Synergistic Effects of Elevated CO₂ and Fertilization on Net CO₂ Uptake and Growth of the CAM Plant *Hylocereus undatus*

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ABSTRACT. This study examined the response of the crassulacean acid metabolism (CAM) vine-cactus fruit crop species *Hylocereus undatus* to two CO₂ regimes [enrichment (1000 $\mu\text{mol}\cdot\text{mol}^{-1}$) vs. ambient control (380 $\mu\text{mol}\cdot\text{mol}^{-1}$)] and to two fertilization regimes [0.5- vs. 0.1-strength Hoagland's solution (designated high and low, respectively)]. CO₂ enrichment increased total daily net CO₂ uptake, nocturnal acid accumulation, shoot elongation, and total dry mass by 39%, 24%, 14%, and 6% (averaging the two fertilization regimes) versus ambient CO₂ treatment, respectively. Plants exposed to high fertilization demonstrated 36%, 21%, 198%, and 79% (averaging the two CO₂ regimes) increases versus those receiving the low fertilization regime in total daily net CO₂ uptake, nocturnal acid accumulation, stem elongation, and total dry mass, respectively. Plants exposed to high fertilization and elevated CO₂ demonstrated 108%, 77%, 264%, and 111% increases versus those receiving the low fertilization regime at the ambient CO₂ concentration in total daily net CO₂ uptake, nocturnal acid accumulation, stem elongation, and total dry mass, respectively. This response was 25% to 71% higher than the summed effects of the separate responses to each factor, indicating a synergistic effect of elevated CO₂ and high fertilization. Thus, it is apparent that *H. undatus* crops grown under a high-fertilization agromanagement regime may benefit from elevated CO₂ to a greater extent than those grown with low fertilization.

Most studies on the physiological responses of plants to increasing CO₂ concentrations have focused on C₃ and C₄ photosynthetic pathway plants. It has been found that CO₂ enrichment enhances net CO₂ uptake and growth of C₃ plants by 30% to 40% (Kimball, 1983), whereas in C₄ photosynthetic pathway plants, the effect is less marked, being about 10% (Newton, 1991). Studies on the response of CAM plants to elevated CO₂ are far more limited than those on C₃ and C₄ plants (Poorter and Navas, 2003). Among the CAM species that have been studied, the findings are not uniform: some reports found no significant change in CO₂ uptake and growth in response to elevated CO₂ levels (Holtum et al., 1983; Szarek et al., 1987), whereas other studies reported an increase in these parameters (Drennan and Nobel, 2000; Raveh et al., 1995).

In C₃ and C₄ plants, a sustained positive response to CO₂ enrichment may be attenuated by environmental conditions such as irradiance [including ultraviolet (UV)-B], temperature, ambient ozone concentrations, and the availability of water and nutrients. These factors, alone or in combination, could lead to acclimation during long-term exposure to elevated CO₂ concentrations (Poorter, 1993). In particular, it has been found that high nutrient concentrations usually amplify the response of plants to CO₂ enrichment (Poorter and Perez-Soba, 2001; Reich et al., 2006). Most of the studies on the response of CAM plants to elevated CO₂ were conducted under conditions of low fertilization [0.1- to 0.3-strength Hoagland's solution (Drennan and Nobel, 2000)], but there have been no systematic studies of

the effect of high-fertilization regimes on the response of CAM plants to CO₂ enrichment.

A small number of studies have investigated the response to CO₂ of the CAM species *H. undatus* (commonly known as pitahaya or dragon fruit), a vine cactus that is indigenous to the tropical and subtropical regions of Central America and that is grown as a crop in a number of countries, including Mexico, some countries of Southeast Asia, and currently in Israel (Mizrahi et al., 1997). Raveh et al. (1995) showed that CO₂ enrichment of *H. undatus* resulted in a 34% increase in total daily net CO₂ uptake, which enabled the plants to overcome various abiotic stresses. Nobel and De la Barrera (2002) reported stimulation of CO₂ uptake by *H. undatus* grown at high N concentrations and ambient CO₂; maximal nocturnal net CO₂ uptake rates were 2.5 and 9.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 0.16 and 16 mM N, respectively.

The specific objective of this study was to test the hypothesis that increased levels of fertilization amplifies the effect of CO₂ enrichment on net CO₂ uptake and growth in *H. undatus*.

