

Review

Ecophysiological responses of *Opuntia* to water stress under various semi-arid environments

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Abstract

In this review, information relating to the ecophysiological response of *Opuntia robusta* plants in wild populations and cultivated plants of *O. ficus–indica* under various environments is presented. The succulence of *O. ficus–indica* allowed plants to maintain photosynthetic activity for five months after rains had come to an end. The highest values of carbon gain were observed in the autumn and at the beginning of winter, coinciding with fresh temperatures and an increase in irradiance. The combined effect of photosynthetic plasticity, the association with mycorrhizal fungi and the avoidance and tolerance mechanisms such as the cessation in the formation of both new cladode and new roots allowed wild plants of *O. robusta* growing in a rocky environment to maintain carbon gain after being exposed to prolonged drought. The development of daughter cladodes on mother cladodes of *O. ficus–indica* during the dry spring season, lead to physiological drought which was noticeable not only in the reduction in photosynthesis and chlorophyll content but also in the relative water content. Daughter cladodes caused physiological stress on mother cladodes during the spring dry period even on well-watered plants and their effects were exacerbated by the decrease in irradiance.

Key words: Drought, mycorrhizas, *Opuntia*, photosynthesis, plasticity.

Resumen

En esta revisión se presenta información relacionada con la respuesta ecofisiológica de plantas de *Opuntia robusta* en poblaciones silvestres y de plantas cultivadas de *O. ficus-indica*, en diferentes ambientes. La suculencia en *O. ficus-indica* permite mantener la actividad fotosintética cinco meses después de que cesan las lluvias. Los valores máximos de ganancia de carbono se observaron en el otoño y al inicio del invierno, coincidiendo con temperaturas frescas y aumento en irradiación. El efecto combinado de plasticidad fotosintética, asociación con hongos micorrícicos y mecanismos de evitación y tolerancia a la sequía, como la supresión en formación de raíces y cladodios, permitieron a las plantas silvestres de *O. robusta* mantener la ganancia de carbono después de estar expuestas a una sequía prolongada. El desarrollo de cladodios hijos en cladodios madre de *O. ficus-indica* durante el periodo seco en primavera causó sequía en éstos y se manifestó por la reducción en la fotosíntesis, contenido de clorofila y en el contenido relativo de agua. Estos también causaron estrés fisiológico en los cladodios madre durante el periodo seco cuando fueron irrigadas y el efecto de la sequía se aumentó por la disminución en la irradiación.

Palabras clave: Fotosíntesis, micorrizas, *Opuntia*, plasticidad, sequía.

Introduction

Unlike C₃ and C₄ plants, only a few species showing the Crassulacean acid metabolism (CAM) are cultivated crops (Loomis and Connor, 1992). Their high resistance to drought conditions, low rates of water consumption and energy make them important crops for sustainable agriculture for semi-arid environments (Pimienta-Barrios and Nobel, 1994). Seasonal studies on photosynthesis in cultivated species as *Agave tequilana* (Weber), *Opuntia ficus-indica* (L) Miller and *Stenocereus queretaroensis* (Weber) Buxbaum have shown that these species have the capacity to maintain the fixation of atmospheric CO₂ during the dry season, from November to March as few wild and cultivated C₃ and C₄ plants can (Pimienta-Barrios *et al.*, 2000; 2001; Nobel *et al.*, 2002). Like other CAM plants, opuntias shows a wide range of geographic distribution, as a result of their remarkable physiological plasticity (Keeley and Rundel, 2003; Winter *et al.*, 2008; Herrera, 2009). However, most of the responses of *Opuntias* to environmental stress, particularly abiotic stress as drought, have been performed under controlled conditions (i.e. greenhouse, growth chambers; Nobel *et al.*, 2002) and a few studies have been performed in field conditions particularly in semi-arid lands of central México, one of the most important native environments for *Opuntias*. Studies performed under controlled conditions, regularly exclude the complex interaction of plants with their natural environment (Cushman, 2001; Dodd *et al.*, 2002; Herrera, 2009).

Thus, ecophysiological studies under field conditions will be helpful to comprehend the ecophysiological mechanisms that underlie the plastic responses of *Opuntia* to drought, particularly their photosynthetic plasticity (Pimienta-Barrios *et al.*, 2003). For that reason, in this paper we summarize our work, conducted from 1997 to 2006, on gas exchange of wild and cultivated species under different semiarid environments in Mexico with emphasis on the response to drought in the spring when drought is the most intense during the year, because the reduction in soil water content is accompanied by high air temperatures with emphasis on: 1) the response to seasonal changes in temperature, irradiance and soil water content in cultivated *O. ficus-indica*; 2) the effect of the mycorrhizal symbiosis on the physiology of a wild population of *O. robusta* (Wendl.) growing in a rocky environment exposed to both extreme water stress and low mineral availability conditions; and 3) the combined effect of daughter cladodes, drought stress and reduced irradiance on the physiological response in basal mature cladodes of *O. ficus-indica*.

Gas exchange responses of mature plants of *Opuntia ficus-indica* in a sub-tropical environment

Our first gas-exchange experiment was conducted on plants from a cultivated population of *O. ficus-indica* located in Techaluta, Jalisco, Mexico during 1997 and 1998. Techaluta is located in a semi-arid, sub-tropical region, within the Sayula Basin of the southern region of the Jalisco state, 20° 05' N, 103° 32' W, and 1380 m above sea level. The average annual rainfall is nearly 700 mm, most of which occurs in the summer (Pimienta-Barrios and Nobel, 1994), and the daily mean extreme temperature that varies from 10 to 34°C, with an annual average of 22°C. Air temperatures during the spring can reach 40°C and the arid index of the environment can be higher than that found in the semi-arid southern region of state of Zacatecas, where this species is native and widely cultivated (Pimienta, 1994; Pimienta-Barrios *et al.*, 2000).

