) ocurre durante la noche cuando los estomas están abiertos y las pérdidas de agua por transpiración son bajas. Esta fijación de CO_2 durante la noche forma ácidos orgánicos que son acumulados en prominentes vacuolas. Durante el día estos ácidos salen de la vacuola y liberan CO_2 , al incrementarse el

CO₂ interno se estimula el cierre de estomas y se concentra el CO₂ alrededor de la enzima Rubisco, suprimiendo la actividad de esta enzima como oxigenasa, y por lo tanto se reduce la transpiración diurna cuando las temperaturas del aire son más altas que en la noche (Nobel, 1995; Pimienta-Barrios et al., 2003). El efecto de esta estrategia de concentración de CO₂ en las plantas CAM constituye una alta eficiencia en el uso del agua, que puede ser hasta cinco veces más alta que las plantas con metabolismo fotosintético C₃ y C₄ (Drennan y Nobel, 2000). Esta ventaja selectiva de alta eficiencia en el uso del agua ayuda a explicar la extensa diversificación y especiación entre estas plantas, principalmente en ambientes con baja disponibilidad de agua. Estudios ecológicos recientes han revelado que las plantas representan aproximadamente el 7% de las especies de plantas vasculares, y es más alto que el porcentaje de especies de plantas C₄ (Winter y Smith, 1996; Lambers et al., 1998). Llama la atención de que el metabolismo CAM se encuentra también en plantas vasculares acuáticas, donde se ha demostrado que mejora la toma de carbono, pero la disponibilidad de CO₂ se vuelve un factor limitante para la fotosíntesis (Keeley, 1996; 1998). De esta manera las limitaciones durante el día en la disponibilidad de CO₂, parece ser un factor común responsable de la evolución de las plantas CAM, como consecuencia de la conservación del agua por el cierre de estomas en habitats áridos terrestres o por la competencia con otras especies y la alta resistencia en la difusión de CO₂ que limitan la toma de este gas en plantas CAM acuáticas (Keeley y Rundel, 2003).

Los usos de las plantas CAM en México y el mundo tienen una gran historia (Barbera, 1995), sin embargo poco es lo que se ha estudiado sobre aspectos

mofológicos y ecofisiológicos en condiciones de campo (Nobel et al., 2002). Los primeros estudios sobre la respuesta fisiológica en plantas CAM de importancia económica en México como es el caso de las opuntias, fueron realizados en condiciones controladas en invernaderos o cámaras de crecimiento (Nobel et al., 2002), por otro lado los estudios en campo son escasos (Szarek y Ting, 1975; Hanscom y Ting, 1978; Sutton et al., 1981) y estos fueron realizados en ambientes que difieren notablemente de las regiones en que se han originado y cultivado la mayor parte de estas especies. Sin embargo, la limitante en los estudios en condiciones controladas es que las condiciones ambientales en que se llevan a cabo las evaluaciones del funcionamiento de las plantas son estandarizadas, permitiendo la variación en la mayoría de los casos de sólo una variable ambiental (i.e luz, temperatura, el estado del agua en el suelo, etc.). Bajo estas condiciones la complejidad del desarrollo ontogénico de la planta no se manifiesta como en el campo ya que variables ambientales abióticas y bióticas como la estacionalidad climática, la microbiología del suelo y la competencia e interacciones planta-animal son excluidas (Körner y Bazzaz, 1996).

En esta tesis se presentan evidencias experimentales apoyadas en trabajos previos de que la fotosíntesis, en particular la ganancia diaria de carbono en cladodios subyacentes madres de *O. ficus-indica* es afectada por el estrés hídrico particularmente cuando se permite el desarrollo de cladodios jóvenes en estos. También se encontró que los cladodios jóvenes, aunque en menor grado, igualmente afectan la fotosíntesis en cladodios madres cuando estos crecen sin estrés edáfico, al regarlos continuamente. La reducción en la toma neta de CO₂ se relacionó con el incremento en el número de cladodios hijos, especialmente

cuando su número es mayor a cinco por planta. Además se demuestra que esta reducción en la toma diaria de carbono en los cladodios madres se acompañó de la reducción del contenido de agua, lo cual se atribuye a la redistribución de agua de los cladodios subyacentes a los cladodios jóvenes.

Por otro lado, la expresión del metabolismo CAM es gobernado por factores genéticos, ontogénicos y ambientales como la intensidad luminosa, humedad relativa, además de la disponibilidad de agua en el suelo. De estas variables la disponibilidad de agua es el factor ambiental que más se ha estudiado (Mattos et al., 1999; Dodd et al., 2002; Keeley y Rundel, 2003), y la que menos se ha estudiado es el efecto de factores ontogénicos (Lüttge, 2004), por lo que probablemente los trabajos reportados en esta tesis sean los primeros en que se demuestra que un factor ontogénico, en este caso los cladodios jóvenes, tengan un alto grado de control sobre la fotosíntesis en plantas de *Opuntia ficus-indica*, siendo así esta la principal aportación de esta tesis.

Una segunda aportación importante de este trabajo, tiene relación con el concepto de fuente-demanda, aspecto fisiológico aparentemente no estudiado en plantas CAM. En plantas C_3 y C_4 domesticadas creciendo en condiciones controladas, se ha observado que la fuerza de la demanda influye en la fuerza de la fuente (fotosíntesis), por lo que la remoción de los tejidos demandantes y en crecimiento (i.e tubérculos, semillas, hojas jóvenes), estimula significativamente la fotosíntesis (Turgeon, 1989; Gifford y Evans, 1981; Salisbury y Ross, 1992). En esta tesis se encontró que la fotosíntesis en la planta CAM *O. ficus-indica* no es estimulada por la fuerza de la demanda (el incremento en el número de cladodios

jóvenes que crecen sobre cladodios maduros), por lo que se concluye que la fuerza de la fuente es afectada por factores ambientales que por la fuerza de la demanda.

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2. CAPÍTULO I

YOUNG DAUGHTER CLADODES AFFECT CO₂
UPTAKE BY MOTHER CLADODES OF

Opuntia ficus-indica

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ABSTRACT

Drought affects C₃ and C₄ crops as well as cultivated CAM plants in the semiarid lands of central Mexico. Damage by drought to cultivated CAM species of *Opuntia* is commonly observed when mother cladodes are planted during the dry spring season, particularly when they develop young daughter cladodes that behave like C₃ plants, with daytime stomatal opening and water loss. In contrast, wild populations of *Opuntia* are less affected by drought, because they do not develop daughter cladodes under extreme drought conditions. The main objective of this work is to evaluate the effects of the number of daughter cladodes on gas exchange parameters of mother cladodes of *Opuntia ficus-indica* exposed to varying soil water contents. Rates of net CO₂ uptake, stomatal conductance, intercellular CO₂ concentration, chlorophyll content, and relative water content were measured in mature mother cladodes with a variable number of cladodes growing in spring under dry and wet conditions. Daily carbon gain by mother cladodes was reduced as the number of daughter cladodes increased to eight, especially during drought. This was accompanied by decreased mother cladode relative water content, suggesting movement of water from mother to daughter cladodes. CO₂ assimilation was most affected in phase IV of CAM (late afternoon net CO₂ uptake) by the combined effects of daughter cladodes and drought. Rainfall raised the soil water content, decreasing the effects of daughter cladodes on net CO₂ uptake by mother cladodes. Daughter cladodes significantly hasten the effects of drought on mother cladodes by competition for the water supply and thus decrease daily carbon gain by mother cladodes mainly by inhibiting phase IV of CAM.

Key words: Chlorophyll, Crassulacean acid metabolism, drought, gas exchange, net CO₂ uptake, *Opuntia ficus-indica*.

INTRODUCTION

The semiarid lands of central northwestern Mexico experienced an unusually dry period during the last part of the 20th century, with annual rainfall below 400 mm (Pimienta-Barrios et al., 2002). Such prolonged drought causes severe damage not only to cultivated C₃ and C₄ crops, but also to cultivated CAM species (Pimienta-Barrios, 1990). The greatest damage by drought to cultivated species of *Opuntia* occurs to mother cladodes from which young cladodes develop during spring. In contrast to cultivated opuntias, wild opuntias generally do not develop young cladodes when extreme drought conditions prevail; this has been considered an avoidance strategy to prevent water loss (Pimienta-Barrios et al., 2002, 2003). During the early stages of development, daughter cladodes exhibit C₃ photosynthesis with daytime stomatal opening (Osmond, 1978; Acevedo et al., 1983) and require water import from the mother cladodes (Nobel et al., 1994a; N Wang et al., 1997). Classically, CAM plants endure drought by maintaining nighttime CO₂ uptake together with morphological succulence and anatomical modifications, such as thick cuticles and low stomatal frequency (Nobel, 1995). The role of photosynthetic plasticity in response to drought has received less attention (Mattos et al., 1999; Cushman, 2001; Dodd et al., 2002; Keeley and Rundel, 2003).

Sink strength can affect the strength of the source, as the removal of actively growing sinks (e.g. tubers, seeds, and young leaves; Gifford and Evans, 1981; Salisbury and Ross, 1992) generally decreases photosynthesis. These observations are essentially only from studies of domesticated C₃ and C₄ plants (Loomis and Connor, 1992; Z Wang et al., 1997), while studies relating source and sink for CAM plants are rare. The main objective of the present study was to evaluate the physiological effects of daughter cladodes on the physiology of mature mother cladodes of *Opuntia ficus-indica* under both wet and dry conditions. An increase in sink strength (greater number of daughter cladodes that develop on a mother cladode) was hypothesized to reduce the photosynthetic capacity of the mother cladodes, especially during drought, because daughter cladodes import water and organic matter from the mother cladodes.

MATERIALS AND METHODS

Site description, plant material, and experimental design

The experiment was performed during spring 2003 at an experimental field of the Departamento de Ecología of the Universidad de Guadalajara. The site is in central Jalisco, Mexico, at 20° 5' N, 103° 32' W, and 1420 m above sea level. The climate is temperate-subtropical. Daily air temperature and rainfall were obtained from a weather station maintained at the Fifth Military Base of the Mexican Air Force. On the dates of gas exchange measurement, the photosynthetic photon flux (PPF, wavelengths of 400-700 nm) on a horizontal plane was recorded hourly from sunrise to sunset with a LI-250 quantum sensor (LI-COR, Lincoln, NE, USA)

and then integrated to get the total daily PPF. Air temperature was recorded every hour using a mercury thermometer. Air relative humidity was recorded hourly using a digital humidity gauge (63-101, RadioShack, Los Angeles, CA, USA).

Mature 12-month-old cladodes (flattened stem segments) of *Opuntia ficus-indica* (L.) Miller (Cactaceae) averaging 35 cm long, 19 cm wide, and 1.8 cm thick were harvested from a cultivated population at Nextipac, Jalisco, Mexico. They were planted on 13 March 2003 in plastic pots (19 L) filled with a vermiculite/sand mixture (1:1, v:v) and watered weekly with 3 L per pot to promote the sprouting of young cladodes at the beginning of the spring. Daughter cladodes were allowed to develop freely on the mature cladodes; 32 days after planting (15 April 2003) young cladodes were selectively removed, leading to zero, one, two, four, or eight daughter cladodes per mother cladode. Each of these five treatments was replicated 16 times, within a completely randomized design. 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. Total rainfall before the last gas exchange measurement on 20-21 June was 110 mm. The length of daughter cladodes was measured weekly using a ruler with the base of the cladode as reference.