Materials and Methods

PLANT MATERIAL. Rooted shoot cuttings of *H. undatus* were used in this study. Shoots of uniform size (60 $\pm$ 2 cm in length) were planted (positioned at random) in 10-L pots, placed 60 cm apart, and filled with volcanic gravel (Tuff Merom Golan, Merom Golan, Israel). These conditions were chosen to ensure that rooting volume did not limit growth (Mizrahi et al., 2007).

EXPERIMENTAL DESIGN. The experiments were conducted in Beer-Sheva, northern Negev Desert, Israel (lat. 31°15'N, long.

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34°48'E, 315 m above sea level). Plants ($n = 10$ for each treatment) were grown for 1 year, from Aug. 2006 to Aug. 2007, under conditions of ambient CO_2 [380 ± 10 (SE) $\mu\text{mol}\cdot\text{mol}^{-1}$] or elevated CO_2 [1000 ± 70 (SE) $\mu\text{mol}\cdot\text{mol}^{-1}$]. The plants were grown in a cooled greenhouse in two vented chambers (each 220 cm high, 120 cm wide, and 1000 cm long), one for each CO_2 concentration. Each chamber contained plants receiving two different fertilization regimes, as described below. The locations of the pots and the chambers were changed three times during the experiment to reduce the effect of location. For the chamber with the enriched CO_2 atmosphere, pure CO_2 (cylinders of compressed CO_2 ; Maxima, Beer-Sheva, Israel) was supplied through a flow meter that controlled the flow rate at about $0.8 \text{ L}\cdot\text{min}^{-1}$. Ambient air entered each chamber through a port in the lower panel of the chamber wall opposite to the wall with the vent. The chamber air was changed three times per hour, with the air being vented via a duct to the outside by means of a centrifugal fan. The CO_2 concentration inside each chamber was monitored at 30-min intervals with an automatic four-channel monitoring system (IRGA PTM-48M; PhyTech, Rehovot, Israel). A detailed description of the system is given in Weiss et al. (2009) and references therein.

Plants were fertigated twice a week via a drip irrigation system ($8 \text{ L}\cdot\text{h}^{-1}$; one dripper per plant) with 2 L of 0.5-strength Hoagland's solution [high ($120 \text{ mg}\cdot\text{L}^{-1} \text{ N}$, $50 \text{ mg}\cdot\text{L}^{-1} \text{ P}$, and $90 \text{ mg}\cdot\text{L}^{-1} \text{ K}$)] or 0.1-strength Hoagland's solution [low ($24 \text{ mg}\cdot\text{L}^{-1} \text{ N}$, $10 \text{ mg}\cdot\text{L}^{-1} \text{ P}$, and $18 \text{ mg}\cdot\text{L}^{-1} \text{ K}$)]. The N:P:K ratio was the same for high and low nutrient treatments to control for negative physiological responses caused by interaction of the nutrient elements (Zhang et al., 2006). The high nutrient treatment was the same as that giving maximum stimulation of CO_2 uptake observed by Nobel and De la Barrera (2002) under ambient CO_2 conditions. It is also the nutrient treatment recommended in commercial fields. The low values were the same as those used in most of the studies previously performed on CAM plants (Drennan and Nobel, 2000).

Air temperatures at the midplant height in the chambers were monitored at 30-min intervals with a data logger (MicroLog™ EC650; Fourier Systems, New Albany, IN). Average values for monthly maximum and minimum air temperatures were similar for the two chambers and were therefore combined in the graphs shown in Fig. 1A. The monthly maximum/minimum air temperature averages for the coldest season (December–February) were $19/7^\circ\text{C}$, and those for the hottest season (May–September) were $32/19^\circ\text{C}$ (Fig. 1A). The experiment was conducted under natural photoperiod. The instantaneous photosynthetic photon flux (PPF) inside the chambers was measured at wavelengths of 400 to 700 nm with a TIR-4 quantum sensor (PhyTech) connected to an IRGA PTM-48M system. Monthly average values for total daily PPF were similar for the two chambers and were therefore combined in the graphs shown in Fig. 1B. The monthly average of total daily PPF for the coldest season was $13 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, and the average for the hottest season was $20 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (Fig. 1B), the latter value being the optimal total daily net CO_2 uptake for *H. undatus* (Raveh et al., 1995).

