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Ecophysiology of a wild platyopuntia exposed to prolonged drought

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Abstract

The effects of reduced annual rainfall over a 7-year period on the daily patterns of gas exchange were analyzed for a wild platyopuntia (*Opuntia robusta*) growing in rocky soil. In spite of the prolonged water stress, stomata opened at night and net carbon gain was observed for eight 1-day sampling periods during 2000. Daily net CO₂ uptake increased more than five-fold from the end of the dry spring season to the middle of the summer wet season. Curtailment in the formation of both new cladodes and new roots is considered to be an avoidance strategy to water scarcity, which in combination with net CO₂ uptake responses to temperature and relative humidity at night, and perhaps association with arbuscular-mycorrhizal fungi, were the most important adaptations for *O. robusta* to the prolonged drought conditions that prevailed in its rocky environment. Such adaptations of *O. robusta* are in addition to the classical physiological and structural modifications of CAM plants to tolerate prolonged drought. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: CO₂ uptake; Drought; *Opuntia robusta*; Rocky soil

1. Introduction

During a 7-year period (1994 to 2000), the temperate semiarid lands of central Mexico, which normally receive about 500 mm annually,

have experienced an unusually prolonged dry period with annual rainfalls below 400 mm (Fig. 1). In the last two of the 7 years, the rainfall amount averaged only 200 mm, resembling conditions for arid environments (Fischer and Turner, 1978). Such prolonged drought has caused severe damage to cultivated C₃ and C₄ crops but not to cultivated and wild *Opuntia* populations, even when they are growing in rocky environments. Rocky environments exacerbate the effects of drought, because soil is scarce. Moreover, the soils dry relatively rapidly after rainfall, leading to

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water stress even during the rainy season. It was hypothesized that the lack of damage to *Opuntia robusta* during the prolonged drought reflected continuing carbon gain at night during the dry season. Such carbon gain could be supported by stem succulence and may be sustained by a symbiosis with mycorrhizal fungi that improves the ability of roots to forage water in rocky soils during the summer wet season, thereby replenishing water to the succulent stems.

CAM plants are commonly considered as drought avoiding, because they store considerable water in their shoots as well as fix CO₂ at night, thereby reducing transpiration as air temperatures, and hence the force for water loss, are lower at night than during the daytime (Nobel, 1994, 1995). When some CAM plants are severely water stressed, they can shift from CAM to CAM-idling, and the stomata remain closed during both day and night (Szarek and Ting, 1974; Ting and Rayder, 1982). CAM-idling is the extreme of a continuum between the use of CO₂ entirely derived from respiration and having nearly all fixed from the air (Lambers et al., 1998). Studies of adaptations to drought under field conditions are scarce in the native habitats of opuntias, such as the semiarid highlands of central Mexico. To explain the success that *O. robusta* displays in facing prolonged drought conditions, it was fur-

ther hypothesized that it has unique ecophysiological responses to a multi-year period of reduced rainfall.

2. Materials and methods

This research was conducted from April to December 2000 at a rocky community in the southwestern corner of the Chihuahuan Desert on highlands of the semiarid region of north central Mexico (Medellín-Leal, 1982). The study site near El Rayo, Zacatecas, at 21°58' N latitude, 101°35' W longitude, and 2190 m above sea level is located in a plateau physiographic region known as the Llanos de Ojuelos (INEGI, 1981). The annual rainfall is generally about 500 mm and occurs primarily in the summer; the average annual temperature is 16–18 °C (Pimienta-Barrios, 1994). Annual rainfall data were obtained from an official weather station maintained by the Instituto Nacional de Investigaciones Forestales y Agropecuarias near Ojuelos, Jalisco (21°52' N, 101°37' W, 2100 m above sea level).

Opuntia robusta Wendl. var. *robusta* (nopal tapon) is a perennial shrubby cactus 0.5–1.0 m tall, with a poorly defined trunk made up of thick, orbicular, brownish–green cladodes, and is widely distributed on the semiarid lands of central Mexico (Bravo-Hollis 1978; Pimienta, 1993). It has a minimally branched root system that is restricted to rock crevices, where a shallow soil layer occurs. At the study site, *O. robusta* is the dominant perennial species. It grows isolated or in association with other species, resulting a patchy distribution of the vegetation, interspersed with rock outcrops.