Gas exchange measurements

Instantaneous net CO₂ uptake, stomatal conductance, and intercellular CO₂ concentration were measured on all mother cladodes every 2 h over 24-h periods on 9-10 May, 30-31 May, and 20-21 June 2003 with a LI-COR LI-6200 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). A 0.25-L leaf chamber was

modified by replacing the distal half-cylinder with a narrowed opening (2 cm × 4 cm) lined with a closed-pore foam gasket that was firmly pressed against an approximately southwest-facing surface of the cladodes.

Chlorophyll content

Five samples for determination of chlorophyll content were removed, using a cork borer 1 cm in diameter, from the centre of mother cladodes in each treatment on the dates on which gas exchange was measured. Chlorophyll was extracted by homogenizing frozen material in cold acetone (80%). 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 ($\mu\text{g cm}^{-2}$) was calculated from spectrophotometric measurements at 645 and 663 nm (Bruinsma, 1961).

Soil water potential, soil water content, and cladode relative water content

Soil water potential was determined with a WP4 dew point potentiometer (Decagon Devices, Pullman, WA, USA) using five samples removed from the center of the root zone (a depth of 10-15 cm) on the same dates as measurement of gas exchange. Soil water content from the center of the root zone was also determined for ten soil samples dried at 105°C to constant mass (generally within 72 h); data are expressed as percentage water content: (fresh mass - dry mass)/dry mass × 100 (Torres, 1984). The relative water content (RWC) for mother cladodes was determined between 10 and 11 a.m. for 5 segments (3 cm × 3 cm) by immediately determining their fresh mass and then oven drying at 80°C to

constant mass. RWC was calculated (Koide *et al.*, 2000). RWC was also determined for daughter cladodes on 30-31 May and 20-21 June only.

Statistical analysis

Data were analyzed using a hierarchical ANOVA (Zar, 1999); means were separated by a least significant difference (LSD) test (Little and Hill, 1975). Data are presented as means \pm SE (n = number of measurements).

RESULTS

For 2003, the total daily PPF was higher on 9-10 May ($57 \text{ mol m}^{-2} \text{ d}^{-1}$), and 30-31 May ($55 \text{ mol m}^{-2} \text{ d}^{-1}$) than on 20-21 June ($50 \text{ mol m}^{-2} \text{ d}^{-1}$). Mean day/night temperatures varied little between the three dates: $26/18^\circ \text{C}$ for 9-10 May, $25/18^\circ \text{C}$ for 30-31 May, and $24/19^\circ \text{C}$ for 20-21 June. Day/night relative humidity was highest on 20-21 June (51/57%), lowest on 9-10 May (31/45 %), and intermediate on 30-31 May (40/68%).

Elongation of daughter cladodes started in early May 2003, showing low rates during the first and second week of May (0.10 cm d^{-1}); rates increase substantially (0.45 cm d^{-1}) from the third week of May to the end of the first week of June before rainfall started. Once rainfall started, daughter cladode growth rate decreased (0.31 cm d^{-1}) (Fig. 1A, B). Rainfall was 91 mm during the second week of June, 56 during the third week, and 105 mm during the fourth week, totaling 252 mm for June (Fig. 1B). Both soil water content and soil water potential decreased substantially from the first week to the fourth week of May, but because of rainfall,

soil water content increased six-fold and soil water potential increased over 1450 fold from 30-31 May to 20-21 June (Fig 1C, D).

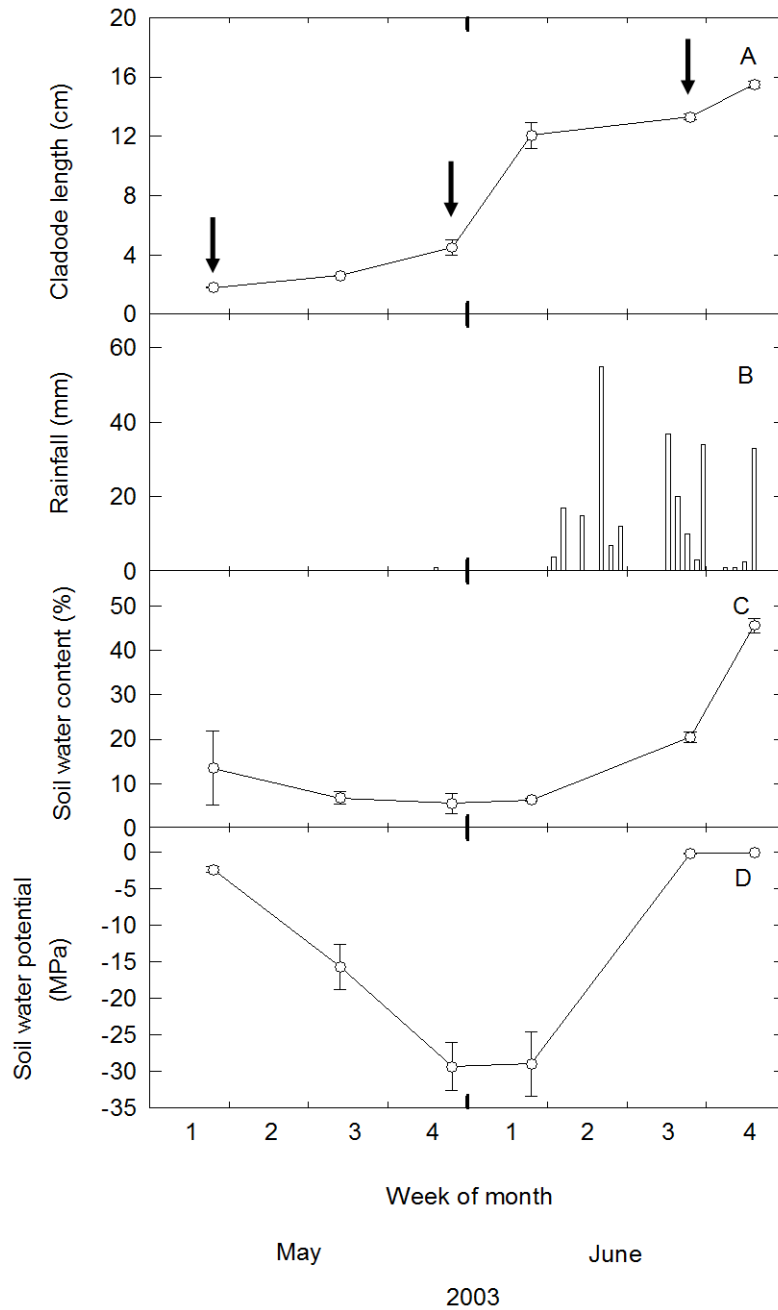


FIG.1. Elongation of developing daughter cladodes of *Opuntia ficus-indica* (A), rainfall (bars) (B), soil water content (C), and soil water potential (D). Arrows indicate dates of measurement of gas exchange. Data are means \pm SE ($n = 32$ cladodes, 8 from each treatment for cladode growth; $n = 10$ samples for both soil water content and soil water potential).

The first measurement of photosynthesis, RWC and chlorophyll coincided with the initial stages of daughter cladode growth, when the spring dry period started. The second measurement of photosynthesis was at the beginning of the linear phase of cladode growth, having the lowest values of soil water content, soil water potential, and RWC in mother cladodes that had developing daughter cladodes (Fig. 1A, C, D; Table 1). The third date of measurement occurred 10 days after the first noteworthy rainfall, when the values of RWC increased substantially in mother cladodes with and without daughter cladodes. A less pronounced increase in RWC was observed for daughter cladodes (Table 1).

As the number of daughter cladodes increased, the RWC of the mother cladodes decreased progressively and substantially on 9-10 and 30-31 May (Table 1); it was lower, particularly with no or few daughter cladodes on 30-31 May than on 9-10 May ($P < 0.05$). Once rainfall occurred, the RWC increased for all the treatments on 20-21 June, but the increase was statistically higher in mother cladodes with zero, four and eight cladodes (Table 1). On 30-31 May the RWC for daughter cladodes was lower when four or eight cladodes grew on mother cladodes, compared with no or fewer daughter cladodes. The RWC for daughter cladodes increased on 20-21 June, but only with two, four and eight daughter cladodes (Table 1).

Table 1. Relationships between number of daughter cladodes and relative water content and chlorophyll content for *Opuntia ficus-indica* on dates of gas exchange measurement.

Date	Number of daughter cladodes	Mother cladode relative water content (%)	Daughter cladode Relative water Content (%)	Mother cladode chlorophyll content ($\mu\text{g cm}^{-2}$)
9-10 May 2003	0	97a		79a
	1	90b		69b
	2	84c		55c
	4	69d		52c
	8	59e		53c
30-31 May 2003	0	84a		69a
	1	73b	93a	46b
	2	67c	88b	39c
	4	61d	81c	42c
	8	58d	80c	42c
20-21 June 2003	0	90a		32a
	1	85b	89b	34a
	2	83b	91a	25a
	4	93a	88b	29a
	8	91a	88b	32a

Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

The chlorophyll content of mother cladodes decreased progressively from zero to two cladodes, but it did not decrease further with four and eight cladodes on 9-10 and 30-31 May (Table 1); such mother cladodes showed yellowing and visible symptoms of dehydration. On 20-21 June the chlorophyll content was lower than in May and was not statistically different among treatments (Table 1).

Gas exchange patterns of mother cladodes varied with the measurement date and the number of daughter cladodes (Fig. 2). For all treatments, positive net CO₂ uptake began earlier in the afternoon (phase IV for CAM plants; Osmond, 1978) and lasted about four h for the wettest conditions (20-21 June) and was shortest during drought (30-31 May). Indeed, during drought CO₂ uptake became substantially positive in the late afternoon for mother cladodes with no daughter cladodes (Fig. 2A), but only early in the night with one daughter cladode (Fig. 2B), at midnight with two or four daughter cladodes (Figs. 2 C, D), and not until after midnight with eight daughter cladodes (Fig. 2E). The treatment with no daughter cladodes tended to have the highest rates of net CO₂ uptake, while the lowest night-time rates (phase I) occurred during drought for the mother cladodes with the greatest number of daughter cladodes (Fig. 2E).