The aim of this measurement was to evaluate the ecophysiological responses of plants to the contrasting environments that prevail in the wet summer and during the progressive drought conditions that began in late fall and end at the begin of the summer season. The seasonal effect of fluctuations in air temperature, photosynthetic photon flow (PPF) and soil water content on, daily net CO₂ assimilation, stomatal opening and intercellular CO₂ mole fraction was evaluated.

The observations in the spring were performed at the end of this season (late May), because in the early spring (March) *Opuntias* are slightly affected by drought mainly due to the water conserved in the stems (succulence) alleviates the effects of drought. It is not until the end of the dry spring period when the effects of drought are more intense and noticeable. We found that the highest maximal instantaneous rates and highest total daily net CO₂ uptake for *Opuntia* occurred in December 1997, reflecting the moderate day/night air temperatures (26/16°C) and the increase in PPF due to reduced cloudy conditions. Moderate night temperature favors PEP case activity and CO₂ uptake by CAM succulents (Hanscom and Ting, 1978; Kluge and Ting 1978; Nobel, 1988; Israel and Nobel, 1995). In particular, the mean day/night air temperatures in December 1997 (26/16°C) and February 1998 (29/15°C) were close to those optimal for net CO₂ uptake by *O. ficus-indica* under controlled environmental conditions (25/15°C; Nobel and Hartsock, 1984; Israel and Nobel, 1995).

The carbon gain continued after the wet season and extended through the autumn of 1997, diminishing at the end of the winter season, when the soil water content was lower than 5%. The values of carbon gain in the middle of the dry season were higher than values observed in the summer. Net CO₂ uptake during the dry season was facilitated by the succulence of photosynthetic mature cladodes of *O. ficus-indica* that allowed photosynthetic stems to maintain stomatal aperture and coincide with an increase in light availability because of reduced cloudy conditions.

Early work revealed that light limits photosynthesis for *O. ficus-indica* as net CO₂ uptake increases linearly with total daily PPF of up to 20 mmol m⁻² d⁻¹ (Nobel and Hartsock, 1984; Nobel and Bobich, 2002). These observations explain in part why the lowest positive daily net CO₂ uptake over 24 h occurred at the beginning of the rainy season (July 1997), when plants began their recovery from the drought and irradiance was reduced.

In addition to night-time CO₂ uptake, the water stored in the stems also allowed diurnal CO₂ assimilation by metabolism photosynthetic C₃, particularly during the early morning (phase II) and late afternoon (phase IV), in the dry months of autumn and winter, when the light and temperature conditions favored photosynthetic activity (Figure 1).

The net carbon gain registered at the end of May, 1998, when the drought and air temperatures reached their maximum levels, was negative ($-40 \text{ mmol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$) (Pimienta-Barrios *et al.*, 2000), indicating that plants of *O. ficus-indica* were exposed to a severe drought at the end of the spring, becoming a substantial source of CO_2 to the atmosphere during the dry season. This result could be important for environments that have been poorly studied worldwide in future scenarios caused by global warming in subtropical deciduous forests.