Vegetation at the study site is of the crassicauleous brushwood type (Rzedowski, 1978) and is composed mainly of fleshy succulent plants; platyopuntias, barrel cacti, agaves, and a *Yucca* species are the most conspicuous elements. The shrub layer is dominated by *O. robusta* and *Opuntia leucotricha*, mixed with *Opuntia imbricata*, *Opuntia joconostle*, and *Senecio praecox*. A subshrub stratum is dominated by *Baccharis* sp., *Jatropha dioica*, and some species of Asclepiadaceae and Loganiaceae. In the herbaceous layer, species of

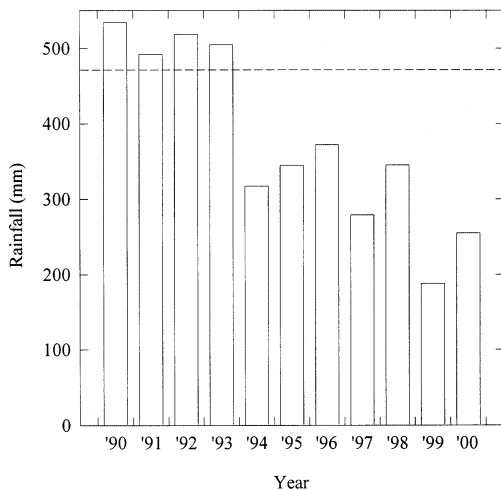


Fig. 1. Annual rainfall at Llanos de Ojuelos, Zacatecas, Mexico. Dashed line indicates the historical average.

Graminae, Liliaceae and Compositae are dominant. A resurrection plant (*Sellaginella lepidophylla*) forms compact masses surrounding the trunks of the opuntias, thereby contributing nutrients and organic materials to the understory soils. The soil is very shallow, brown, and of the haplic planosol type, with a sandy loam texture, a pH of 7.3, 3.3% organic matter on a dry mass basis, 240 $\mu\text{g g}^{-1}$ carbonate-extractable phosphorus, 0.16% total nitrogen, and 980 $\mu\text{g g}^{-1}$ potassium (Pimienta-Barrios, 1990).

Net CO_2 uptake was measured every 2 h over 24-h periods on 13–14 April, 19–20 May, 28–29 June, 2–3 August, 5–6 September, 5–6 October, 1–2 November, and 13–14 December 2000 on 6–15 plants with a LI-6200 portable photosynthesis system (Li-Cor, Lincoln, NE). A 0.25-L leaf chamber was modified by replacing the distal half-cylinder with a narrowed opening ($2 \times 4 \text{ cm}^2$) lined with a closed-pore foam gasket that was firmly pressed against an approximately south-west-facing surface of the cladodes. Photosynthetic photon flux (PPF, wavelengths of 400–700 nm) on a horizontal plane was recorded hourly from sunrise to sunset with a Li-Cor LI-250 quantum sensor and then integrated to get the total daily PPF.

Soil water content was determined for eight soil samples removed from the center of the root zone (a depth of 10 cm) on the dates for gas exchange. Soils were dried at 105 °C to constant mass (generally within 72 h); data are expressed as a percentage water content: (fresh mass – dry mass)/fresh mass \times 100. Field soil capacity was estimated using the method of Palmer and Troeh (1995). Air temperature was recorded every hour with a mercury thermometer. The relative humidity was recorded every 2 h with a digital humidity gauge. The cladode water content and relative water content (RWC) were determined for 15 segments ($3 \times 3 \text{ cm}^2$) that were immediately weighed to determine their fresh mass and then oven-dried at 80 °C until no further mass change occurred (Beadle et al., 1993). The fully turgid mass was obtained using stem segments 2 cm in diameter and 4 mm thick equilibrated with water-saturated filter paper at 30 °C for 3 h.

Thirty mature terminal cladodes were collected to measure the volume/surface area ratio. From the same cladodes, tissue samples were removed and fixed in FAA (formalin: acetic acid: ethanol: water, 10:5:50:35). Stomatal frequency was obtained using epidermal plus hypodermal peels examined at $40 \times$ using bright field optics with a Zeiss Sinoptic microscope (Oberkochen, Germany). To evaluate cuticle thickness, hand-cut transverse sections were stained in 0.5% (w/w) aqueous toluidine blue and viewed at $40 \times$.