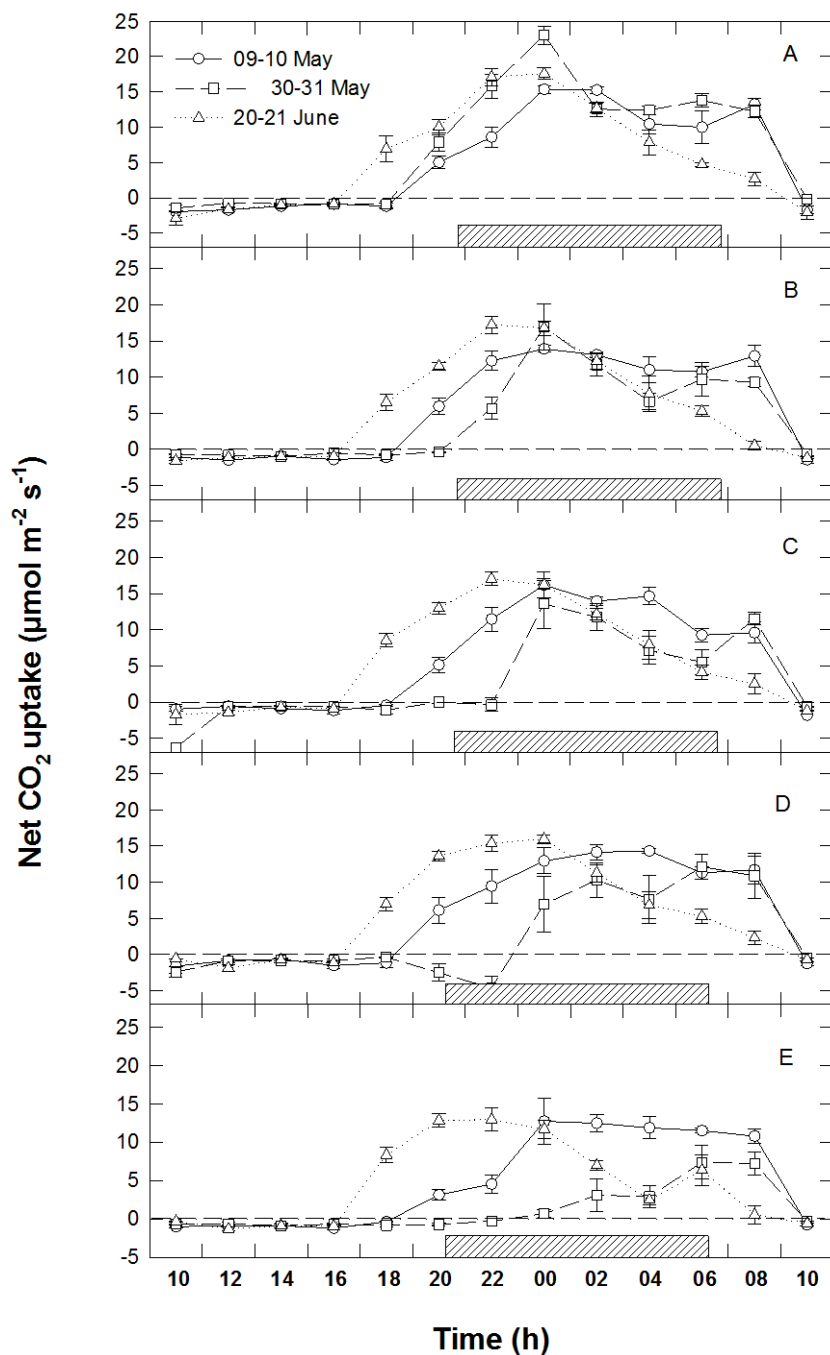


FIG. 2. Net CO₂ uptake rates over 24-h periods for mother mature cladodes of *O. ficus-indica* with no daughter cladodes (A), one daughter cladode (B), two daughter cladodes (C), four daughter cladodes (D), and eight daughter cladodes (E) on 9-10 May 2003 (○), 30-31 May 2003 (□), and 20-21 June 2003 (△). Hatched bars indicate night-time. Data are means \pm S.E. ($n = 16$ plants).

Carbon gain during phase I for mother cladodes on May 9-10 depended only slightly on the number of daughter cladodes (Table 2). In contrast, during drought (30-31 May) carbon gain sharply decreased with the increase in the number of daughter cladodes (Table 2). For 20-21 June, carbon gain in phase I was lowest with eight daughter cladodes. Phase II (early morning net CO₂ uptake) did not vary significantly with the number of daughter cladodes on May 9-10. On 30-31 May carbon gain was lowest with eight cladodes. On June 20-21 carbon gain during phase II was lower than in May. Phase III (daytime period of net CO₂ release) was little affected by the number of daughter cladodes or the measurement date. Carbon gain in phase IV in mother cladodes did not vary significantly in relation with the number of daughter cladodes on 9-10 May, and was absent during drought (30-31 May) when daughter cladodes were present. On 20-21 June after rainfall, carbon gain increased considerably, with no statistical differences among treatments (Table 2).

Total daily carbon gain was reduced significantly only by eight daughter cladodes on 9-10 May. On 30-31 May total daily carbon gain progressive decreased as the number of daughter cladodes increased, reaching a reduction of 81% with eight daughter cladodes compared with no daughter cladodes. The maximum rates of net CO₂ uptake (Fig. 2) tended to decrease as the number of daughter cladodes increased on 30-31 May only. On 20-21 June the lowest maximal rates of net CO₂ uptake was with eight cladodes. The average night-time intercellular CO₂ mole fraction was the same among treatments for 9-10 May and 20-21 June; on 30-31 May, it increased with the number of daughter cladodes ($P < 0.05$; Table 2).

Table 2. Total net CO₂ uptake during CAM phases, highest rates of CO₂ uptake, total daily net CO₂ uptake and mean night-time intercellular CO₂ mole fraction for mother cladodes in response to number of daughter cladodes on different dates. Data are means ($n = 16$ plants).

Date	Number of daughter cladodes	Total net CO ₂ uptake for indicated phases (mmol m ⁻²)				Highest rates of CO ₂ uptake (μmol m ⁻² s ⁻¹)	Total daily net CO ₂ uptake (mmol m ⁻² d ⁻¹)	Mean night-time intercellular CO ₂ mole fraction (μmol mol ⁻¹)
		I	II	III	IV			
9-10 May 2003	0	430b	143a	-36a	28a	15a	565a	222 a
	1	439b	139a	-31a	35a	14a	582a	233 a
	2	472a	104a	-23a	34a	16a	589a	223 a
	4	447ab	126a	-29a	36a	14a	580a	224 a
	8	383b	117a	-25a	20a	13a	495b	245 a
30-31 May 2003	0	559a	132a	-25a	50a	23a	716a	184 b
	1	364b	100a	-27a	0b	17ab	437b	237 ab
	2	289bc	124a	-59a	0b	14b	354bc	313 ab
	4	208c	117a	-51a	0b	12b	274bc	328 ab
	8	86d	78b	-30a	0b	7c	134c	430 a
20-21 June 2003	0	432a	29a	-29a	122a	18a	554a	308 a
	1	426a	3b	-26a	130a	17a	533a	305 a
	2	414a	27a	-26a	155a	17a	570a	298 a
	4	394a	25a	-32a	148a	16a	535a	315 a
	8	291b	4b	-26a	152a	13b	421a	333 a

Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

Stomatal conductance for mother cladodes during phase I showed not significant changes with the number of daughter cladodes on 9-10 May and 20-21 June, although on 30-31 May, the treatment with eight daughter cladodes had the highest value of stomatal conductance. For all treatments stomatal conductance did not vary significantly during phase II on 30-31 May and 20-21 June, but on 9-10 May the treatments with two and eight daughter cladodes showed the highest values. Stomata conductance in phase III did not vary significantly on 9-10 May and 30-31 May among treatments, but it was highest with zero daughter cladodes on 20-21 June. Phase IV did not vary significantly on 9-10 May and 20-21 June, but it was highest with zero daughter cladodes on 30-31 May. The highest values of stomatal conductance were observed in phase II for the three dates of measurement, and the lowest values were recorded in on 9-10 May during phase III, and in phase IV during the driest date of measurement (30-31 May), principally with mother cladodes when daughter cladodes were present (Table 3).

Table 3. Stomatal conductance during CAM phases for mother cladodes in response to number of daughter cladodes on different dates. Data are means ($n=16$ plants).

Date	Number of daughter cladodes	Conductance for indicated phases ($\text{mol m}^{-2} \text{s}^{-1}$)			
		I	II	III	IV
9-10 May 2003	0	0.160 a	0.452 b	0.005 a	0.048 a
	1	0.187 a	0.573 ab	0.007 a	0.018 a
	2	0.196 a	0.661 a	0.004 a	0.017 a
	4	0.193 a	0.571 ab	0.006 a	0.012 a
	8	0.188 a	0.617 a	0.006 a	0.035 a
30-31 May 2003	0	0.280 ab	1.744 a	0.019 a	0.053 a
	1	0.258 ab	1.303 a	0.012 a	0.016 b
	2	0.192 b	0.836 a	0.012 a	0.007 b
	4	0.278 ab	0.897 a	0.013 a	0.003 b
	8	0.328 a	1.326 a	0.015 a	0.004 b
20-21 June 2003	0	0.305 a	0.576 a	0.022 a	0.090 a
	1	0.288 a	0.897 a	0.011 b	0.096 a
	2	0.364 a	0.764 a	0.005 b	0.093 a
	4	0.387 a	0.983 a	0.008 b	0.087 a
	8	0.387 a	1.562 a	0.015 b	0.093 a

Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

DISCUSSION

The total daily carbon gain for mother cladodes of *O. ficus-indica* with daughter cladodes was significantly reduced during drought. The RWC of the mother cladodes decreased in parallel with the reduction in daily net CO₂ uptake, suggesting that water moved from them to the developing daughter cladodes (Barcikowski and Nobel, 1984; Nobel et al., 1994a; Herrera et al., 2000; Rabas and Martin, 2003). This observation is supported by the fact that mother cladodes with four or eight cladodes showed the greatest yellowing and most dehydration (RWC of about 60% on 30-31 May versus 80% for their daughter cladodes). Actually, the second measurement date (30-31 May) coincides with the time that daughter cladodes start increasing their rates of growth and hence increase their demand for assimilates (N Wang et al., 1997, 1998). Near the beginning of the experiment (9-10 May) a gradual decrease in RWC of mother cladodes was correlated with the increase in the number of daughter cladodes. Hence in addition to importing assimilates, daughter cladodes also import large volumes of water from mother cladodes (in a dilute phloem solution; Nobel et al., 1994a), causing a physiological drought condition in mother cladodes that was hastened by a reduction in soil water availability. These observations suggested that an ontogenic factor (daughter cladodes) acts in synergism with the abiotic drought with respect to the mother cladodes.

As indicated, daughter cladodes in the early stages of development import significant volumes of water from mother cladodes. Interestingly, the progressive reduction in RWC of mother cladodes was less during drought (30-31 May) than earlier, suggesting that mother cladodes had reached RWC values for which water

is tightly held by matric potentials particularly mucilage and the apoplast (Barcikowski and Nobel, 1984; Goldstein et al., 1991; Nobel et al., 1992), and most of the water available in the capacitance system of mother stems have been exhausted by both transpiration of mother cladodes and by daughter cladodes restricting its movement to daughter cladodes. After rainfall had begun (measurement date of 20-21 June), the RWC of daughter cladodes increased, but such an increase was less pronounced than for mother cladodes. Also, daughter cladodes of *O. ficus-indica* exhibit C_3 photosynthesis with daytime stomatal opening, leading to a high water demand from the mother cladodes (Ting, 1985; Nobel et al., 1994a; N Wang et al., 1997). Such a redistribution of water is considered an adaptive response to drought stress (Herrera et al., 2000; Rabas and Martin, 2003). The presence of daughter cladodes hastened the effects of drought, even under the moderate day/night temperatures prevailing during the measurement dates. Indeed, mother cladodes of some cultivated varieties of *O. ficus-indica* suffer excessive dehydration and yellowing—and may even die—if daughter cladodes develop on them during extreme drought that regularly occurs in the semiarid lands of central Mexico (Pimienta-Barrios, 1990; Pimienta-Barrios et al., 2002).

In addition to water availability, the relative expression of the four CAM phases (Osmond, 1978) is governed by genotypic, ontogenic, and environmental factors, such as light intensity and relative humidity (Mattos et al., 1999; Cushman, 2001; Borland and Griffiths, 1996). Because water availability is often most crucial, the flexibility of CAM phases is commonly related to changes from wet to dry conditions or vice versa, either artificially or under natural conditions (Nobel, 1985;

Mattos et al., 1999; Dodd et al., 2002; Nobel et al., 2002; Keeley and Rundel, 2003). The flexibility of the expression of CAM phases for mother cladodes of *O. ficus-indica* also depended on the presence of daughter cladodes, an example of photosynthetic plasticity in response to an ontogenic factor (Cushman, 2001). Photosynthetic plasticity through the regulation of CAM phases allowed *O. ficus-indica* to increase its net CO₂ uptake under favorable conditions and to maintain carbon gains in mother cladodes when they were stressed by the combined effects of daughter cladodes and drought, as cultivated opuntias do not stop forming daughter cladodes but wild opuntias do (Pimienta-Barrios et al., 2002, 2003).