GAS EXCHANGE MEASUREMENTS. Gas exchange was measured, starting from day 212 of the experiment, on third-order shoots ($n = \text{eight}$ for each treatment) with an IRGA PTM-48M monitoring system. Measurements were taken over a period of 4 d (15–18 Mar. 2007) and were recorded throughout the day and night at 30-min intervals. To measure net gas exchange of

Fig. 1. Monthly averages of (A) maximum [T_{max} (○)], mean [T_{mean} (Δ)], and minimum [T_{min} (□)] daily air temperature and (B) total daily instantaneous PPF (◇) in the growth chambers situated in a cooled greenhouse during the period 15 Aug. 2006 to 15 Aug. 2007. Values are means $\pm$ SE.

the shoot, the original chamber of the IRGA PTM-48M, which is suitable for measuring flat leaves, was replaced with a chamber enclosing 20 cm^2 ($2 \times 10 \text{ cm}$) of shoot surface area. The chamber, which was made of 0.2-cm-thick polyethylene, had a volume of 4 cm^3 ; the airflow through the chamber was $11.6 \text{ cm}^3\cdot\text{s}^{-1}$. The chamber was attached directly to the shoot with two pieces of cellophane tape. The air, which entered the chamber through two identical openings, one on each side of the chamber, was pumped through the center of the chamber via a 0.4-cm-diameter tube to the IRGA PTM-48M. This set-up created a constant turbulent flow that minimized leaf boundary resistance and permitted a restricted amount of air to be drawn into the chamber. A detailed description of the chamber and the gas exchange calculation is given in Weiss et al. (2009).

NOCTURNAL ACIDITY ASSAY. Nocturnal acid accumulation (ΔH^+), a measure that reflects nighttime CO_2 uptake in CAM plants (Osmond, 1978), was determined 90 d ($n = \text{five}$ for each treatment) and 150 d (data not shown) after the beginning of the experiment. Shoot tissue was sampled with a cork borer (0.9 cm in diameter) at dusk [1800 HR (H^+_{dusk})] and at dawn [0530 HR

(H^+_{dawn})]. All tissue samples were frozen immediately in liquid N_2 and stored at -20°C for subsequent analysis. For acid analysis, the shoot tissue was homogenized with 10 mL of double-distilled water with an ice-cold pestle and mortar. The homogenate was titrated against 0.01 N NaOH to pH 7.4. ΔH^+ was then estimated as $\Delta H^+ = H^+_{\text{dawn}} - H^+_{\text{dusk}}$ (Nobel and Israel, 1994).

SHOOT NUTRIENT ASSAY. Third-order shoots ($n = \text{eight}$ for each treatment) were selected at random at the end of the experiment. Samples were washed with doubly distilled water and were then oven dried at 70°C to constant mass (48–72 h). The dry mass was ground in a grinder to a fine powder, which was used for further analyses. Samples were acid digested (with concentrated H_2SO_4) and analyzed for reduced nitrogen content by the Kjeldahl method (Bradstreet, 1965). Phosphorus and potassium concentrations were assayed by coupled plasma atomic emission spectrometry (ICP-AES Optima 3000; Perkin Elmer, Norwalk, CT).

MEASUREMENTS OF GROWTH AND BIOMASS. Total shoot lengths were measured with a measuring tape on 17 Sept. 2006, 27 Nov. 2006, 3 Feb. 2007, and 15 Aug. 2007. At the end of the experiment, the plants were harvested, and shoots and roots were separated. Roots were washed gently to remove soil and were blotted ($n = 10$ for each treatment). To determine root dry mass, roots were oven dried at 70°C to constant mass (48–72 h). Shoot dry mass was determined by multiplying total shoot length by the average percentage of dry mass to length ratio of six shoot segments (each 30 cm long) sampled from each of the measured plants.

Table 1. Probability values of analysis of variance and Tukey's honestly significant difference test for the effects of CO_2 , nutrient and their interactions on total daily net CO_2 uptake, maximal net CO_2 uptake rate, nocturnal acid accumulation, shoot nutrient concentration (N, P, and K), total shoot length, shoot dry mass, root dry mass, total dry mass, and root/shoot dry mass ratio of *Hylocereus undatus* plants grown at elevated and ambient CO_2 (1000 and $380\ \mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively).