Fine rain-induced roots were collected from July to September 2000, fixed in FAA, and cut into 1.5 cm segments. The segments were washed, cleared in 10% (w/w) KOH, and stained with trypan blue (Phillips and Hayman, 1970). Stained segments were mounted on slides, and the percentages of root length containing hyphae, vesicles, and arbuscules was assessed following the magnified intersection method (McGonigle et al., 1990) using the Zeiss Sinoptic microscope.

Data were subjected to ANOVA and where significant differences existed, means were separated by a least significant difference (LSD) test. Correlation analysis was used to test for relations between nocturnal CO_2 uptake and air relative humidity, air temperature, soil water content, stem water content, and stem relative water content. Data are presented as mean \pm SE (n = number of measurements).

3. Results

Annual rainfall data from 1990 to 2000 revealed that the study site had experienced a prolonged drought beginning in 1994 (Fig. 1). 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. The first rainfall in 2000 thus occurred in May, a month that received 29% of the annual total; soil water content rose from 1.7% in May to 11.1% in June, a value that was just less than the field capacity of 13% (Table 1). In response to the soil water content, the cladode water content was also lowest in May, increasing during the summer (Table 1). The values of cladode RWC were lower

Table 1

Mean soil and cladode water contents, cladode relative water content, total daily photosynthetic photon flux (PPF), temperatures, and relative humidity over 24-h periods for *Opuntia robusta* near El Rayo, Zacatecas, México

Date	Soil water content (%)	Cladode water content (%)	Cladode relative water content (%)	Total daily PPF (mol m ⁻² per day)	Day/night air temperature (°C)	Day/night relative humidity (%)
13–14 April 2000	1.95c	80.4c	66.9c	54	23/12	32/45
19–20 May 2000	1.68c	77.4c	66.1d	48	25/19	36/66
28–29 June 2000	11.07a	84.5b	68.0b	65	21/14	48/74
2–3 August 2000	5.28b	88.0a	68.9a	58	21/15	34/68
5–6 September 2000	3.52bc	86.2ab	68.5ab	62	22/15	33/59
5–6 October 2000	11.98a	86.1b	68.4b	38	23/14	45/97
1–2 November 2000	3.26bc	85.4b	68.2b	38	21/15	36/48
13–14 December 2000	3.32bc	85.8b	68.4b	35	19/10	44/44

Data are means for soil water content ($n = 8$ samples), cladode water content ($n = 15$ plants), and cladode relative water content ($n = 15$ plants). Values within a column that are followed by different letters are significantly different at $P < 0.05$ by LSD's multiple test.

than the values of cladode water content, varying from 66.1 to 68.9, and showed less variation than the cladode water content among measurement dates (Table 1).

Total daily PPF, obtained by averaging hourly instantaneous values, was highest in the summer (June–September) and lowest in the winter, ranging from 35 to 65 mol m⁻² per day (Table 1). The average day/night air temperatures had small variations among the measurement dates for gas exchange, with the lowest in December (19/10 °C) and the highest in May (25/19 °C; Table 1). The average relative humidity of the ambient air during the daytime also had relatively small variations for the eight measurement dates and was high during the night, especially after rainfall events (e.g. 5–6 October; Table 1).

Plants in the *O. robusta* population under study did not form new cladodes during spring 2000, in contrast to the usual condition for most *Opuntia* species at the study site. New cladode formation seemed to have been arrested since 1997, because the peripheral cladodes of the approximately 200 plants examined on a 1 ha region appeared to be older than 3 years based primarily on scars from new organs; also, new flowers and fruits were scarce. In particular, cladodes older than 3 years typically have at least five flower or fruit scars (cladode scars are less common), whereas 1-year-old cladodes do not have such scars and 2-year-old cladodes have one or two scars. In addition, cladodes older than 3 years are over 2.5 cm thick with 4–5 brownish spines per areole, whereas 1-year-old cladodes are less than 1.2 cm thick with two translucent spines per areole. The root system was poorly developed, with individual roots being highly suberized and restricted to rock crevices. Only a few, fine, rain-induced roots were formed during the summer rainy season. Such new roots were colonized by vesicular arbuscular mycorrhizal (VAM) fungi, and the plants are apparently obligately mycorrhizal. Root colonization by VAM fungi increased slightly from July to August and then decreased in September 2000. Hyphae, vesicles, and arbuscules were common in July, but in August and September only hyphae and vesicles were observed (Table 2). Anatomical observations on the experimental plants revealed a low