Cultivated *O. ficus-indica* is more sensitive to biotic and abiotic stressors than are wild opuntias (Pimienta-Barrios, 1990), which have developed physiological and morphological traits by adaptation, acclimation, and genetic selection/speciation that allowed them to withstand various biotic and abiotic stresses, leading to the development of both tolerance and avoidance mechanisms (Pimienta-Barrios et al., 2002). In contrast, during *Opuntia* domestication specific trait combinations that maximized yield under optimum conditions were promoted, which apparently reduced their capacity to cope with environmental stresses through avoidance or tolerate mechanisms (Orcutt and Nilsen, 2000; Grime, 2001).

The higher sensitivity of phase IV during the dry period was mainly because this phase occurs in the late afternoon (Black and Osmond, 2003; Dood et al., 2002), coinciding with higher temperatures and high irradiance that favour photorespiration (Osmond, 1978; Maxwell et al., 1997; Takeba and Kozaki, 1997; Lambers et al., 1998). Frequently severe drought stress in CAM plants led to the

loose of phase IV and reduction of phase II (Nobel, 1995; Mattos et al., 1999; Griffiths, 2002). During phase II both of CO₂ uptake and stomatal aperture by *O. ficus-indica* was maintained even during prolonged drought in the mother cladodes with daughter cladodes, in contrast phase IV was curtailed, coinciding with lowest values of conductance. Regularly when dehydration of photosynthetic tissue is well advanced as occurs on 30-31 May the zero point for gas exchange is determined by the total or near total closure of stomatal (Larcher et al., 2003). However, there are evidences that drought stress affect processes of photosynthetic metabolism also, in addition to stomatal aperture (Tezara et al., 2003).

Commonly water deficits affects stomatal conductance diminishing photosynthetic rate, because it decreases CO₂ availability in the mesophyll, and not ATP (Tezara et al., 2003). However, our observations showed that drought stress increases CO₂ availability on mother cladodes particularly with the higher number of daughter cladodes. However, there is evidences that drought also processes in photosynthetic metabolism which may impair by water stress include Rubisco enzyme activity, RuBP regeneration, ATP supply and electron transport rate (Tezara et al., 2003). Drought might also affect the photosynthetic metabolism because the increase solute concentration (Barcikowski and Nobel, 1984). CAM plants tend to accumulate high levels of mineral particularly calcium, in their long lived photosynthetic stems (Nobel, 1985), compared with other photosynthetic organs.

The stress on mother cladodes of *O. ficus-indica* caused by drought and exacerbated by daughter cladodes apparently increased night-time respiration and reduced the activity of PEP carboxylase, leading to higher intercellular CO₂ mole

fractions (Maxwell et al., 1997). The increase in intercellular CO₂ under water stress indicates mesophyll (non-stomatal) limitation to photosynthesis (Srinivasa-Rao et al., 2000). The highest intercellular CO₂ mole fraction (399 μmol mol⁻¹) for mature plants of *O. ficus-indica* and other CAM plants in the field is observed in the spring when soil moisture is extremely low and day/night air temperatures are high (Pimienta-Barrios et al., 2000; Nobel et al., 2002). High intercellular CO₂ mole fractions during drought can help prevent damage by photooxidation in CAM plants (Pieters et al., 2003). High levels of irradiance were common in the study site, particularly during the driest measurement date. The simultaneous occurrence of high irradiance and drought stress increase photo damage in addition to reducing carbon gain (Long et al., 1994), explaining in part the physiological significance of high values of intercellular CO₂ concentration observed for mother cladodes with four and eight cladodes on 30-31 May.

Daily net CO₂ uptake was reduced because daughter cladodes and drought shortened the periods of positive net CO₂ uptake and reduced the maximum rates of CO₂ uptake. These effects became more pronounced as the number of daughter cladodes increased to eight, causing the highest rates of net CO₂ uptake to occur late in the night when temperatures were lower and the relative humidity was higher. This pattern reflects an avoidance strategy to maintain carbon gain with reduced water loss and constitutes an example of physiological plasticity, which is also observed for *O. robusta* in the field during the driest months (Pimienta-Barrios et al., 2002, 2003) and for unrooted cladodes of *O. ficus-indica* eight weeks after detachment (Raveh and Nobel, 1999). Under wet conditions and without the presence of daughter cladodes, carbon gain during the day for mature

cladodes of *O. ficus-indica* contributed 27 % of the total daily net CO₂ uptake. Indeed, the flexibility of CAM phases in mature cladodes of *O. ficus-indica*, such as stomatal opening in the daytime during wet periods with moderate temperatures (Cushman, 2001; Pimienta-Barrios et al., 2001), can extend carbon gain daily and seasonally, leading to a more efficient use of resources (Nobel, 1995; Mattos and Lüttge, 2001; Pimienta-Barrios et al., 2001).

The chlorophyll content of photosynthetic tissue can be an important indicator of its photosynthetic integrity. In this regard, the synthesis of precursors of chlorophyll is affected by small reductions in tissue water potential (Hsiao, 1973). Perhaps the reduction in the content of these photosynthetic pigments during drought helped reduce daily net CO₂ uptake by the mother cladodes, but might increase the susceptibility of photosynthetic tissue to photodamage (Horton, 2000), an effect exacerbated by the presence of daughter cladodes. The average thickness of chlorenchyma tissue in cultivated CAM plants such as *Opuntia* varies from 3 to 6 mm (Nobel et al., 1994b; Nobel et al., 2002), while in C₃ and C₄ it varies from 100 to 300 µm (Nobel, 1999); as a consequence, the chlorophyll content per unit area is significantly higher in CAM plants (Cui et al., 1993) than in C₃ plants (Larcher, 2003). Apparently, the relatively lower chlorophyll content in mother cladodes observed after rainfall on June was still sufficient to carry out the light reactions related to CO₂ fixation that lead to carbon gain by *O.ficus-indica*. In *Hedera canariensis* a 60% reduction of chlorophyll content resulted in only a 10 to 13% reduction in leaf absorptance. Therefore, a change in chlorophyll content per unit area is not always a major factor in regulating light interception (Björkman and

Demmig-Adams, 1995), particularly for species with a thick chlorenchyma such as *O. ficus-indica*.

Consistent with the hypothesis, the increase in the number of daughter cladodes decreased the photosynthetic activity of the mother cladodes. Indeed, the substantial decrease in the RWC of the mother cladodes and the conservation maintenance of highest values of RWC in daughter cladodes suggests that water moves from mother to daughter cladodes of *O. ficus-indica* (N Wang et al., 1997, 1998). After drought stress had been alleviated by rainfall the mother cladodes became rehydrated and substantial daily net CO₂ uptake and stomatal conductance occurred in phase IV.

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3. CAPÍTULO II

EFFECTS OF YOUNG CLADODES ON THE GAS
EXCHANGE OF BASAL CLADODES OF *Opuntia*
ficus-indica (CACTACEAE) UNDER WET AND DRY
CONDITIONS

International Journal of Plant Sciences, en prensa

ABSTRACT

CAM plants such as the cultivated *Opuntia ficus-indica* are resistant to drought, but their responses can be affected by development of new organs, such as young developing cladodes. The effect of a variable number of daughter cladodes on net CO₂ uptake, night-time intercellular CO₂ concentration, stomatal conductance, and relative water content for mother cladodes exposed to wet and dry conditions was evaluated. Daily net CO₂ uptake by mother cladodes of *O. ficus-indica* was significantly reduced as the number of daughter cladodes increased under dry conditions but was less affected under wet conditions. Stress caused by daughter cladodes and by dry soil affected the expression of CAM phases in mother cladodes, curtailing CO₂ uptake in the late afternoon (phase IV) and reducing carbon gain during the night (phase I) and early morning (phase II), the reduction being most marked under dry conditions. Dry soil and daughter cladodes reduced the relative water content of mother cladodes, decreased the net CO₂ uptake during the day and at night, and increased the internal cellular CO₂ concentration. Under wet conditions the RWC of mother cladodes was only slightly affected by daughter cladodes; nevertheless, daughter cladodes affected the recovery of phases I, II and IV after substantial rainfall interrupted drought, and affected also the net CO₂ uptake in phases II and IV; this is one of the first times that an ontogenic factor has been shown to affect net CO₂ uptake under well watered conditions. The increase in the number of daughter cladodes did not increase the photosynthetic capacity of mother cladodes under wet or dry conditions.

Keywords: Cactaceae, CO₂ uptake, Crassulacean acid metabolism, drought, gas

exchange, *Opuntia ficus-indica*

INTRODUCTION

Crassulacean acid metabolism (CAM) photosynthesis is a mechanism involving traits that reduce transpirational water loss, such as night-time CO₂ uptake, thick cuticle, low stomatal frequency, and a water storage system that allows CAM plants to tolerate prolonged drought during periods of low soil water availability (Szarek and Ting 1975; Nobel 1995; Smith et al. 1997; Lüttge 2004). However, severe prolonged drought that occurs in the arid lands of Mexico (Pimienta-Barrios 1990; Pimienta et al. 2002) and Brazil (Felker 1995) can severely damage cultivated *Opuntia* species. CAM plants often avoid desiccation in dry environments through a mechanism that resembles a desiccation–delay response to drought stresses (Tyree et al. 2002). Desiccation–delay confers tolerance to drought on cultivated species of *Opuntia*, which is disrupted if mother cladodes develop young daughter cladodes under dry conditions (Pimienta-Barrios et al. 2002). In contrast to cultivated opuntias, mother cladodes of wild opuntias generally do not develop young daughter cladodes during drought, which has been considered an avoidance strategy to prevent desiccation and ultimately damage to mother cladodes (Pimienta-Barrios et al. 2002). Daughter cladodes can increase the sensitivity of mother cladodes to drought because during the early stages of development, daughter cladodes exhibit C₃ photosynthesis with daytime stomatal opening (Osmond 1978; Acevedo et al. 1983) and hence require substantial water import from the mother cladodes (Nobel et al. 1994; N Wang et al. 1997). In addition to water availability, the expression of CAM phases is governed by other environmental factors, such as irradiation, temperature and relative humidity, as

well as by genotypic and ontogenic factors (Cushman 2001). Experimental evidence for the effect of an ontogenic factor on the expression of CAM phases is scarce (Lüttge 2004). The main objective of present study is to evaluate the effects of daughter cladodes on the gas exchange and water relations of mother basal cladodes of *O. ficus-indica* under both wet and dry conditions. An experimental increase in the number of daughter cladodes (i.e., an increase in sink strength) that develop on mother cladodes was hypothesized to reduce the relative water content of mother cladodes, especially during drought, and as a consequence to reduce the photosynthetic capacity of the mother cladodes. Under well-watered conditions, on the other hand, an increase in the number of daughter cladodes was predicted to increase the photosynthetic capacity of mother cladodes, as occur for various C₃ and C₄ species (Gifford and Evans 1981; Z Wang et al. 1997).

MATERIAL AND METHODS

Site description

Measurements were made during the spring of 2004 at an experimental field of the Departamento de Ecología of the 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 (data provided by the Fifth Military Base of the Mexican Air Force).

Climatic and microclimatic data

Rainfall data were obtained from a weather station maintained by the Fifth Military Base of the Mexican Air Force at 20° 45' N, 103° 27' W; and 1625 m above sea level. On the dates of gas exchange measurement, the photosynthetic photon flux (PPF, wavelengths of 400-700 nm) on a horizontal plane at the experimental site was recorded hourly from sunrise to sunset with a LI-250 quantum sensor (LI-COR, Lincoln, NE, USA) and then integrated to get the total daily PPF. Air temperature was recorded hourly using a mercury thermometer. Air relative humidity was recorded hourly using a 63-101 digital humidity gauge (RadioShack, Los Angeles, CA, USA).