Source of variance	CO_2	Nutrient	$CO_2 \times \text{nutrient}$
	Probability		
Total daily net CO_2 uptake	0.0006	0.0139	0.0467
Maximal net CO_2 uptake	0.0092	0.0215	0.1201
Nocturnal acid accumulation	0.0024	0.0082	0.0112
Shoot N concentration	0.0031	0.0004	0.0389
Shoot P concentration	0.0076	0.0003	0.0293
Shoot K concentration	0.7955	0.1284	0.2399
Total shoot length in			
Sept. 2006	0.1370	<0.0001	0.3354
Total shoot length in			
Nov. 2006	0.0197	<0.0001	0.5819
Total shoot length in			
Feb. 2007	0.0004	<0.0001	0.0446
Total shoot length on			
Aug. 2007	<0.0001	<0.0001	0.0109
Shoot dry mass	0.0198	<0.0001	0.0401
Root dry mass	0.0091	0.0919	0.0495
Total dry mass	0.0147	<0.0001	0.0385
Root/shoot dry mass ratio	0.2058	0.0679	0.4358

STATISTICAL ANALYSIS. Statistical analyses were performed with *JMP IN 5.0*[®] software (SAS Institute, Cary, NC), using analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test, with significance set at $P < 0.05$. Statistical analyses of the effect of CO_2 and fertilization and their interaction are summarized in Table 1.

Results

NET CO_2 UPTAKE. For *H. undatus*, CO_2 uptake occurred mostly at night (Fig. 2). At the high nutrient concentration, net CO_2 uptake rate peaked earlier in the night under the elevated CO_2 concentration than under the ambient concentration (2200 vs. 0100 HR). For the low nutrient treatment, maximal CO_2 uptake rate was recorded earlier in the night (at 2000 HR), regardless of the CO_2 treatment. However, for these plants, CO_2 uptake rate toward the end of the night (0330–0800 HR) was higher in plants exposed to the ambient CO_2 concentration than in those grown with CO_2 enrichment (Fig. 2). High nutrient treatment significantly increased the total daily net CO_2 uptake by 36% and 49% under the ambient and elevated CO_2 treatments, respectively (Fig. 3A, Table 1). CO_2 enrichment

Fig. 2. Daily course of rates of net CO_2 uptake in *Hylocereus undatus* plants grown for 212 d at elevated CO_2 [$1000\ \mu\text{mol}\cdot\text{mol}^{-1}$ (●)] and ambient CO_2 [$380\ \mu\text{mol}\cdot\text{mol}^{-1}$ (○)] under high (A) and low (B) nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Measurements were taken every 30 min over a period of 4 d (15–18 Mar. 2007). Average day/night air temperature was $25/13^\circ\text{C}$, and total daily instantaneous PPF was $18\ \text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$. The shaded area at the bottom of the figure represents the nighttime hours. Values are means $\pm$ SE ($n = \text{eight}$ plants).

Fig. 3. Total daily net CO₂ uptake (A) and maximal net CO₂ uptake rate (B) in *Hylocereus undatus* plants grown for 212 d at elevated and ambient CO₂ (1000 and 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Values are means $\pm$ SE (n = eight plants). Total daily net CO₂ uptake data were obtained by integrating the instantaneous rates in Fig. 2. Different letters represent significant differences between treatments at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test).

significantly increased the total daily net CO₂ uptake by 39% and 52% under the low and high nutrient treatments, respectively. Under CO₂ enrichment, the maximal values of net CO₂ uptake rates were 5.2 and 10.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at low and high nutrient treatments, respectively (Fig. 3B). Under the ambient CO₂ concentration, maximal net CO₂ uptake rate was 3.0 and 4.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at low and high nutrient treatments, respectively. The elevated CO₂/high nutrient combination raised the total daily net CO₂ uptake by 108% in comparison with the ambient CO₂/low nutrient combination (Fig. 3A, Table 1). There was a significant synergistic effect (CO₂ $\times$ nutrient interaction; Table 1) of the elevated CO₂/high nutrient combination on total daily net CO₂ uptake. There was no significant effect of the elevated CO₂/high nutrient combination on maximal net CO₂ uptake rate (Fig. 3B, Table 1).