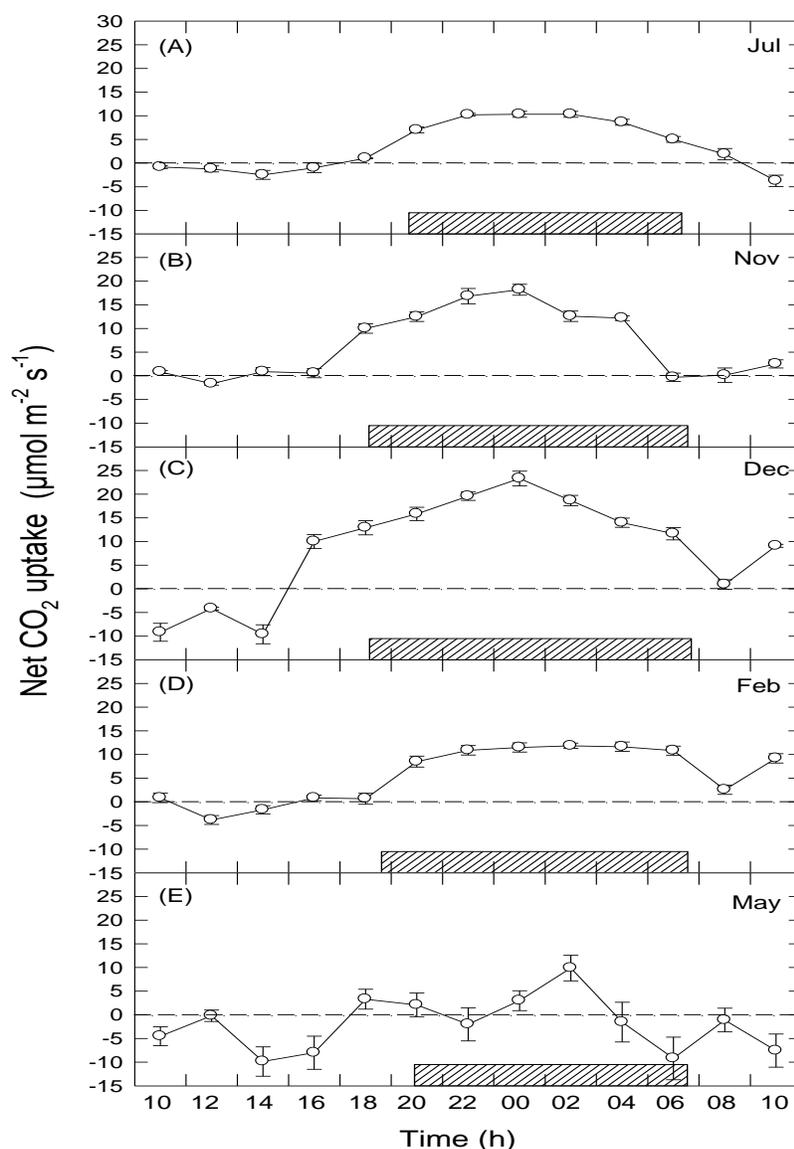


Figure 1. Net CO_2 uptake rates over 24 h periods for *O. ficus-indica* on 25–26 July 1997 (A), 7–8 November 1997 (B), 17–18 December 1997 (C), 12–13 February 1998 (D) y 9–10 May 1998 (E), at Techaluta, Jalisco, Mexico. Data are means \pm SE, except when are little of symbol ($n=10$) (Pimienta-Barrios *et al.*, 2000; Licence number 2426630074173). Hatched bars indicate nighttime.

The highest rates of net CO₂ uptake coincided with low values of stomatal conductance at night (e.g. 1.9 mol m⁻² s⁻¹ in July, 1997; 0.15 mol m⁻² s⁻¹ in December, 1997 and 0.21 mol m⁻² s⁻¹, in February, 1998). This reduction occurred concomitantly with a lowering of the molar fraction of intracellular CO₂, which fluctuated from 334 μmol mol⁻¹ in July, 1997 to 301 μmol mol⁻¹ in December of the same year, to 237 μmol mol⁻¹ in February, 1998 (Pimienta–Barrios *et al.*, 2000). Such behavior suggests that PEPc was active during these months of the year, thereby allowing the maintenance of substantial carbon gain in the dry season, when the rates of stomatal conductance are low.

Growth and gas exchange of *Opuntia robusta* after prolonged drought in a rocky stressful environment

This study was performed in a wild population of *O. robusta* growing in a rocky environment located in El Rayo, state of Zacatecas, Mexico, after a prolonged drought. Annual rainfall data from 1990 to 2000 revealed that the study site had experienced a prolonged drought beginning in 1994. The 258 mm of rainfall during 2000 occurred as follows: May, 75 mm; June, 11 mm; July, 68 mm; August, 64 mm; September, 15 mm; and October, 25 mm (Pimienta–Barrios *et al.*, 2002). This region is characterized by temperate climate, with a shallow soil that dries–out rapidly after the rain as a consequence the soil water content regularly reach values less than 5%, even during the wet season.

The response of *O. robusta* to this extreme dry environment was manifested mainly through their physiological plasticity, expressed by changes in the activity of CAM phases. Since Osmond (1978), the assimilation of carbon in CAM plants is usually separated into four distinct phases (Cushman, 2001; Dodd *et al.*, 2002; Lüttge, 2006). In phase I, CO₂ fixation occurs during the night to produce malic acid which accumulates in the vacuole. Phase II occurs in early morning and represents the transition of the carboxylation of PEPc to Rubisco. During Phase III, the stomata close during the day and the malic acid is released from the vacuole, is decarboxylated and increases the cellular levels of CO₂, which is refixed by Rubisco. In late afternoon, phase IV begins and the activation of the PEPc initiates (Figure 2). The plasticity of *O. robusta* was evident by changes in the CAM phases. These changes regulated the transition between the wet and dry periods, and vice versa (Figure 3). For example, the CO₂ fixation phase was registered during the dry months only, the late afternoon (phase IV), as opposed to the early morning (phase II). The highest rates of assimilation CO₂ fixation in phase I in these months occurs predominantly after midnight, a period which coincided with fresh temperature and higher relative humidity conditions that favored water conservation by the plant (Pimienta–Barrios *et al.*, 2002). It has been found that CAM plants growing under extreme water–stress conditions demonstrate a metabolic physiotype known as CAM–idling, in which stomata remained closed even during the nighttime and the organic acid cycle is only maintained by re–fixation of CO₂ generated during mitochondrial respiration (Lüttge, 2006). Interestingly, CAM–idling was not observed in *O. robusta* plants, despite the fact that they were exposed to 7–year extreme drought conditions (Pimienta–Barrios *et al.*, 2002). Instead, *O. robusta* coped with drought by maintaining CAM metabolism with nighttime net CO₂ uptake, even during part of the day, although with low rates of CO₂ fixation coupled with morphological (succulence) and anatomical (thick cuticles, low stomatal frequency) adaptations that allow carbon uptake to proceed with a substantial reduction in water loss compared to daytime stomatal opening (Nobel, 1995). Thus, stomata were found to open at night and even during part of the day, although with low rates of CO₂ fixation.

When the study started, *O. robusta* plants had ceased to grow as observed by the reduction in fine root or “rain roots” and daughter cladodes formation. This response is typical in water–stressed C₃

and C_4 plants in dry environments (Smith *et al.*, 1997). We suggest that growth cessation in terms of reduced cladode emergence was a strategy to avoid water loss in stressed *O. robusta* plants. Young cladodes require water import from the underlying cladode organs (Wang *et al.*, 1997; Pimienta-Barrios *et al.*, 2005a), opening stomata during the daytime and thus leading to considerable water loss into the surrounding air (Pimienta-Barrios *et al.*, 2005a, b). The root system of *O. robusta* growing in rocky soil was only slightly affected by the absence of rainfall in the spring and in the early summer, as few new roots grew during the summer rainy season. Fine root formation reduction is also part of the water-stress avoidance strategy employed by these plants during the extremely dry soil conditions prevalent during the spring season (-40.0 MPa), which are considerably lower than those found in the root tissue (-0.8 MPa) and can therefore, like daughters, release water from the fine roots into a dry soil, and ultimately will drain water loss from mother cladodes, that occurs in stems and fruits of *O. robusta* in controlled conditions (-0.62 and -0.42 MPa, respectively; Nobel and de la Barrera, 2000). We conclude that the suppression of the formation of both new cladodes and fine roots is a strategy that avoids both dry air and dry soil (Pimienta-Barrios *et al.*, 2002).