Experimental design

Mature 12-month-old cladodes (flattened stem segments) of *Opuntia ficus-indica* (L.) Miller (Cactaceae) averaging 35 cm long, 19 cm wide, and 1.8 cm thick were harvested from a cultivated plantation at Nextipac, Jalisco, México. They were planted on 3 March 2004 in plastic pots (19 L) filled with a vermiculite/sand mixture (1:1, v:v). To promote development of young cladodes, the planted cladodes were watered weekly with 3 L per pot. On 23 March 2004 daughter cladodes were selectively removed, leading to zero, one, two, four, or eight daughter cladodes per mother cladode. Each of these five treatments was replicated six times, within a completely randomized design. From 23 March to 26 June 2004, the effect of water supply on daughter cladode growth was monitored for 30 well-watered plants (“wet”) that continued to receive 3 L per pot until rainfall was substantial, and for 30 non-watered plants (“dry”). The length of daughter cladodes was measured weekly using a ruler with the base of the cladode as

reference. Gas exchange was monitored for all 60 plants on 22-23 April, 14-15 May, 28-29 May, and 25-26 June 2004.

Soil water potential and cladode relative water content

Soil water potential was determined with a WP4 dew point potentiometer (Decagon Devices, Pullman, WA, USA) using five samples removed from the center of the root zone (a depth of 10-15 cm) from 23 March to 26 June 2004. The relative water content (RWC) for mother cladodes was determined for 5 segments (1.1 cm × 3 cm) from 10 to 11 a.m. 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 stems sections obtained with a corkborer 11 mm in diameter and equilibrated with water-saturated filter paper at 30°C for 1 hour. RWC was calculated as: $RWC (\%) = (\text{fresh mass} - \text{dry mass}) / (\text{saturated mass} - \text{dry mass}) \times 100$ (Koide et al. 2000).

Gas exchange measurements

The rates of instantaneous net CO₂ uptake, stomatal water conductance, and intercellular CO₂ concentration (C_i) were measured on mother cladodes every 2 h over 24-h periods with a LI-COR LI-6200 portable photosynthesis system. A 0.25-L leaf chamber was modified by replacing the distal half-cylinder with a narrow opening (2 cm × 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 analyzed using a hierarchical ANOVA (Zar 1999); means were separated by a least significant difference (LSD) test (Little and Hill 1975). Correlation analysis was used to test relations between night-time rates of net CO₂ uptake and C_i or stomatal conductance (Zar 1999). Data are presented as means \pm S.E. (n = number of measurements).

RESULTS

Daughter cladodes growth

Elongation of daughter cladodes started in early March 2004, showing a linear phase of growth from the first week of March to the last week of April (fig. 1A). The initial rates of daughter cladode growth were similar during March for both watered and non-watered plants (0.39 and 0.37 cm d⁻¹, respectively). In April, the rate of daughter cladodes growth was higher for watered plants (0.39 cm d⁻¹) than for non-watered plants (0.30 cm d⁻¹; $P < 0.05$). From early May to late June, rates of daughter cladode growth declined and were similar under both wet and dry conditions. Final daughter cladode length was greater under wet (22.4 cm) compared with dry (18.2 cm) conditions.

Climatic and microclimatic conditions

Rainfall started during the last week of May and continued through June 2004 (fig. 1B). The soil water potential remained constant and relatively high under wet conditions, varying from -0.16 MPa on 22-23 April to -0.01 MPa on 25-26 June

(fig. 1C). Under dry conditions, the soil water potential was extremely low under dry conditions before rainfall began, varying from -66 MPa on 22-23 April to -80 MPa on 14-15 May; once rainfall started at the end of May, it increased sharply, reaching -0.02 MPa at the end of June (fig. 1C). The total daily PPF was higher on 14-15 May ($60 \text{ mol m}^{-2} \text{ d}^{-1}$) than on 22-23 April, 28-29 May and 25-26 June (44, 46, and $46 \text{ mol m}^{-2} \text{ d}^{-1}$, respectively). Mean day/night temperatures were lowest for 22-23 April and 25-26 June ($23/14^\circ\text{C}$ and $23/17^\circ\text{C}$, respectively) and highest for 14-15 May and 28-29 May ($28/19^\circ\text{C}$ and $26/21^\circ\text{C}$, respectively). Mean day/night relative humidity was highest on 25-26 June (65/91%), lowest on 14-15 May (23/43%), and intermediate on 28-29 May and 22-23 April (34/59% and 48/89%, respectively).

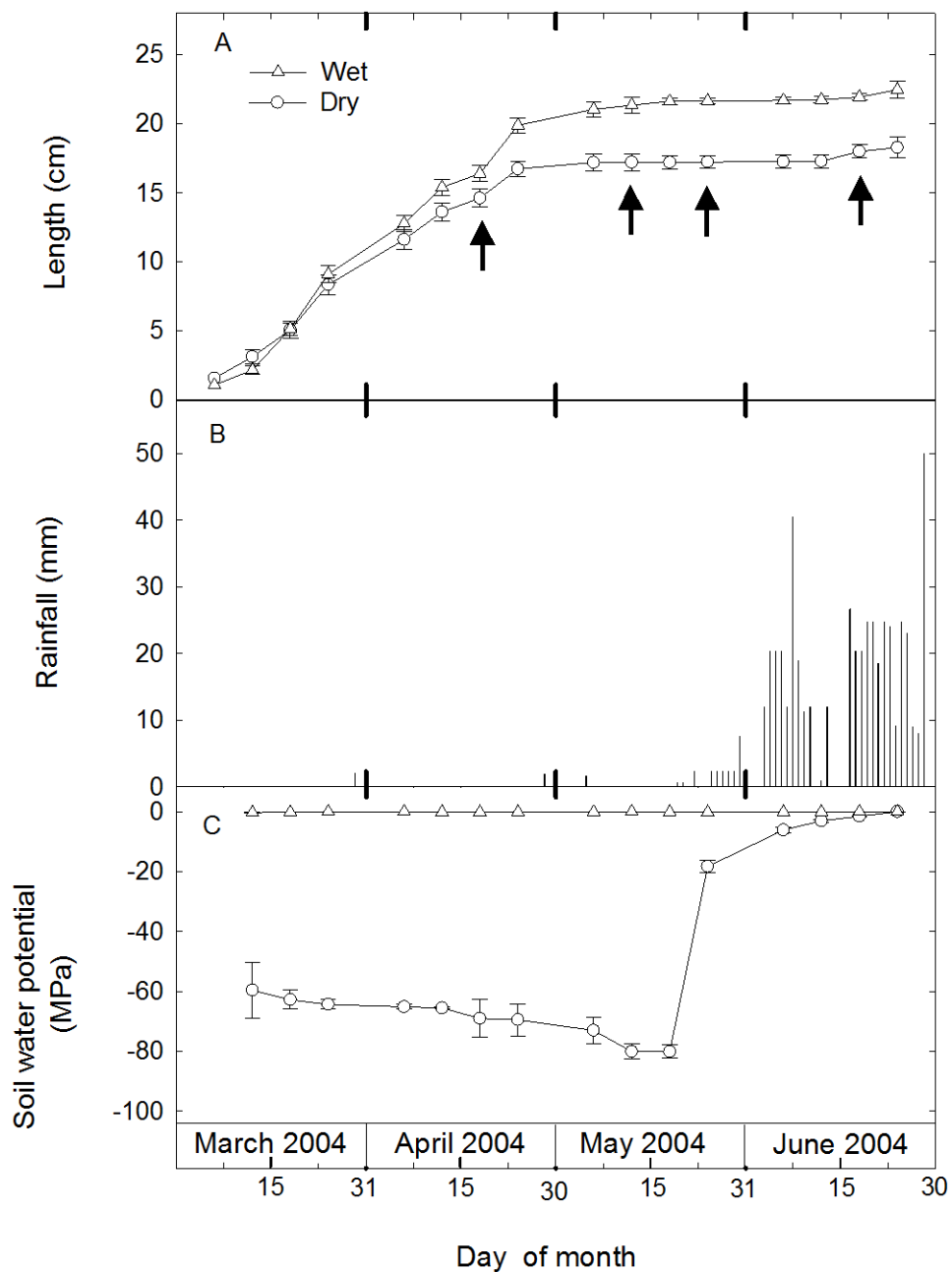


Fig. 1. Elongation of developing daughter cladodes of *Opuntia ficus-indica* under wet and dry conditions (A), daily rainfall (B), and soil water potential (C). Arrows indicate dates of gas exchange measurement. Data are means \pm SE ($n = 24$ cladodes, six from each treatment for cladode growth; $n = 10$ samples for soil

water potential). “Wet” refers to plants watered weekly and “dry” to those that received only rainfall.

Mother cladode relative water content

The first measurement of net CO₂ uptake coincided with the end of the linear phase of cladode growth in late April (fig. 1A). At this time, the RWC for mother cladodes with daughter cladodes under dry conditions was lower than for the wet treatment; the RWC for mother cladodes with daughter cladodes under wet conditions were similar for all daughter cladodes numbers (fig. 2). The second date of measurement, 14-15 May, was also during the dry period, with similar RWC values, including the mother cladodes with no daughter cladodes having a higher RWC (fig. 2). The third date of measurement, 28-29 May, occurred at the beginning of the rainy season (fig. 1) and also had similar RWC values, except that mother cladodes with eight daughter cladodes were lower (fig. 2). The last measurement of gas exchange was on 25-26 June after substantial rainfall had occurred, causing the RWC for mother cladodes previously under dry conditions to increase substantially (especially for two or fewer daughter cladodes). On that date the RWC of mother cladodes tended to decrease with increasing number of daughter cladodes for both water treatments (fig. 2).