NOCTURNAL ACID ACCUMULATION. High nutrient treatment increased the total nocturnal acid accumulation by 21% and 42% under the ambient and the elevated CO₂ treatments, respectively (Fig. 4). CO₂ enrichment increased the nocturnal acid accumulation by 24% and 46% under the low and high nutrient treatments, respectively. The elevated CO₂/high nutrient combination raised nocturnal acid accumulation by 77% in comparison with the ambient CO₂/low nutrient combination (Fig. 4, Table 1). After 90 d, there was a significant synergistic effect of the elevated CO₂/high nutrient combination on nocturnal acid accumulation (Fig. 4, Table 1). Similar results were obtained for the 150-d measurement (data not shown).

SHOOT NUTRIENT CONCENTRATION. High nutrient treatment significantly increased shoot N concentration in comparison with low nutrient treatment (by 171% for the ambient CO₂ group and by 105% for the elevated CO₂ treatment). At the low nutrient concentration, elevated CO₂ did not have any significant effect on shoot N concentration, whereas at the high nutrient concentration, elevated CO₂ caused a significant reduction of as much as 41% in shoot N concentration (Fig. 5A). The elevated CO₂ treatment in combination with the high nutrient treatment significantly increased shoot P concentration by 50% to 102% in comparison with plants grown under ambient and low nutrient conditions (Fig. 5B). Thus, the nature of the elevated CO₂/high nutrient combination had a significant effect on shoot N and P concentration. There was, however, no

significant effect of either factor, alone or in combination, on shoot K concentration (Fig. 5C, Table 1).

SHOOT LENGTH. Total shoot length was significantly increased by high nutrient treatment at ambient and elevated CO₂ concentrations for all the sampling dates (Fig. 6). At the end of the experiment, total shoot length under the high nutrient treatment had increased by 198% at ambient CO₂ and by 218% at elevated CO₂ (Fig. 6, Table 1) in comparison with the values for low nutrient treatment. Total shoot length was significantly increased (22%) by elevated CO₂ only under the high nutrient regime (e.g., in February and Aug. 2007). The elevated CO₂/high nutrient combination increased total shoot length by 224% and 264% in Feb. 2007 and Aug. 2007, respectively, in comparison with the ambient CO₂/low nutrient combination (Fig. 6, Table 1). These

effects were found to be synergistic (Table 1). During January/February, the response of shoot elongation to elevated CO₂ was about 3-fold higher for the high nutrient treatment than for low nutrient treatment [increases of 49% vs. 18%, respectively (Fig. 6, Table 1)].

BIOMASS. High nutrient treatment promoted significant increases in total dry mass and shoot dry mass under ambient and elevated CO₂ treatments (Fig. 7, A and C). The effect of nutrient treatment on dry mass was more marked than the effect of CO₂ treatment. For example, at elevated CO₂, the total dry mass increased by 197% at high nutrient versus low nutrient concentrations. However, only under the high nutrient treatment did elevated CO₂ significantly increase total dry mass (18% at elevated CO₂ vs. ambient CO₂) and shoot dry mass (Fig. 7, A and C; Table 1).

Root dry mass was significantly increased (by 56%) under elevated CO₂ compared with ambient CO₂, but only for the high nutrient treatment (Fig. 7B, Table 1). There was no significant

Fig. 4. Effect of elevated CO₂ on the nocturnal acid accumulation of *Hylocereus undatus* plants grown for 90 d at elevated and ambient CO₂ (1000 and 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Values are means $\pm$ SE (n = five plants). Different letters represent significant differences between treatments at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test).

Fig. 5. Mean shoot N (A), P (B), and K (C) concentrations (% of dry mass) of *Hylocereus undatus* plants grown for 1 year at elevated and ambient CO₂ (1000 and 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Values are means $\pm$ SE (n = five plants). Different letters represent significant differences between treatments at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test).

effect of elevated CO₂ or high nutrient treatment on root/shoot dry mass ratios (Fig. 7D, Table 1). The elevated CO₂/high nutrient combination caused shoot dry mass, root dry mass, and total dry mass to increase by 110%, 100%, and 111%, respectively, in comparison with the ambient CO₂/low nutrient combination (Fig. 7, Table 1). These effects were characterized by a synergistic pattern (Table 1).