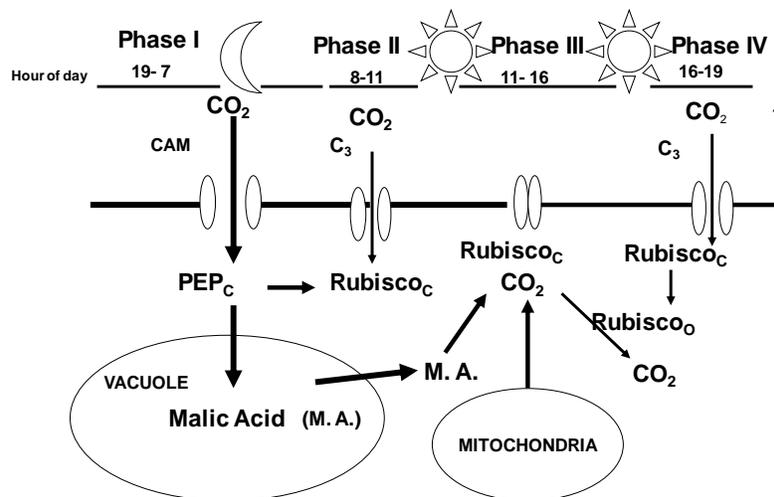


Figure 2. Diagram of CAM metabolism phases, showing the gas exchange processes related with times of stomatal aperture during the day and nighttime and occurrence of photosynthesis C_3 and CAM.

On the other hand, tolerance may be interpreted as the ability of the cells of both chlorenchyma and pith to lose up to 50% of their water during drought (Larcher, 1995). In this regard, approximately 80% of the water loss for cacti comes from the water storage parenchyma (Barcikowski and Nobel, 1984; Goldstein *et al.*, 1991). For *O. robusta*, RWC ranged from 66 to 68% during the year, indicating the prolonged multi-year drought (Pimienta Barrios *et al.*, 2002).

Opuntia robusta plants also presented tolerance response to water stress, considering that they maintained positive values of CO_2 assimilation even under conditions in which mother cladodes were found to lose up to 34% of their relative water content (RWC), particularly at the end of the dry season. Such behavior could be interpreted as the ability of the pith parenchyma to lose up to 50% of its water content during the dry period while the chlorenchyma remained turgid and photosynthetically active (Goldstein *et al.*, 1991). On the other hand, when the environmental conditions were favorable, the daily net CO_2 fixation rate increased three-fold in the driest month

(160 mmol m⁻² d⁻¹ in May, 2000) compared to the most humid (420 mmol m⁻² d⁻¹ in August, 2000) (Pimienta-Barrios *et al.*, 2002).

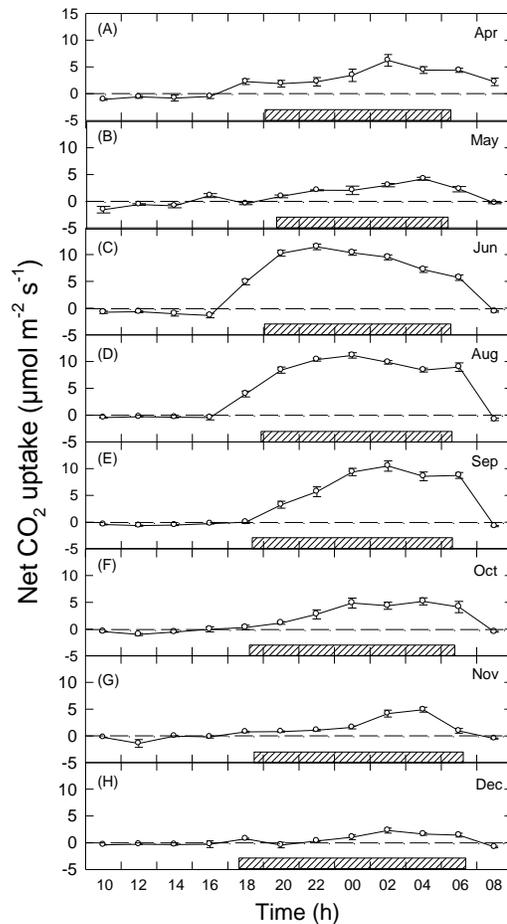


Figure 3. Net CO₂ uptake rates over 24 h periods for *O. robusta* on 13–14 April (A), 19–20 May (B), 28–29 July (C), 2–3 August (D), 5–6 September (E), 5–6 October (F), 1–2 November (G) and 13–14 December (H), 2000 at El Rayo, Pinos, Zacatecas. Data are means ± SE, except when are little of symbol (n= 6–15 plants) (From Pimienta-Barrios *et al.*, 2002; License number 2422051494654). Hatched bars indicate nighttime.

Response of *Opuntia robusta* to mycorrhizas

The presence of arbuscular mycorrhizal fungi (AMF) in the roots of *O. robusta* was determined in parallel with the gas-exchange measurements discussed above. We study the relevance of this symbiosis exploring the possibility that the uptake of water and nutrients in this rocky environment, characterized by shallow soils and low water availability, could be enhanced by their association with AMF (Smith and Read, 1997; Pimienta-Barrios *et al.*, 2003). The reduction in the number of AMF in the roots of *O. robusta* following treatment with the fungicide benlate® (+B) was directly related to the small number of fine roots that were formed in the summer of 2000. Conversely,

AMF colonization increased when the drought period ended in 2001, as evidenced by a higher number of AMF structures (hyphae, vesicles and arbuscules) detected during the summer of 2001 in plants without Benlate® (-B). In contrast, AMF structures decreased significantly in +B treatment, principally arbuscule abundance, observation which could be physiologically significant considering that these structures are believed to be where water, minerals and photosynthates are exchanged between the AMF and the plant (Orcutt and Nilsen, 2000).