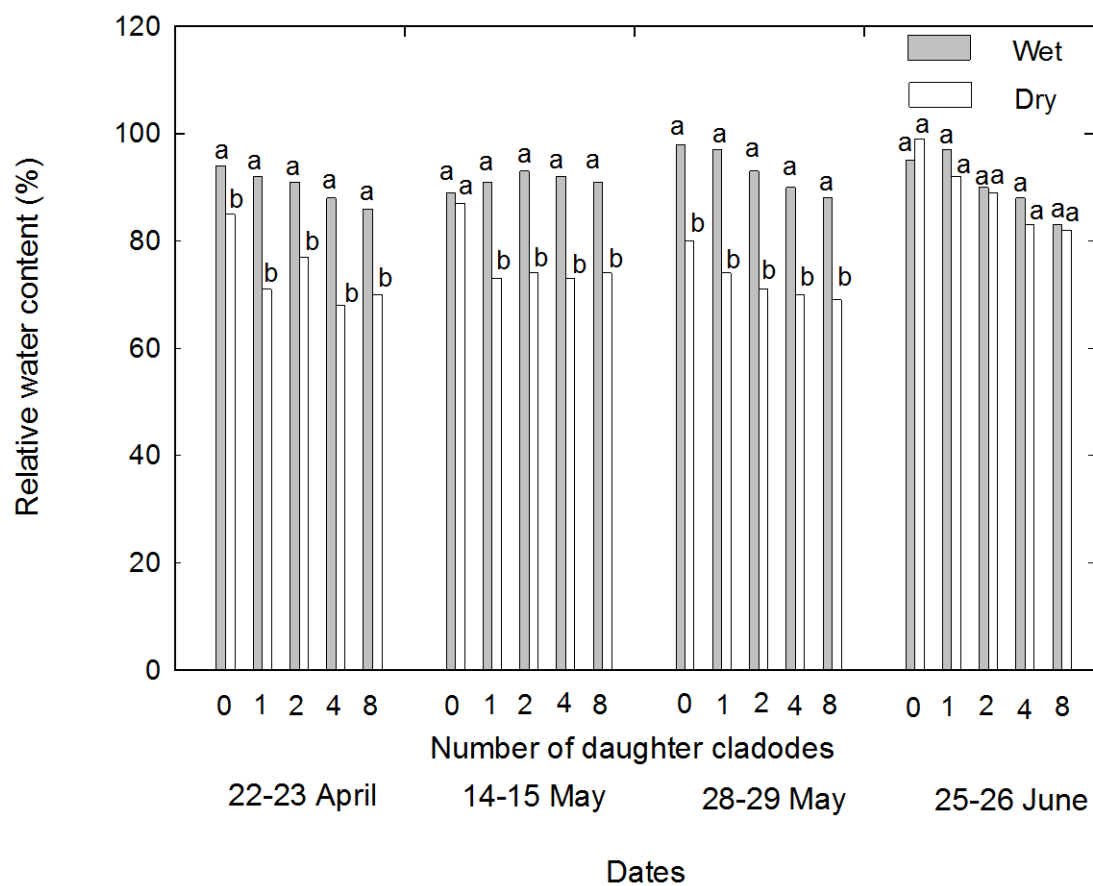


Fig. 2. Relative water content for mother cladodes of *O. ficus-indica* with variable number of daughter cladodes under wet and dry conditions on the dates of gas exchange measurement. Data are means \pm S.E ($n = 6$ plants). Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

Daily patterns of net CO₂ uptake

Under wet conditions for all measurement dates and daughter cladode numbers, most of the net CO₂ uptake by mother cladodes occurred at night (phase I), with some daytime net CO₂ uptake in the late afternoon (phase IV) and early morning (phase II); the highest rates of net CO₂ uptake were observed from midnight to 02:00, declining steadily afterward (fig. 3). The overall highest rates of net CO₂ uptake were on 25-26 June, and the lowest rates on the driest measurement date, 14-15 May, particularly for mother cladodes with eight daughter cladodes (fig. 3).

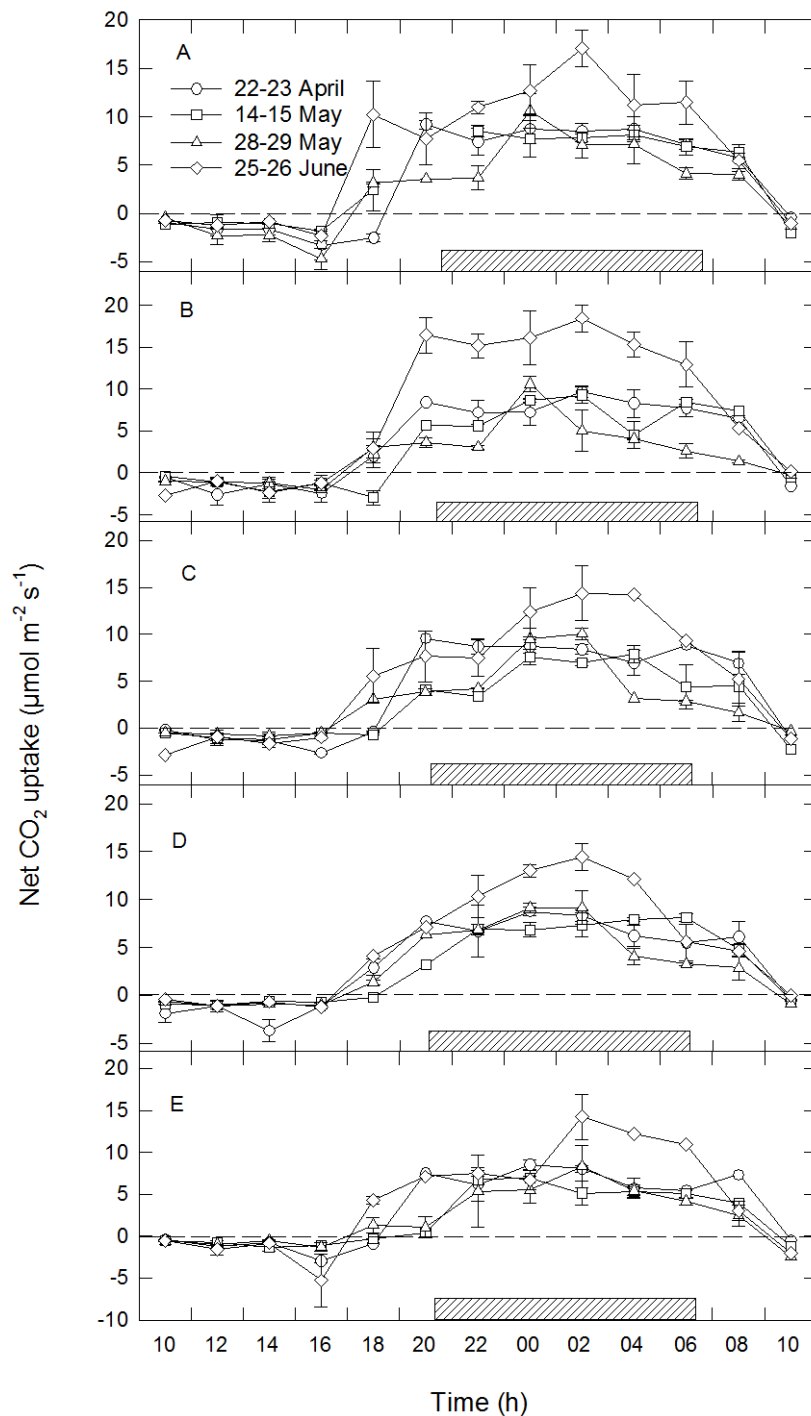


Fig. 3. Net CO₂ uptake rates over 24-h periods under wet conditions for mother cladodes of *O. ficus-indica* with no (A), one (B), two (C), four (D), and eight (E)

daughter cladodes. Hatched bars indicate night-time. Data are means \pm S.E ($n = 6$ plants).

Under dry (non-watered) conditions (fig. 4), net CO₂ uptake rates for mother cladodes were lower than under wet conditions (fig. 3) and uptake occurred predominantly at night, except on 25-26 June following appreciable rainfall. The rates of net CO₂ uptake for the three driest dates tended to decrease as the number of daughter cladodes increased. For these cases, net CO₂ uptake rates were low at the beginning of the night, increasing after midnight (fig. 4A-E). The time with positive net CO₂ uptake at the beginning of the night was less on 14-15 May and 28-29 May, particularly with eight daughter cladodes (fig. 4E). For the two driest dates (22-23 April and 14-15 May), no late afternoon net CO₂ uptake occurred with two or more daughter cladodes (fig. 4C-E), and no early morning net CO₂ uptake was observed with eight daughter cladodes (fig. 4E). Considerable recovery in both daytime and night-time net CO₂ rates was observed on 25-26 June, after significant rainfall had occurred, especially for mother cladodes with four or eight daughter cladodes (fig. 4D; E).

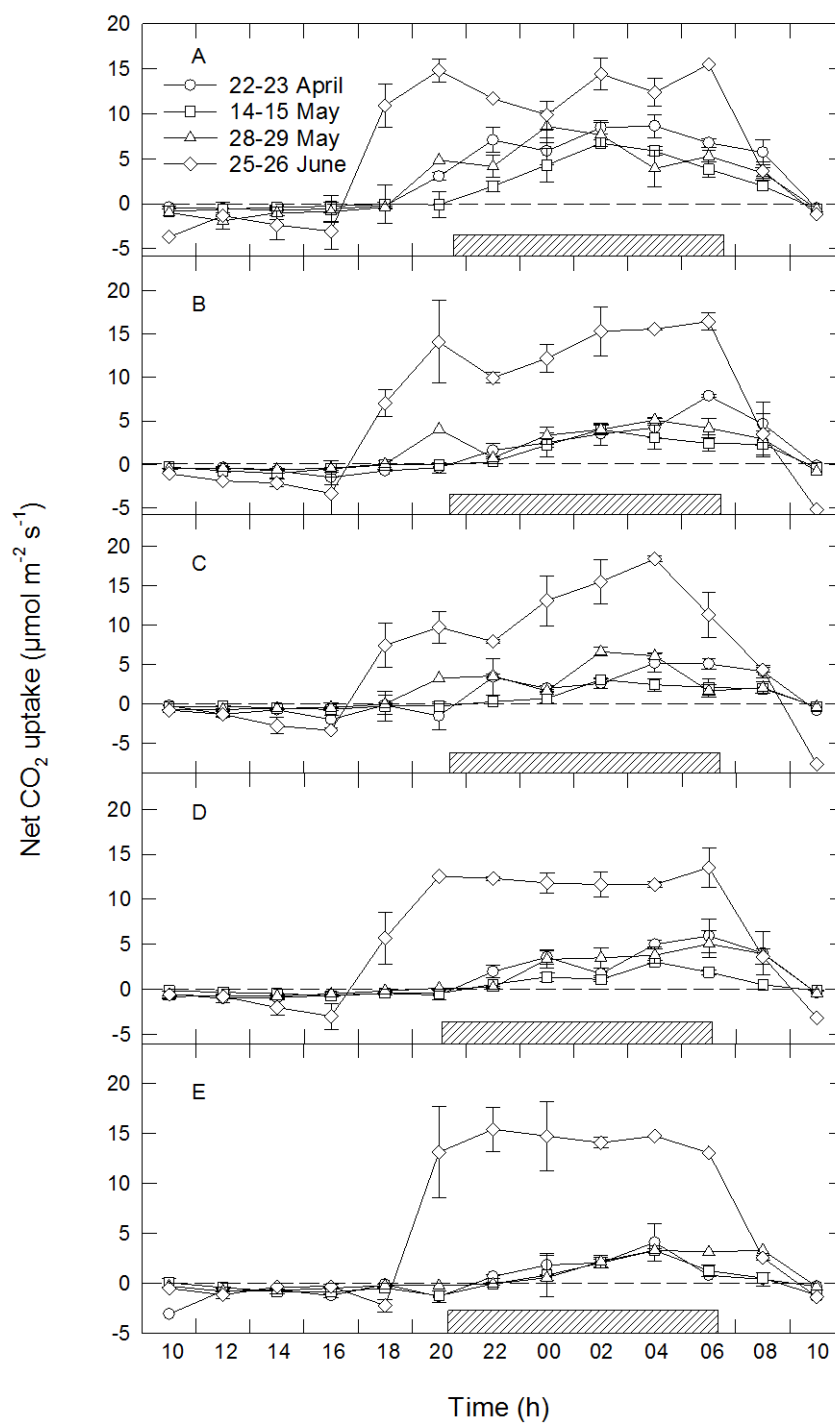


Fig. 4. Net CO₂ uptake rates over 24-h periods under dry conditions for mother cladodes of *O. ficus-indica* with no (A), one (B), two (C), four (D), and eight (E)

daughter cladodes. Hatched bars indicate night-time. Data are means \pm S.E ($n = 6$ plants).

Total daily net CO₂ uptake

Under wet conditions, the total daily net CO₂ uptake by mother cladodes was greatest on 25-26 June for all numbers of daughter cladodes compared with the other three measurements dates (fig. 5A). Also daily carbon gain did not increase as the number of daughter cladodes increased, but rather tended to decrease. Under dry conditions (non-watered plants on 22-23 April, 14-15 May, and 28-29 May), the total daily net CO₂ uptake was lower than under wet conditions and steadily decreased as the number of daughter cladodes increased (fig. 5B). Surprisingly, after rainfall had occurred (25-26 June), mother cladodes formerly under dry conditions with more than two daughter cladodes actually had greater total daily net CO₂ uptake than mother cladodes continuously under wet conditions.