Table 2

Percentage of root length colonized by arbuscular mycorrhizal fungi hyphae, arbuscules, and vesicles for *O. robusta* during the summer

Date	Hyphae (%)	Arbuscules (%)	Vesicles (%)
8 July 2000	48.9 ± 8.9	4.9 ± 3.3	5.8 ± 5.2
3 August 2000	52.6 ± 6.6	0.00	1.2 ± 0.6
6 September 2000	42.0 ± 9.3	0.00	1.6 ± 0.9

Data are means ± standard error ($n = 9–15$ plants).

stomatal frequency ($40 ± 3$ stomata mm⁻²) and a thick cuticle ($7.1 ± 0.4$ μm); the volume/surface area ratio was $0.89 ± 0.03$ cm ($n = 30$) for the terminal cladodes of *O. robusta*.

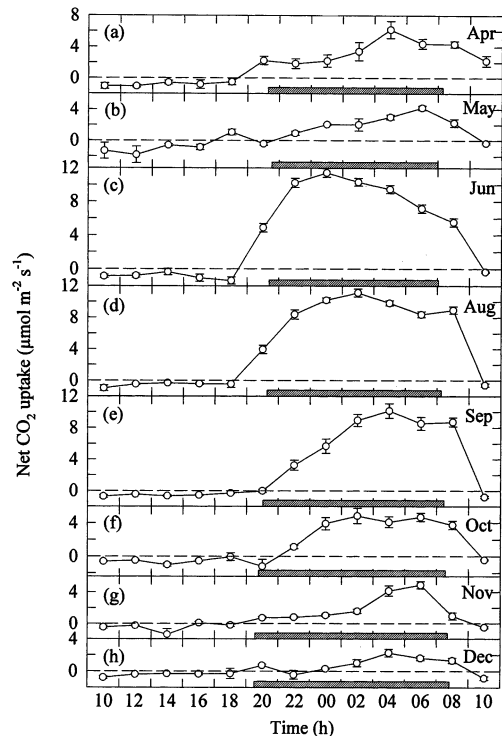


Fig. 2. Net CO₂ uptake rates over 24-h periods for *Opuntia robusta* on: (a) 13–14 April 2000; (b) 19–20 May 2000; (c) 28–29 June 2000; (d) 2–3 August 2000; (e) 5–6 September 2000; (f) 5–6 October 2000; (g) 1–2 November 2000; and (h) 13–14 December 2000 near El Rayo, Zacatecas, Mexico. Data are means ± standard error ($n = 6–15$ plants).

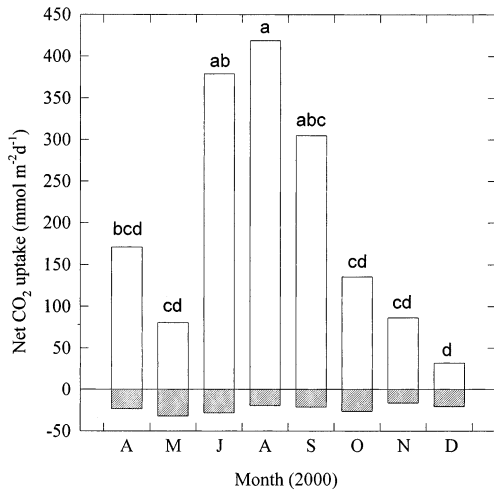


Fig. 3. Daily positive (open bars) values of net CO₂ uptake and negative (hatched bars) for *O. robusta* obtained from data in Fig. 2. Different letters above a bar indicate significant difference at $P < 0.05$ by LSD's multiple test.

Most net CO₂ assimilation by *O. robusta* occurred at night (Fig. 2). Except for June, the highest instantaneous rates occurred after midnight. In April, June, and August, late afternoon net CO₂ uptake was observed; early morning assimilation was observed for all months. The rates of instantaneous net CO₂ assimilation were highest in June and August, decreasing later in the summer and autumn (Fig. 2). Total daily net CO₂ uptake, obtained by integrating the instantaneous rates over 24 h, was 171 mmol m⁻² per day in April (Fig. 3). It decreased in May, increased in June, and was maximal in August, steadily decreasing from September to October to November and December. From August to December, the daily net CO₂ uptake decreased 13-fold, as the soil became drier. Averaged over the 8 months of measurement, total daily net CO₂ uptake was 203 mmol m⁻² per day for *O. robusta*, corresponding to an annual CO₂ uptake of 74 mol m⁻². Daily net CO₂ efflux, which occurred during the daytime for the 24-h periods of measurement, was relatively low for all the measurement dates (Fig. 3).