The daily net CO₂ uptake in both treatments was found to increase progressively during the summer of 2001 and to subsequently decrease in the autumn (Figure 4). Also, night CO₂ uptake was higher during the months of July, August and October in plants subject to the -B treatment, although the difference was statistically different only in October.

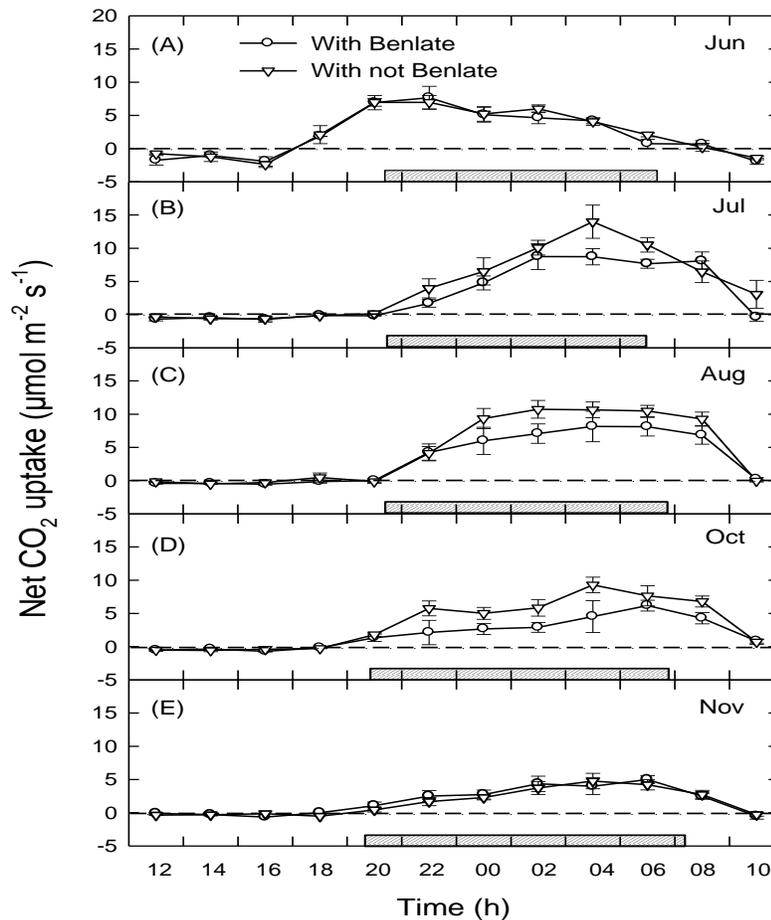


Figure 4. Net CO₂ uptake rates over 24 h periods for *O. robusta* plants with not Benlate® (∇) and with Benlate® (○) on 28–29 June (A), 19–20 July (B), 2–3 August (C), 18–19 October (D), and 29–30 November (E) of 2001 at El Rayo, Pinos, Zacatecas. Data are means ± SE, except when are little of symbol (n=5 plants) (Data not published). Hatched bars indicate nighttime.

Water content, as determined by the water potential (Ψ) and phosphorus (P) increased in cladodes of *O. robusta* in response to the rain in both treatments (Figure 5). However, no significant differences could be found between the -B and +B treatments. On the other hand, AMF inhibition

was found to affect C gain at the beginning of autumn 2001, but without modifying both Ψ and P content in plants +B; therefore, the increase in CO₂ uptake (sink strength) in –B treatment was not associated with their water or nutritional status. This suggests that the process of AMF colonization increased the sink strength, and consequently the demand for photosynthates in the roots of these plants, similarly to what has been described by Wright *et al.* (1998).

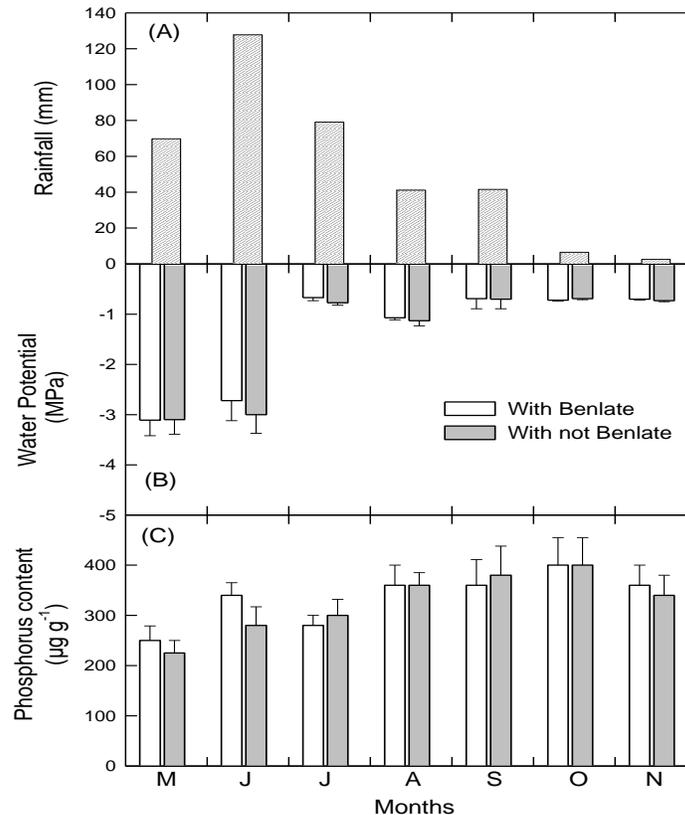


Figure 5. Monthly rainfall at El Rayo, Pinos, Zacatecas (A), and seasonal variation of water potential (B) phosphorus content (C) on cladodes of plants of *O. robusta* with not Benlate[®] and with Benlate[®]. Data are means \pm SE (n=5 plants). Not differences significantly between treatments by the test *t* ($p < 0.05$) (modified of Pimienta–Barrios *et al.*, 2003; Licenses numbers 2422081377660 and 2422080722680).