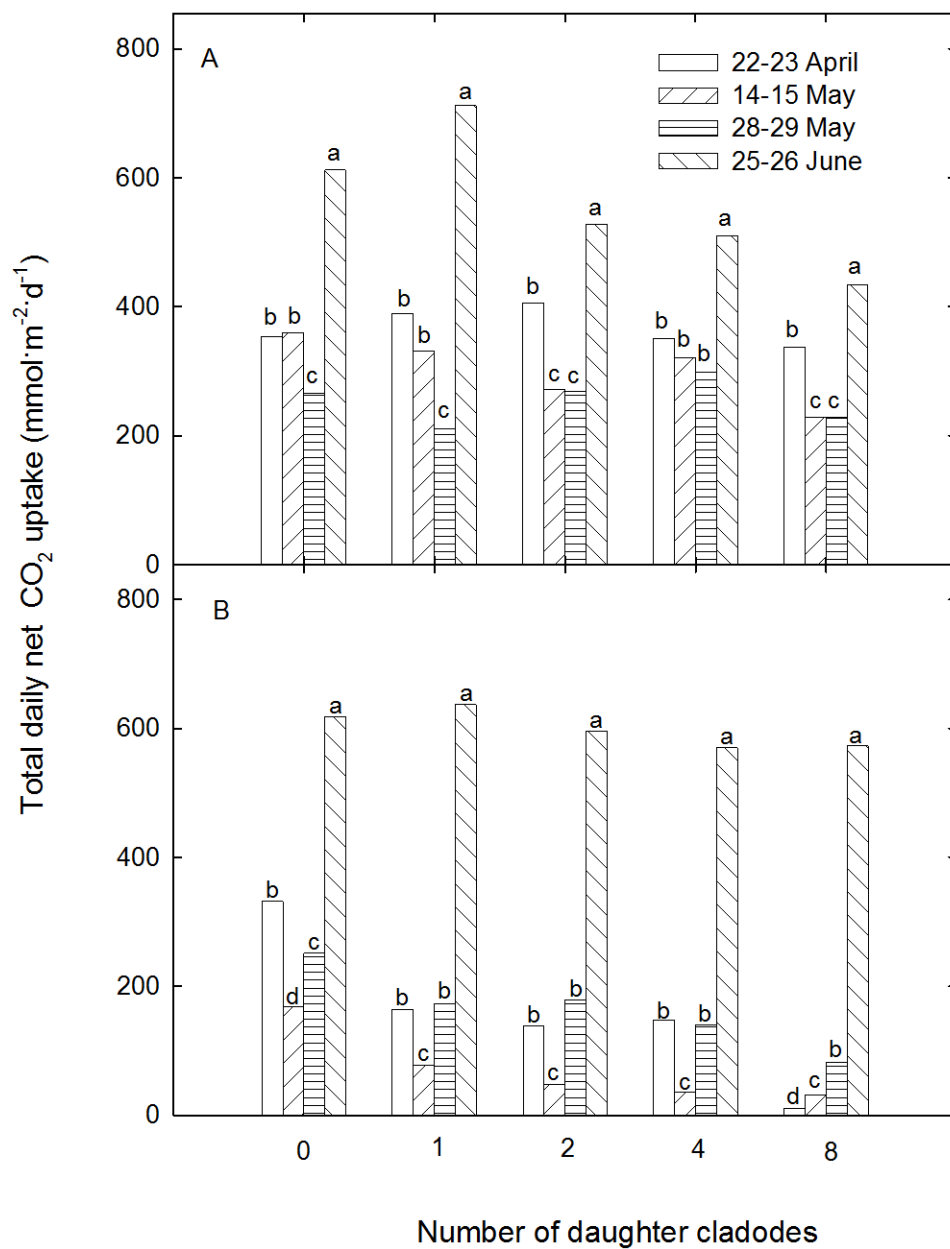


Fig. 5. Total daily net CO₂ uptake for mother cladodes of *O. ficus-indica* with a variable number of daughter cladodes under wet (A) and dry (B) conditions. Data are means \pm S.E ($n = 6$ plants). Values within columns for a particular treatment followed by different letters are significantly different at $P < 0.05$.

Intercellular net CO₂ concentrations

Mother cladodes with daughter cladodes had a higher C_i at night under dry compared with wet conditions at the beginning of the dry season (22-23 April; fig.6). On 14-15 May, C_i had increased and was higher under dry compared with wet conditions for all treatments (fig. 6). No differences were observed on 28-29 May and 25-26 June. The C_i for mother cladodes did not vary with the number of daughter cladodes under wet conditions (fig. 6A). Under dry conditions, C_i was highest with four and eight daughter cladodes. For the four measurements dates, C_i at night was negatively correlated with the net CO₂ uptake rate under wet conditions ($r = -0.65$; $P < 0.05$), but no significant relation occurred under dry conditions ($r = -0.25$; $P > 0.05$). Similarly the stomatal conductance at night was correlated with the CO₂ rate under wet ($r = 0.73$, $P < 0.05$) but not under dry ($r = 0.22$, $P > 0.05$) conditions.

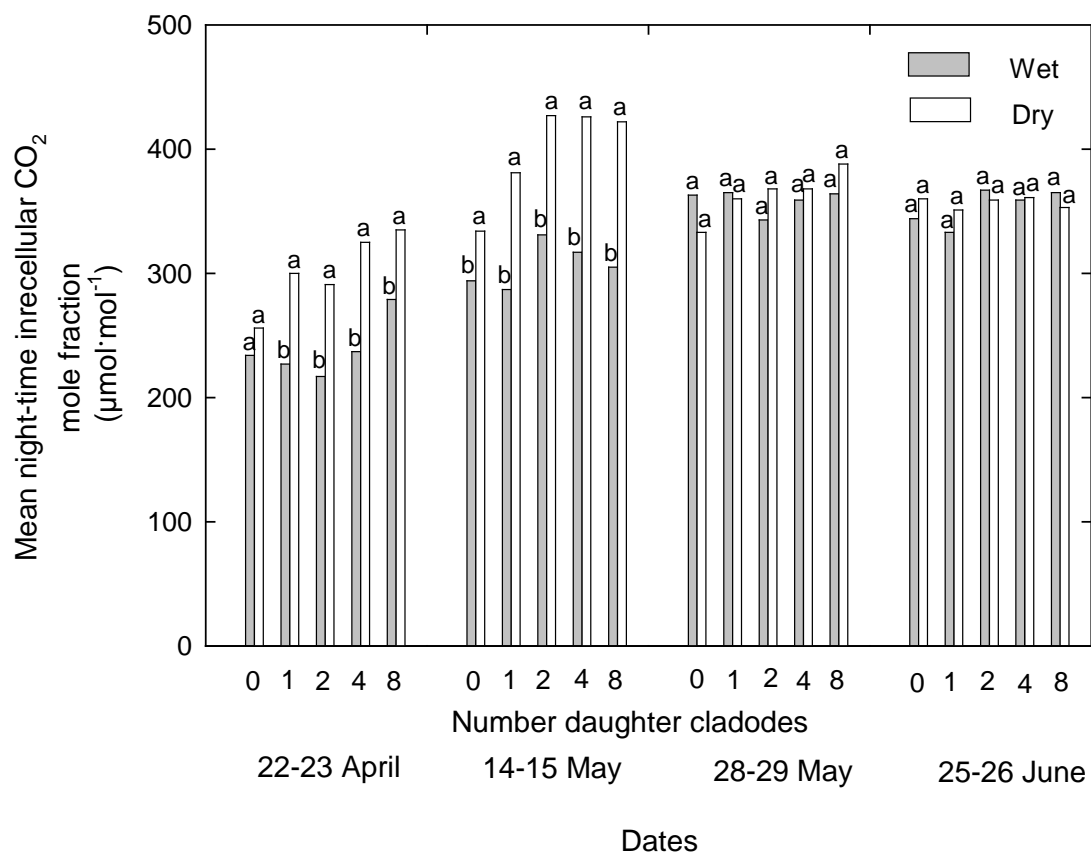


Fig. 6. Mean night-time intercellular CO₂ mole fraction for mother cladodes of *O. ficus-indica* with a variable number of daughter cladodes under wet (A) and dry (B) conditions. Data are means \pm S.E ($n = 6$ plants). Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

Net CO₂ uptake in CAM phases

Under wet conditions, net CO₂ uptake in phase I (night-time) by mother cladodes was highest on 25-26 June, when it was statistically lowest for two or more daughter cladodes, (table 1). Net CO₂ uptake in phase II (early morning) under wet conditions was much less than in phase I (night-time) and tended to decrease in mother cladodes with more than two daughter cladodes on 14-15 May and with more than one cladode on 28-29 May. Net CO₂ uptake increased on 25-26 June and tended to be lowest for 8 daughter cladodes (table 1). Net CO₂ loss in phase III (mid-day) was also low, without a consistent pattern with the number of daughter cladodes (table 1). Except under the wettest conditions when it was the highest, net CO₂ uptake under wet conditions in phase IV (late afternoon) was similar to that in phase II; it tended to decrease as the number of daughter cladodes increased, except on 22-23 April.

For the first three measurement dates under dry conditions, net CO₂ uptake in phase I was less than under wet condition and sharply decreased as the number of daughter cladodes increased (table 1); on 25-26 June, net CO₂ uptake did not vary with the number of daughter cladodes and was actually higher than under continuously wet conditions for two or more daughter cladodes. Under dry conditions on 22-23 April and 14-15 May, net CO₂ uptake in phase II decreased as the number of daughter cladodes increased, becoming vanishingly small for 8 daughter cladodes on 22-23 April, and for 4 and 8 daughter cladodes on 14-15 May; after rainfall net CO₂ uptake in phase II increased sharply on 28-29 May, especially for mother cladodes with 4 and 8 daughter cladodes (fig. 1). After

substantial rainfall (25-26 June), relatively small increases in net CO₂ uptake were observed for mother cladodes with 0, 1, and 2 daughter cladodes; and decreased with more than 4 daughter cladodes (table 1). Also, under dry conditions, net CO₂ uptake in phase IV was small and often negative; on 25-26 June, it became significant, exceeding that under wet conditions except with eight daughter cladodes. Thus, under dry conditions both phases I and IV were drastically reduced by the combined effect of daughter cladodes and dry soil (table 1).

TABLE 1. Net CO₂ uptake during CAM phases under wet and dry conditions by mother cladodes in response to number of daughter cladodes on different dates. Data are means ($n = 6$ plants). Values within columns for a particular date followed by different letters are significantly different at $P < 0.05$.

