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Net CO_2 uptake rates for *Hylocereus undatus* and *Selenicereus megalanthus* under field conditions: Drought influence and a novel method for analyzing temperature dependence

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Abstract

Net CO_2 uptake rates (P_N) were measured for the vine cacti *Hylocereus undatus* and *Selenicereus megalanthu*s under relatively extreme climatic conditions in Israel. Withholding water decreased rates and the daily amount of CO_2 uptake by about 10 % per day. Compared with more moderate climates within environmental chambers, the higher temperatures and lower relative humidity in the field led to a more rapid response to drought. The upper envelopes of scatter diagrams for P_N *versus* temperature for these Crassulacean acid metabolism species, which indicate the maximal rates at a particular temperature, were determined for both night time CO_2 uptake in Phase I (mediated by phospho*enol*pyruvate carboxylase, PEPC) and early morning uptake in Phase II (mediated by ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO). As stem temperature increased above 13 °C, the maximal P_N increased exponentially, reaching maxima near 27 °C of 12 and 8 μmol m^{-2} s^{-1} for Phases I and II, respectively, for *H. undatus* and 6 and 4 μmol m^{-2} s^{-1}, respectively, for *S. megalanthus*. Based on the Arrhenius equation, the apparent activation energies of PEPC and RuBPCO were 103 and 86 kJ mol^{-1}, respectively, for *H. undatus* and 77 and 49 kJ mol^{-1}, respectively, for *S. megalanthus*, within the range determined for a diverse group of species using different methodologies. Above 28 °C, P_N decreased an average of 58 % per °C in Phase I and 30 % per °C in Phase II for the two species; such steep declines with temperature indicate that irrigation then may lead to only small enhancements in net CO_2 uptake ability.

Additional key words: activation energy; CAM plants; cacti; optimal temperature; phospho*enol*pyruvate carboxylase; ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

The vine cacti *Hylocereus undatus*, *H. polyrhizus*, and *Selenicereus megalanthus* are cultivated in 20 countries for their fruits, which are known as 'pitahayas' (Mizrahi and Nerd 1999, Nobel and De la Barrera 2004). The plants have rapid growth, precocious yielding, and utilize Crassulacean acid metabolism (CAM), but little is known about their rates of net CO_2 uptake (P_N) under field conditions. Physiological studies have indicated that vine cacti require shading for maximal P_N and high productivities (Raveh *et al*. 1995, 1997, 1998). Under wet, shaded conditions, these plants can have a net daily P_N of 300 mmol m^{-2} d^{-1} (Nobel and De la Barrera 2004), which is in the lower part of the range for the prickly pear cactus *Opuntia ficus-indica* and certain other highly productive CAM species (Nobel 1988, 1994, Winter and Holtum 2002). Growth chamber experiments indicate that *H. undatus* can maintain substantial daily CO_2 uptake for 2 weeks after withholding water (Raveh *et al*. 1995, Nobel and De la Barrera 2002a, 2004). However, little is known about P_N under current cultivation conditions.

Based largely on studies with conventional C_3 and C_4 crops, vine cacti in Israel and California are irrigated the year round (except during rainy periods), but how rapidly P_N decreases during drought has not been determined in

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the field. Thus one objective of the present study was to determine the effect of water stress under extreme climate on P_N for *H. undatus*. Another objective was to continuously monitor P_N for *H. undatus* and *S. megalanthus* to measure the rates in the various phases of CAM (Osmond 1978, Nobel 1988) and to determine their temperature dependence under field conditions.

A technique based on the upper envelopes of the data was used to determine the maximal P_N at various temperatures, even when other factors, such as water stress or irradiance, would concomitantly limit net CO_2 uptake. Based on this technique, the apparent activation energies were determined for positive net CO_2 uptake at night in Phase I (mediated by phospho*enol*pyruvate carboxylase, PEPC; Osmond 1978) and in the early morning in Phase II (mediated by ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO). Above the optimal temperatures, the upper envelopes were used to characterize the inhibition of P_N by increasing temperature (Yurista 1999, Bernacchi *et al*. 2002). Enzyme activation energies are usually estimated using an Arrhenius plot (Nobel 2005) when temperature is the only independent variable under controlled laboratory conditions (Cornish-Bowden 1995, Gutfreund 1995). Laboratory results may not always correctly predict enzyme activity under natural conditions when multiple environmental variables are changing simultaneously. Indeed, optimal temperatures for PEPC and RuBPCO activities are rarely determined under field conditions. The uniqueness of the data presented here are that (*1*) they were obtained under field conditions, and (*2*) they were collected for two CAM species for which little is known concerning the activity of the enzymes involved.