Relevance of daughter cladodes in the physiology of mother Cladodes of *Opuntia ficus-indica*

In previous sections we discuss that in contrast to cultivated *Opuntias*, wild *Opuntias* generally do not develop young cladodes in prevailing extreme drought conditions. Damage by drought observed in cultivated species of *Opuntia* occurs because they develop young cladodes during spring and therefore become more sensitive to drought (Pimienta–Barrios *et al.*, 2002, 2003). This observation lead us to conduct several experiments with *Opuntia ficus-indica*, one of the most important species cultivated in México and worldwide (Pimienta, 1994).

In the first experiment, we evaluated the physiological response of mother cladodes of *O. ficus-indica* to drought without daughter cladodes and emerging cladodes sprouted on mother cladodes selectively, leaving 1, 2, 4 and 8 daughter cladodes. Mother cladodes with non-daughter cladodes and with a variable number of daughter cladodes were exposed to a progressive drought stress during the spring. In the second experiment, the effect of a variable number of daughter cladodes in mother cladodes was compared in both drought-stressed and well-watered plants. In the third experiment the combined effect of a 45% reduction in irradiance, variable number of daughter cladodes and a spring drought stress on the physiology of mother cladodes, was evaluated.

The results from the first experiment demonstrated that daughter cladode growth rates increased at the end of the spring dry period. The effect of drought stress on the physiology of mother cladodes became more evident during the final stages of the dry season (30–31 May, 2003) in all treatments with attached daughter cladodes (Figure 6).

This effect was manifested by a reduction in the rates of net CO₂ uptake and chlorophyll content and was more pronounced in mother cladodes with eight daughter cladodes (Figure 6E), in which dehydration and yellowing were more obvious. The RWC in mother cladodes also decrease as the drought period progressed, with mother cladodes with four and eight daughter cladodes showing a severe reduction of the RWC (approximately 60%) at the end of the dry season (Pimienta-Barrios *et al.*, 2005a). However, in the daughter cladodes, the RWC values showed slight changes and were relatively high (81%); suggesting that water moved from the mother cladodes to the developing daughter cladodes, similarly to what has been observed in other plant species (Herrera *et al.*, 2000).

This response tended to trigger the condition of physiological drought in mother cladodes already affected by the soil drought conditions of the dry spring season. Conversely, mother cladodes without daughter cladodes had the highest rates of CO₂ assimilation, whereas the lowest rates of net CO₂ uptake during the night (phase I) occur in mother cladodes with the highest number of daughter cladodes, particularly in late May when the soil water content reached their slowest values (Pimienta-Barrios *et al.*, 2005a).

The second experiment showed that the daily net CO₂ uptake observed in mother cladodes growing in favorable conditions of irrigation did not increase carbon gain proportionally to the number of daughter cladodes. On the contrary, mother cladodes presenting the highest number of daughter cladodes showed reduced rates of daily net CO₂ uptake (Figure 7, Pimienta-Barrios *et al.*, 2005b), suggesting that an increase in sink strength (higher number of daughter cladodes) did not lead to a higher photosynthetic activity in source organs (mother cladodes), as occurs in C₃ and C₄ plants (Loomis and Connor, 1992).

The discontinuance of the drought conditions with the first rains of 2004 led to a marked increase in the rates of net CO₂ uptake in mother cladodes, particularly in both night (phase I) and day (phases II and IV) periods. This indicates that under favorable conditions carbon gain in *O. ficus-indica* can occur through the combination of CAM and C₃ photosynthetic pathways (Cushman, 2001; Pimienta-Barrios *et al.*, 2006).