Date	Number of daughter cladodes	Net CO ₂ uptake in CAM phases (mmol m ⁻²)							
		I		II		III		IV	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
22-23 April 2004	0	291 a	265 a	62 a	62 a	-47 a	-14 a	48 a	20 a
	1	289 a	142 b	70 a	50 b	-43 a	-20 ab	74 a	-8 b
	2	300 a	132 b	75 a	45 b	-34 a	-26 b	66 a	-12 b
	4	255 a	131 b	66 a	43 b	-48 a	-19 ab	77 a	-8 b
	8	244 a	54 c	79 a	3 c	-33 a	-37 c	47 a	-11 b
14-15 May 2004	0	282 a	163 a	69 a	21 a	-28 a	-13 a	40 a	-1 a
	1	263 a	69 b	80 a	25 a	-25 a	-17 a	20 b	-1 a
	2	218 a	50 c	48 b	21 a	-20 a	-17 a	25 b	-7 a
	4	268 a	46 c	52 b	6 b	-21 a	-10 a	21 b	-6 a
	8	211 a	52 c	40 b	5 b	-23 a	-13 a	1 c	-13 b
28-29 May 2004	0	234 a	213 a	43 a	37 a	-61 c	-30 b	48 a	32 a
	1	182 a	125 b	15 c	32 ab	-34 b	-13 a	48 a	29 a
	2	214 a	141 b	18 c	29 b	-15 a	-15 a	50 a	23 a
	4	234 a	115 b	31 b	43 a	-22 a	-17 a	55 a	-0.13 b
	8	206 a	65 c	27 b	36 a	-21 a	-15 a	17 b	-4 b
25-26 June 2004	0	456 a	460 a	59 a	39 a	-32 b	-66 c	129 a	185 a
	1	561 a	501 a	58 a	38 a	-46 c	-54 c	139 a	152 a
	2	416 b	477 a	57 a	47 a	-40 c	-52 c	95 b	124 a
	4	400 b	439 a	50 a	39 a	-21 a	-40 b	81 b	131 a
	8	371 b	483 a	33 b	27 b	-52 d	-15 a	82 b	78 b

DISCUSSION

Contrary to one of the hypotheses, total daily net CO₂ uptake for mother cladodes of *Opuntia ficus-indica* under wet conditions did not increase as the number of daughter cladodes increased, despite the fact that light availability, average day/night air temperatures, and average day/night relative humidity were favorable for net CO₂ uptake by this species (Nobel and Hartsock 1984; Pimienta-Barrios et al. 2000). As expected, under dry conditions during April and May, total daily net CO₂ uptake by mother cladodes with daughter cladodes showed a marked reduction in daily net CO₂ uptake.

Daughter cladodes can exhibit C₃ photosynthesis with daytime stomatal opening (Osmond 1978; Acevedo et al. 1983), leading to a substantial movement of water from mother to daughter cladodes (Nobel et al. 1994; N Wang et al. 1997). Thus an ontogenic factor, in this case young daughter cladodes, exacerbated the effect of drought on mother cladodes particularly under dry conditions, as indicated by both the drastic reduction in the RWC and in the net CO₂ uptake by mother cladodes. Under wet conditions the RWC of mother cladodes was only slightly affected by daughter cladodes; however, the number of daughter cladodes affected the recovering of phases I, II and IV after substantial rainfall interrupted drought. The net CO₂ uptake of phases II and IV under wet conditions was affected by the highest number of daughter cladodes; apparently one of the first times that an ontogenic factor has been shown to affect net CO₂ uptake under well watered conditions.

As indicated, for the CAM plant *O. ficus-indica* an increasing sink stress (number of young cladodes) did not increase the photosynthetic capacity in the source (mother cladodes), as regularly occurs for domesticated C₃ and C₄ plants (Gifford and Evans 1981; Salisbury and Ross 1992; Z Wang et al. 1997). In fact, the highest values of daily net CO₂ uptake in cultivated CAM plants (e.g., *Agave*, *Opuntia*, *Stenocereus*) in Jalisco, México tends to occur at the beginning and the middle of the dry period (late October to the end of February), coinciding with the increase in light availability because of reduced cloudiness as well as moderate air temperatures (Pimienta-Barrios et al. 2000, 2001). During the dry period, activity of vegetative and reproductive sinks is minimal, indicating that the strength of photosynthesis in these CAM plants is more under environmental rather than ontogenic control, demonstrating that *O. ficus-indica* integrates carbon uptake across the diel cycle in response to the favorable conditions created by rainfall (CO₂ uptake increases in phases I, II and IV), as occurs in the CAM plant *Clusia* when is exposed to dry and wet periods (Lüttge 2004).

Although *O. ficus-indica* might be considered as species that faces drought using a mechanism of desiccation–delay (Tyree et al. 2002), its relatively quick recovery from drought stress resembles the response to drought shown by desiccation–tolerant plants that are adapted to drying and wetting cycles (Tuba et al. 1998). That is, opuntias in their natural habitats show a rapid recovery of their photosynthetic capacity once drought is interrupted by rainfall, despite having RWC values for the photosynthetic stems that are lower than 65% (Pimienta-Barrios et al. 2002). Early observations showed that non-irrigated plants of *Opuntia*

basilaris also had major CO₂ uptake capacity a few days after rainfall (Hanscom and Ting 1978).

During the photosynthetic recovery to rainfall shown by mother cladodes under dry conditions, CO₂ uptake increased considerably at night (phase I) as well as in the morning and the late afternoon (phases II and IV, respectively). The favorable net CO₂ uptake responses of *O. ficus-indica* to rainfall during the day and the night by the combination of C₃ and CAM pathways revealed a photosynthetic plasticity that can optimize water use, plant carbon balance, and light use during both favorable and unfavorable conditions (Mattos and Lüttge 2001; Dodd et al. 2002). Indeed, use of the C₃ pathway commonly allows some CAM plants to maximize carbon gain when water is abundant (Cushman 2001).

The adjustment of CAM phases plays an important role in carbon gain under both dry and wet conditions. Under dry conditions the daytime phases (II and IV) as well as the night-time phase (I) are important for the adaptation of mother cladodes of *O. ficus-indica* to the water stress caused by the combined effect of daughter cladodes and soil drought. Phase IV was the most and earliest affected; in contrast, phase II was reduced under dry conditions but not eliminated. Drought stress also affected phase I for mother cladodes with daughter cladodes under dry conditions, reflected by less time with positive net CO₂ uptake as well as reduced rates of net CO₂ uptake. Net CO₂ uptake by *O. ficus-indica* increased at the end of the night, coinciding with higher air relative humidity and lower air temperatures, revealing a physiological avoidance of drought (Larcher 2003). This response is similar to patterns of gas exchange observed for the CAM plants *Agave deserti*, *Hylocereus undatus*, and *Opuntia robusta* exposed to drought (Nobel 1990; Raveh

et al. 1995; Pimienta-Barrios et al. 2002). Thus, physiological plasticity plays an important role in the response of mother cladodes to drought stress as well as favorable conditions of rainfall, allowing net carbon gain under both wet and dry conditions. Based on the response of photorespiration to environmental stresses, the greater sensitivity of phases IV during the dry period may be mainly because it coincides with higher temperatures and irradiance, which commonly favors photorespiration in C_3 and C_4 plants (Maxwell et al. 1997; Takeba and Kozaki 1997; Lambers et al. 1998). The less time with positive net CO_2 uptake as well as reduced rates of net CO_2 uptake observed in phase I particularly in the driest date of measurement is because the occurrence of high night-time temperatures that regularly affected the activity of PEP-carboxylase in *O. ficus-indica* (Israel and Nobel 1985). Daytime photosynthesis for C_3 and C_4 plants is affected by water stress as a consequence of stomatal closure (Chaves 1991; Yordanov et al. 2000), which reduces CO_2 availability in the mesophyll (Kitao et al. 2003; Warren 2004). Because their stomata open at night when air temperatures tend to be lower than during the daytime, CAM plants in warm climates can have high rates of net CO_2 uptake under environmental conditions that stress C_3 and C_4 plants (Pimienta-Barrios et al. 2000, 2002); also, the succulence of the shoots maintains hydration of the photosynthetic tissue, allowing stomatal opening and carbon gain even during prolonged drought (Nobel 1995; Pimienta-Barrios et al. 2002). The low values of C_i in the photosynthetic tissues of CAM plants such as *O. ficus-indica* may not be related to lower stomatal conductance but perhaps to higher carboxylation efficiency, and an increase in C_i that accompanies reduced CO_2 uptake might probably be related to an increase in respiration as a consequence of

the physiological stress (Lawlor 2002; Llorens et al. 2003; Tezara et al. 2003) caused by the combined effects of both daughter cladodes and dry soil.

The stems of *O. ficus-indicas* have a large capacitance (water storage ability) that allows tissue water potentials to remain relatively high, even when the soil water content is low. For instance, cultivated CAM plants such as *Agave tequilana* and *O. ficus-indica* can maintain and even increase carbon gain during the dry season, six months after rainfall has ceased (Pimienta-Barrios et al. 2000, 2001; Nobel et al. 2002). However, the presence of daughter cladodes causes desiccation of mother cladodes in a relatively short time, significantly reducing net CO₂ uptake by mother cladodes of *O. ficus-indica*, which increases with the number of daughter cladodes, especially during dry conditions. The stress caused by developing cladodes can lead to the redistribution of water from water storage tissue in old stems to young active tissue (Schulte and Nobel 1989; Herrera et al. 2000; Rabas and Martin 2003; Lüttge 2004; Pimienta-Barrios et al. 2005). Translocation of water between organs may be an important mechanism to maintain constant water content in young active tissues during times of drought (Zotz and Hietz 2001), affecting the ecological benefits of succulence in response to drought (Nobel et al. 1994, 2002).

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4. CONCLUSIONES

1. El efecto de la sequía edáfica redujo significativamente la asimilación de CO_2 en las plantas madres de *O. ficus-indica*, fue activado por la presencia de cladodios jóvenes o cladodios hijos.
2. La reducción en la toma de carbono se acompañó de la disminución del contenido de agua en los cladodios madres, debido al movimiento de agua de los cladodios madres a los cladodios hijos.
3. El movimiento de agua de los cladodios madre a los cladodios hijos se atribuye principalmente al hecho de que estos presentan metabolismo fotosintético C_3 , por lo que los estomas se abren durante el día, favoreciendo la salida de agua vía transpiración.
4. Los cladodios hijos también causan sequía fisiológica en las plantas madres aún cuando el suelo se encuentra completamente hidratado.
5. Contrariamente a una de las hipótesis, la asimilación neta diaria de CO_2 en los cladodios madre de *Opuntia ficus-indica* bajo condiciones de humedad no incrementó conforme el número de cladodios hijos aumentó, a pesar de

que la disponibilidad de luz, el promedio de la temperatura día-noche y la humedad relativa día-noche fueron favorables para la asimilación de CO₂ por los cladodios madre.

6. La flexibilidad de la expresión de las fases CAM en los cladodios madre de *O. ficus-indica* también dependieron de la presencia de cladodios hijos, constituyendo esto un ejemplo de la plasticidad fotosintética en respuesta a un factor ontogénico. Esta plasticidad a través de la regulación de las fases CAM permite a *O. ficus-indica* incrementar la asimilación de CO₂ en condiciones favorables y mantener la ganancia de carbono en los cladodios madre cuando estos están estresados por el efecto combinado de la presencia de los cladodios hijos y la sequía.
7. El efecto de la sequía edáfica en la asimilación de CO₂ en las plantas madres de *O. ficus-indica* se incrementa notablemente cuando el número de cladodios hijos es mayor a cinco cladodios, por lo que es recomendable que en las plantaciones cultivadas destinadas a producir “nopalitos”, se realice podas permanentes para no permitir el desarrollo de más de cinco cladodios hijos por planta madre.
8. En los programas de reforestación en zonas áridas en las que se emplean opuntias, es recomendable el uso de variedades silvestres que cultivadas, ya que las primeras presentan mecanismos de evitación a la sequía que aumentan su tolerancia a este estrés ambiental, como es la cesación en la formación de cladodios hijos cuando ocurren sequías prolongadas.
9. Este es uno de los primeros trabajos en que se demuestra que un factor ontogénico afecta la asimilación de CO₂ en condiciones de humedad y de

sequía.

5. APENDICE