Correlation analysis between the instantaneous rates of net CO₂ uptake for *O. robusta* with environmental variables revealed a significant pos-

itive relationship between the rate of instantaneous CO₂ uptake and the relative humidity during the nighttime ($r = 0.52$, $P < 0.001$; Fig. 4a). Although considerable scatter occurred for the data, maximum net CO₂ uptake occurred for air temperatures near 12–13 °C ($r^2 = 0.13$; Fig. 4b). Significant correlation occurred between the total daily net CO₂ uptake and soil water content ($P = 0.04$) and total daily PPF ($P < 0.01$) but not stem water content ($P = 0.3$) or stem relative water content ($P < 0.3$).

4. Discussion

The 7-year drought at the study site did not induce CAM-idling in *O. robusta* during the dry months. Instead, *O. robusta* coped with drought by maintaining CAM metabolism with nighttime net CO₂ uptake, 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 with daytime stomatal opening (Nobel, 1995). These important physiological and morphological adaptations enable CAM

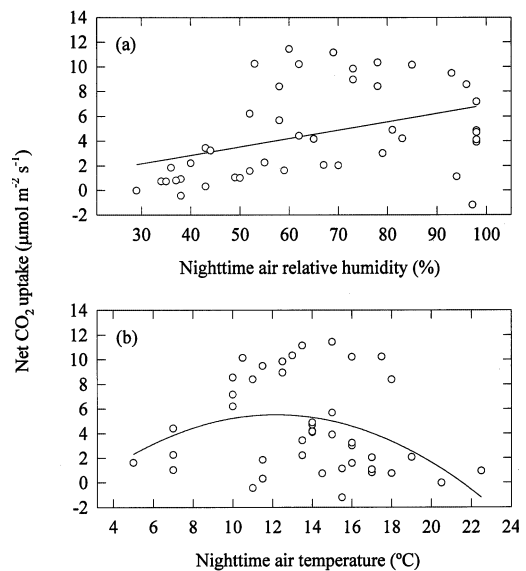


Fig. 4. Relationships between nocturnal net CO₂ uptake rates and (a) air relative humidity and (b) air temperature for *O. robusta* near El Rayo, Zacatecas, Mexico.

plants to be successful in dry environments (Nobel, 1994, 1995; Cushman and Bohnert, 1997).

In addition to the classical morphological and physiological adaptations, *O. robusta* modulated its response to drought through non-classical morphological and physiological adjustments. The cessation of the formation of new cladodes during the dry period was an important avoidance strategy to prevent water loss from the existing plant. Young cladodes require water import from the underlying cladodes (Nobel et al., 1994; Wang et al., 1997) and also behave like C_3 photosynthetic organs, opening stomata during the daytime and thus leading to considerable water loss to the surrounding air (Osmond, 1978; Acevedo et al., 1983; Altesor et al., 1992). Fine roots of most cacti are induced by rain (Gibson and Nobel, 1986), and most cacti respond quickly to soil wetting after drought (Szarek et al., 1973; Nobel et al., 1994). However, the root system of *O. robusta* growing in rocky soil was only slightly affected by spring and summer rains, as few new roots grew during the summer rainy season. The suppression of the formation of both new cladodes and fine roots is a strategy that avoids both dry air and dry soil, resembling in part the classical example of drought avoidance (Salisbury and Ross, 1992).

Tolerance might 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 considerable water loss in response to the prolonged multi-year drought. Also, the extremely low number of fine roots formed during the summer season is consistent with the small increase in cladode water content from May to August.