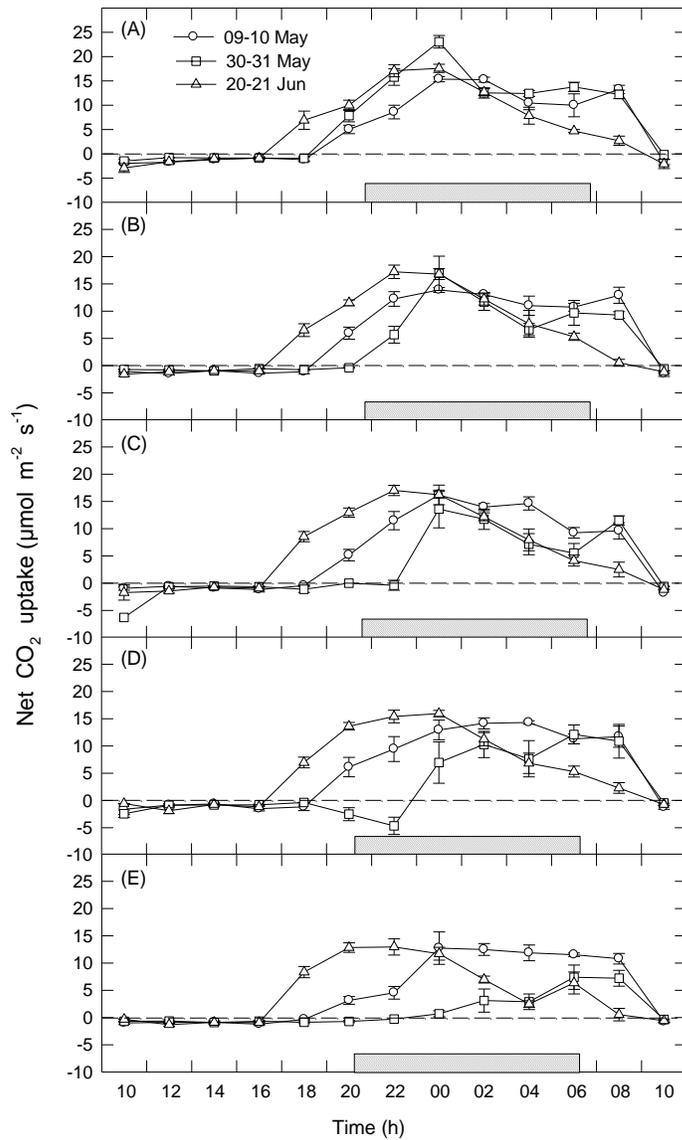


Figure 6. Net CO₂ uptake rates over 24-h periods for mother cladodes of *O. ficus-indica* with no (A), one (B), two (C), four (D), and eight (E) daughter cladodes during spring season of 2003. Data are means \pm SE (n=16 plants). Hatched bars indicate nighttime (From Pimienta-Barrios *et al.*, 2005a; License number 2422090516140).

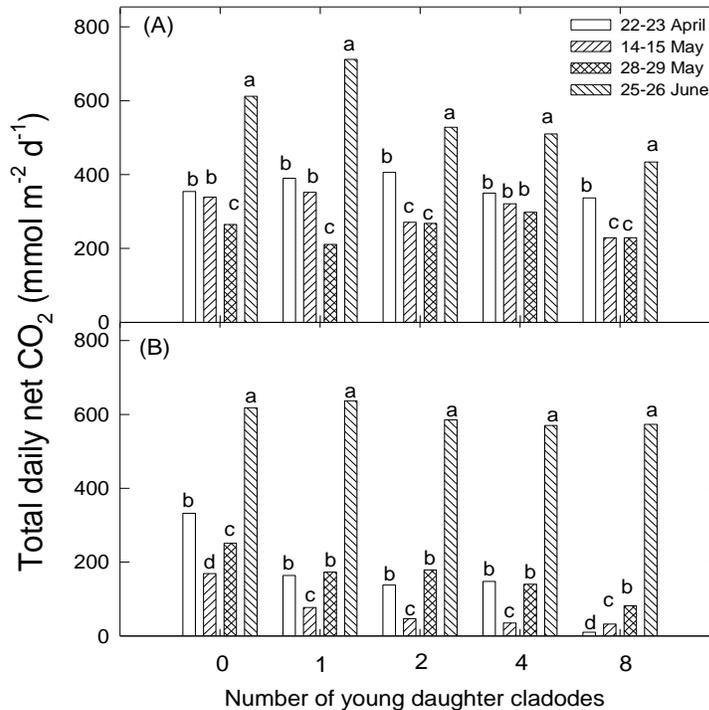


Figure 7. Daily net CO₂ for mother cladodes of *O. ficus-indica* with different number daughter cladodes in water conditions (A) and dry conditions (B). Data are means ± SE (n=6 plants). Different letters indicate statistical significantly (p<0.05) (Pimienta–Barrios *et al.*, 2005b).

The third experiment was designed to evaluate the effects on the reduction of light availability (approximately 45%; 22 mol m⁻² d⁻¹ PPF) on the physiology of mother cladodes with not daughter cladodes and with four or more daughter cladodes (Pimienta–Barrios *et al.*, 2007). Shading reduced the daily net CO₂ uptake in both dry and wet conditions, and was further reduced as the number of daughter cladodes increased. The effects of shading in plants exposed simultaneously to drought conditions are controversial. For example, Valladares and Pearcy (2002) argued that shade exacerbates the drought in the physiology of plants, while other studies have shown that shading mitigates drought stress effects on the plant physiology by reducing leaf and air temperature, water–vapor pressure deficit and photo–inhibition (Quero *et al.*, 2006). In *O. ficus-indica* (Pimienta–Barrios *et al.*, 2007), the stressful effects caused by the combination of shading, lower water content in the soil and the presence of daughter cladodes led to a significant reduction in photosynthetic activity, similarly to the effects observed in *Heteromeles arbutifolia* by Valladares and Pearcy (2002). Therefore, there are two mechanisms through which drought stress negatively affects photosynthesis in mother cladodes with daughter cladodes: firstly, by a reduction in stomatal conductance that limits the diffusion of CO₂ from air to the mesophyll (Cornic, 2000) and secondly, by the metabolic inhibition of the photophosphorylation process, which eventually leads to an inadequate supply of ATP (Lawlor, 2002). The latter effect is of critical importance in CAM plants, since this photosynthetic pathway is energetically more costly than that in C₃ plants (Lüttge, 2004).