Discussion

The findings described above support the hypothesis that the response of *H. undatus* to CO₂ enrichment increases as nutrient availability increases. The results of this study on a CAM species are consistent with those of earlier studies on C₃ and C₄ plants (Coleman et al., 1991; Ghannoum et al., 2000; Poorter and Perez-Soba, 2001): A comparison of the summed relative effect of CO₂ and nutrient on each of the measured parameters

Fig. 6. Effect of elevated CO₂ on total shoot length of *Hylocereus undatus* plants grown for 1 year at elevated CO₂ [1000 $\mu\text{mol}\cdot\text{mol}^{-1}$ (solid symbols)] and ambient CO₂ [380 $\mu\text{mol}\cdot\text{mol}^{-1}$ (open symbols)] CO₂ and under high [0.5-strength Hoagland's solution (circles)] and low [0.1-strength Hoagland's solution (triangles)] nutrient concentrations. Values are means $\pm$ SE (n = 10 plants). Different letters represent significant differences between treatments within a specific sampling date at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test).

(predicted values, calculated from Figs. 3, 4, 6, and 7, A–C) with their observed relative effect showed a synergistic effect of CO₂ and nutrient in most of the measured parameters (Fig. 8). For example, the increases in shoot dry mass in response to elevated CO₂ alone (elevated CO₂/low nutrient vs. ambient CO₂/low nutrient) and to high nutrient alone (ambient CO₂/low nutrient vs. ambient CO₂/high nutrient) were 6% and 79%, respectively, leading to a predicted sum effect of 85%. The measured effect of both treatments (elevated CO₂/high nutrient vs. ambient CO₂/low nutrient) demonstrated an increase of 110%. The difference between the measured and predicted values (25% higher than the predicted sum effect) reflect the percentages of synergism.

Similar results were found previously for C₃ plants (Zhang et al., 2006). The mechanism behind those interactions could be attributed to the fact that elevated CO₂ is expected to enhance photosynthesis, which in turn increases growth, resulting in increased nutrient demand. Therefore, increased nutrient availability can support synergistic increases in plant growth and biomass production at elevated CO₂, as was previously demonstrated (Poorter and Perez-Soba, 2001; Reich et al., 2006). In our study, the nutrient effect on shoot growth was more than 10 times higher than the effect of elevated CO₂, as was found previously for C₃ and C₄ plants (Coleman et al., 1991; Newman et al., 2006). In contrast, the nutrient effect on root growth (Fig. 7B) was significant only under elevated CO₂, as has also been reported for C₃ and C₄ plants (Carswell et al., 2000; Kim et al.,

Fig. 7. Effect of elevated CO₂ on the shoot dry mass (A), root dry mass (B), total dry mass (C) and root/shoot dry mass ratio (D) of *Hylocereus undatus* plants grown for 1 year at elevated and ambient CO₂ (1000 and 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Values are means $\pm$ SE (n = 10 plants). Different letters represent significant differences between treatments at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test).

2001). Increase in root/shoot dry mass ratio in response to elevated CO₂ was previously found in C₃ plants (Carswell et al., 2000), and a similar ($P > 0.05$) trend was also demonstrated in the current study (Fig. 7D, Table 1), probably due to high within-treatment variation. The magnitude of the increase in biomass due to elevated CO₂ for *H. undatus* was in the same range as that found in nine species of CAM plants by Poorter and Navas (2003). These findings demonstrate a CO₂-unsaturated photosynthetic process in CAM plants exposed to ambient CO₂.

For the low nutrient treatment groups, the lack or attenuation of the stimulation of biomass production and total shoot length in response to elevated CO₂, as observed here (Figs. 6 and 7) and in previous studies on C₃ and C₄ plants, was originally explained by a downward acclimation of photosynthesis to elevated CO₂, which is known to be more marked under nutrient deficiency (Reich et al., 2006). Yet this downward acclimation of photosynthesis was not evident in our net CO₂ uptake and nocturnal acid accumulation measurements (Figs. 2, 3, and 4). Therefore, the "downward acclimation" hypothesis was not applicable to the findings of the present study. Thus, it is necessary to obtain multiple measurements over time to assess possible acclimation. In other studies, the lack of response to elevated CO₂ was explained by the limited level of sink strength in the low nutrient treatment, as was also found by Mandre et al. (1995).