Avoidance and tolerance of drought for *O. robusta* are accompanied by a physiological plasticity in response to favorable environmental conditions that allowed net carbon gain in both dry and wet seasons. For instance, total daily net CO_2 uptake increased more than five-fold from May to August, when summer rains start, and the highest

instantaneous rates of net CO_2 uptake by *O. robusta* during the driest months occurred toward the end of the night, coinciding with the highest air relative humidity and lowest stem temperatures, conditions leading to low transpiration and relatively high water-use efficiency, both of which are consistent with the water conserving attributes of CAM plants (Nobel, 1988, 1995). Similar daily patterns of net CO_2 uptake occur for *O. ficus-indica* whose cladodes remain unrooted 8 weeks after detachment (Raveh and Nobel, 1999), for *Agave deserti* subject to 33 days of drought (Nobel, 1990), and for *Hylocereus undatus* subject to 24 days of drought (Raveh et al., 1995). For these three species, the daily net CO_2 uptake also decreases significantly with increasing drought and the maximal instantaneous rates of net CO_2 uptake occur after midnight.

Vesicular arbuscular mycorrhizal fungi are associated with the roots of *O. robusta*, as occurs for cacti in arid environments (Bethlenfalvay et al., 1984; Arceta-González et al., 1999), and may have enhanced its water uptake. By improving the water uptake of the host plant, such fungi can improve the plant performance in environments where water availability is the main environmental factor limiting productivity (Allen, 1991; Titus and Del Moral, 1998). Also, a small root system under severe stress conditions allows a plant to save energy reserves, and perhaps it is less costly to support the mycorrhizal fungi than to develop a higher root biomass (Pimienta-Barrios and Nobel, 1998). However, little is known about the relationship between mycorrhizal colonization and photosynthetic patterns in the field (Gupta, 1991) and data on the association of mycorrhizae with CAM plants are scarce (Arceta-González et al. 1999; Rincon et al., 1993; Smith and Read, 1997). In any case, *O. robusta* sustained a level of succulence that allowed the plants to take up CO_2 at night while maintaining a low investment of carbon through the curtailment in the formation of new roots and new cladodes during prolonged periods of drought.

The shedding of new cladodes by *O. robusta*, which is apparently uncommon among platyopuntias, is a relevant feature in the population under study. Also, the unusual cladode shedding

may contribute to the deposition of organic matter underneath the canopy of *O. robusta* patches, giving rise to ‘islands of fertility’ (García-Moya and McKell, 1970; Cross and Schlesinger, 1999), because the organic matter content was over 3% for those patches, in contrast with open spaces in which the organic matter is less than 1.5% (Pimienta-Barrios, 1990). *Opuntia robusta* can act as a ‘nurse plant’, facilitating the establishment of vegetation under its canopy, as occurs in other communities in arid and semiarid environments (Moro et al., 1997). The vegetation associated with *O. robusta* in the study site occurs in low-cover patches, as is typical of the vegetation in arid ecosystems (Aguiar and Sala, 1999).

Low temperatures at night are commonly considered as one of the main environmental factors that favor net CO₂ uptake and transpiration by CAM plants (Hanscom and Ting, 1978; Israel and Nobel, 1995; Pimienta-Barrios et al., 2000). Net CO₂ uptake at night for *O. robusta* was maximal near 12–13 °C, similar to results for other non-epiphytic cacti (Nobel, 1988). For *O. robusta*, higher rates of net CO₂ uptake were correlated with higher nighttime relative humidity, which could lead to greater stomatal opening without excessive water loss. Similar response of nocturnal net CO₂ fixation to high ambient relative humidity occurs for *Cissus quadrangularis* subjected to water shortage, even when temperatures are maintained constant (Virzo De Santo and Bartoli, 1996).

Low net CO₂ efflux rates were observed during the daytime for *O. robusta* during both dry and wet months. Such low rates may reflect the moderate day/night temperatures at the relatively high elevation semiarid environment of the study site. In hotter subtropical environments, high day/night temperatures increase net CO₂ efflux rates and reduce total daily net assimilation for *O. ficus-indica* and *S. queretaroensis* (Pimienta-Barrios et al., 2000).

For *O. robusta* at the study site, annual drought ended in June, as indicated by the increase in cladode water content by 7% from May to June. After the summer, such drought started for *O. robusta* in October, as indicated by the decrease in its cladode water content, the reduction in the

maximal rate of CO₂ uptake, and the shift in the time of maximal net CO₂ uptake to later in the night. At this time of the year, the plant can no longer obtain water from the soil and hence must rely on its own reserves of stored water to maintain the hydration and activity of the chlorenchyma (Nobel, 1995). As for other CAM plants, stem succulence may be considered as a crucial adaptation that has allowed *O. robusta* to obtain carbon even in a stressful environment, such as rocky soils exposed to prolonged drought.

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