The thickness of parenchyma tissue, where most of the water accumulated in the cladodes is stored, was notably reduced as the drought conditions progressed, especially in mother cladodes exposed to full sunlight and with daughter cladodes (Figures 8C–D). However, the chlorenchyma thicknesses

in mother cladodes present only minor changes during the wet and dry periods (Figures 8A–B), suggesting that the water transport proceeds from the core parenchyma to the chlorenchyma (Goldstein *et al.*, 1991). The lateral movement of water from the parenchyma tissue during the dry period will sustain stomatal opening keeping the photosynthetic tissue active at least during the initial period of drought (Barcikowski and Nobel 1984). These traits become significant for *Opuntias* and other succulent plants (Rabas and Martin, 2003) and highlight the ecological and agronomical success of cacti such as *Opuntia* in semi-arid environments. Both stem succulence and nocturnal carbon assimilation are considered adaptive characteristics that cacti such as *Opuntias* tolerate prolonged drought by extending carbon gain during periods of low soil water availability, while stem succulence acts as an important buffer to maintain turgescence in the photosynthetic tissue (Nilsen *et al.*, 1989, 1990; Nobel, 1995; Pimienta-Barrios *et al.*, 2000).

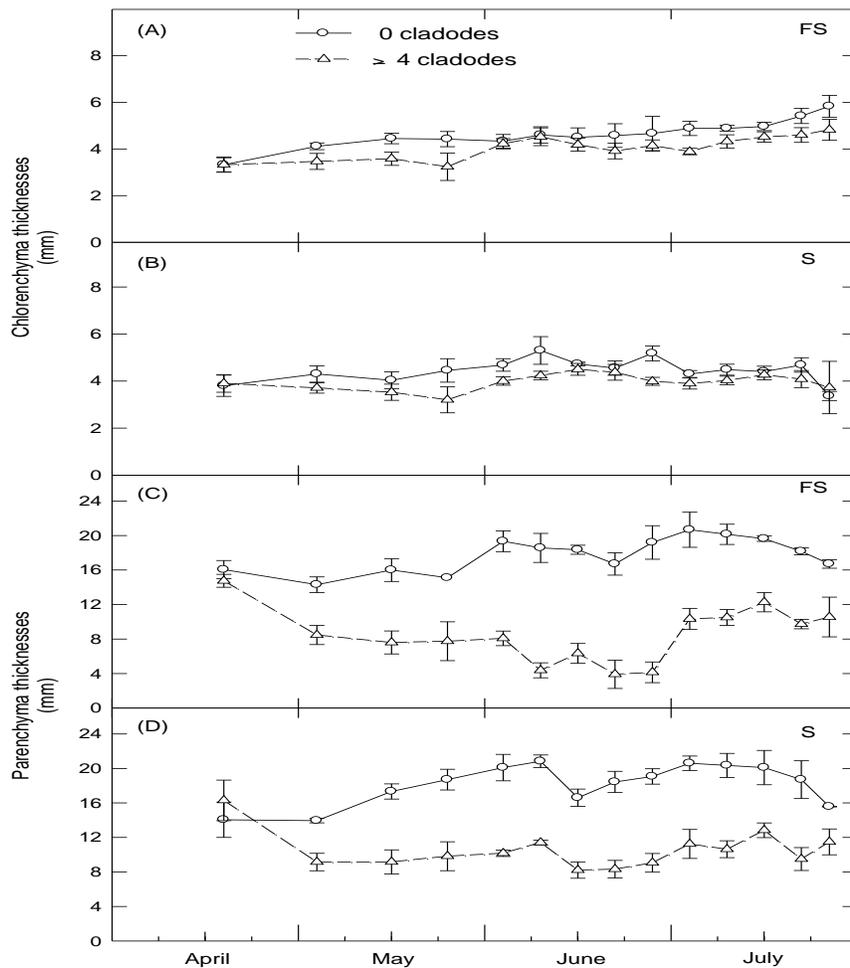


Figure 8. Chlorenchyma (A, B) and parenchyma (C, D) thicknesses in full sunlight (FS) and shaded by 45% (Sh) for mother cladodes without (\circ) and with (Δ) daughter cladodes. Data are means \pm SE (n=20 plants) (From Pimienta-Barrios *et al.*, 2007, License number 2427180778259).

Soil water availability is frequently the most important factor affecting photosynthetic activity and the manifestation of the different phases in CAM plants (Dodd *et al.*, 2002; Keeley and Rundel, 2003). Our results suggest that water stress *per se* is not the only factor leading to a reduced photosynthetic activity in mother plants of *O. ficus-indica*. Ontogenetic factors as the presence of daughter cladodes in combination with low light intensity can significantly affect the physiology of *O. ficus-indica*. 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, which became more pronounced as the number of daughter cladodes increased causing the highest rates of net CO₂ uptake occurring late at night, when temperatures were low and relative humidity high.

This pattern reflects an avoidance strategy to maintain carbon gain with reduced water loss and shows 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). In contrast with *O. robusta* cultivated *O. ficus-indica* develop daughter cladodes in the spring dry periods, which reduce their capacity to cope with drought stress, making them more sensitive to biotic and abiotic stressors than wild *Opuntias* (Pimienta, 1990; Pimienta-Barrios *et al.*, 2005a).

Conclusions

The combined effects of photosynthetic plasticity and the association with AMF and drought avoidance mechanisms, such as the suppression of root and cladode formation, allowed the maintenance of positive values of carbon gain in wild population of *O. robusta*, growing under the extreme conditions inherent to a shallow soil cover in a predominantly rocky environment suffering from an intense long-term drought. The plastic response of *O. ficus indica* and *O. robusta* plants during part of the dry period manifested through the expression of the CAM phases was facilitated by the succulence of photosynthetic organs that allowed extending CO₂ assimilation in the dry period. Finally, cultivated *Opuntias* such as *O. ficus-indica* appear to be more sensitive to the negative effects of drought than wild *Opuntia* species. Possibly, this has occurred as a consequence of the domestication process, which has promoted the development of those traits associated with increased yields in economically important tissues such as the fruit, in detriment of characteristics related to resistance to environmental stress. Both photosynthetic plasticity and succulence will help to safeguard the response of *Opuntias* to global warming, particularly in dry environments.

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