Under high nutrient conditions, shoot N, P, and K levels were similar to those of CAM plants in a commercial orchard (Nobel, 1988). Values for shoot N, P, and K concentrations for the at low nutrient/ambient CO₂ combination (Fig. 5) were

similar to the shoot N, P, and K levels of other CAM plants growing in their natural habitats (Nobel and Pimienta-Barrios, 1995). The values for shoot N concentration for the low nutrient treatment and ambient CO₂ were half of those of Nobel and De la Barrera (2002) for *H. undatus* fertilized with 24 $\text{mg}\cdot\text{L}^{-1}$ N, but our study was characterized by a chronically longer nutrient-deficient period (52 vs. 22 weeks). The findings in our study that elevated CO₂ significantly decreased shoot N concentration (Fig. 5A) under conditions of high nutrient supply are in keeping with those for C₃ and C₄ plants grown under a wide range of nitrogen concentrations (Coleman et al., 1991; Hocking and Meyer, 1985; Larigauderie and Oechel, 1988). Our findings may be explained by a dilution of N concentration as a result of the increase in biomass (Ma et al., 2007). In contrast to this N reduction, our study revealed an elevation of shoot P (Fig. 5B) for elevated CO₂/high nutrient conditions, as was previously found for C₃ plants (Ma et al., 2007). The lack of response of shoot K concentrations to the different treatments (Fig. 5C) may indicate that K availability remained the same for all treatments, and this indicates that the amount of K in the low nutrient treatment was adequate for the needs of the vplants.

In general, total daily net CO₂ uptake was significantly increased by elevated CO₂ and high nutrient concentrations, as was found previously for *H. undatus* by Raveh et al. (1995) and Nobel and De la Barrera (2002), respectively. During the second half of the night, there was a reduction in the stimulation of CO₂ uptake rates by elevated CO₂, but only at low nutrient concentrations (Fig. 2B). Such a pattern was also demonstrated in other CAM species growing under low nutrient conditions and reflects the earlier cessation of acid accumulation (Nobel and Hartsock, 1986). The response to elevated CO₂ measured in terms of net CO₂ uptake and nocturnal acid accumulation was higher than that measured as an increase in total biomass. However, it should be remembered that the biomass measurement reflects the accumulative response, while net CO₂ uptake and nocturnal acid accumulation reflect the effect of CO₂ enrichment during a particular measured period.

In contradiction to the assumption that the predominant initial fixation of CO₂ by phosphoenolpyruvate carboxylase (PEPCase) in CAM plants is saturated under ambient atmospheric CO₂ (Long et al., 2006), our results indicated that most of the stimulation of CO₂ uptake by elevated CO₂ occurred during the nighttime [phase I: (Osmond, 1978); Fig. 2, A and B] when the uptake of atmospheric CO₂ involves the binding of CO₂ by PEPCase. Our findings, together with the recently published similar results of Nogues et al. (2008), indicate the presence of a factor restricting CO₂ uptake during phase I under ambient conditions.

In conclusion, highly fertilized CAM crops may benefit from elevated atmospheric CO₂ to a greater extent than CAM plants grown under low fertilization regimes due to the synergistic effect of high nutrition and elevated CO₂. These results pave the

Fig. 8. Observed and predicted relative effects of the interaction between CO₂ and nutrient treatments on total daily net CO₂ uptake (A), maximal net CO₂ uptake rate (B), nocturnal acid accumulation (C), total shoot length (D, E, F, and G measured in Sept. 2006, Nov. 2006, Feb. 2007, and Aug. 2007, respectively), total dry mass (H), root dry mass (I), and shoot dry mass (J) of *Hylocereus undatus* plants grown for 1 year at elevated and ambient CO₂ (1000 and 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively) under high and low nutrient concentrations (0.5- and 0.1-strength Hoagland's solution, respectively). Predicted values are the summed effects of the separate responses to each factor (CO₂ and nutrient treatments). Points above the 1:1 line demonstrate a positive synergistic effect of CO₂ and nutrient treatments. Asterisks represent significant interaction between treatments at $P < 0.05$ (analysis of variance and Tukey's honestly significant difference test; Table 1).

way for testing the effect of CO₂ enrichment and high fertilization regimes for optimal fruit production. Our findings may be of particular importance in subtropical areas such as Israel, where pitahayas are grown under netting to prevent photoinhibition damage (Raveh et al., 1996; 1998). In this context, it should be noted that stretching a plastic film over the supporting structure during the night to enable enrichment with CO₂ should involve only a minimum extra cost, while making a substantial contribution to increasing fruit yield.

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