Materials and methods

Experiments on mature stems of the vine cacti *Hylocereus undatus* (Haworth) Britton & Rose and *Selenicereus megalanthus* (K. Schumann *ex* Vaupel) Moran (Cactaceae) were conducted in Beer Sheva, Israel, from May through August 2004. The plants were spaced at 1.5 m intervals along rows that were 2.5 m apart using a 1.7-m-high trellis for plant support. Water was applied by a drip system for 2 h every 3 d with two drippers (2 000 cm^{-3} h^{-1} each) placed 20 cm from each side of a plant to keep the soil close to the plants constantly wet in the rooting zone (mean depth of 20 cm). Water was withheld for up to 10 d to study the effect of drought on P_N. The average daily temperature was 33 ºC (maximum 50 ºC, minimum 15 ºC). The average relative humidity was 51 % (maximum 89 %, minimum 13 %). The plants were shaded 70 % by polyethylene netting. The orchards, which were established from approximately 8-month-old, 55-cm-long cuttings planted vertically in the ground on 10 March 2002, were characterized by intensive vegetative growth; measurements were made on the third stem segment from the ground.

P_N by the stems was measured with a *PM48* portable photosynthesis system (*PhyTech*, Rehovot, Israel). The automated system has a chamber that is closed for 2 min and then open for 28 min, when undisturbed gas exchange occurs between the stem and the atmosphere. Accessories included a *TIR-4* sensor for photosynthetic photon flux (PPF; 400–700 nm), *ATH-2* temperature and humidity sensors (all manufactured by *PhyTech*), and two 1.0-mm-diameter, copper-constantan thermocouples inserted 1.5 mm into a stem. The acrylic chamber surrounded a 15-cm section at mid-stem, enclosing 0.014 m^2 of stem surface area; sponge material adjacent to the stem allowed a controlled amount of ambient air to be drawn into the chamber.

The impact of temperature on P_N was analyzed for nocturnal Phase I of CAM mediated by PEPC and early morning Phase II mediated by RuBPCO (Osmond 1978). A scatter diagram of all the positive uptake rates for each phase was determined. The upper envelope, when factors other than temperature (such as irradiance, relative humidity, soil water content, and chemical substrate amounts) would not be limiting net CO_2 uptake, was then fitted for the rising portion of the curve using an exponential based on the Arrhenius equation (Nobel 2005):

$$\text{Rate} = B\, e^{-A/RT} \quad (1)$$

where B is a constant, A is the apparent activation energy [kJ mol^{-1}], R is the gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature [K]. Using an Arrhenius plot [$\ln P_N$ *vs*. $1/T$], A, which represents the minimum energy for the reaction, was estimated for both species.

Above the optimum temperature, P_N values declined with increasing temperature, presumably representing inactivation (or deactivation; Bernacchi *et al*. 2002, Sharkey 2005) of the catalytic properties of the enzymes involved. The decline in rate with increasing temperature was assumed to be proportional to the rate:

$$\text{d(Rate)}\, T^{-1} = -C \times \text{Rate} \quad (2)$$

where C represents the relative fraction of inactivated molecules per unit increase in temperature. Reorganizing Eq. 2 and integrating leads to:

$$\text{Rate} = D\, e^{-CT} \quad (3)$$

where D is a constant of integration that incorporates the particular units used. Eq. 3 was used to fit the upper envelopes of P_N above the optimal temperatures.

Results

PPF reached a maximum near midday (Fig. 1*A,D*) and stem temperature reached a maximum near mid-afternoon (Figs. 1*B,E*) for both *Hylocereus undatus* and *Selenicereus megalanthus*. Nocturnal P_N (Phase I of CAM) were maximal after midnight and were generally higher for *H. undatus* than for *S. megalanthus*. For both species, P_N was positive at the beginning of the daytime (Phase II) and was negative during most of the rest of the daytime (Phase III); little positive P_N occurred at the end of the daytime (Phase IV; Fig. 1).

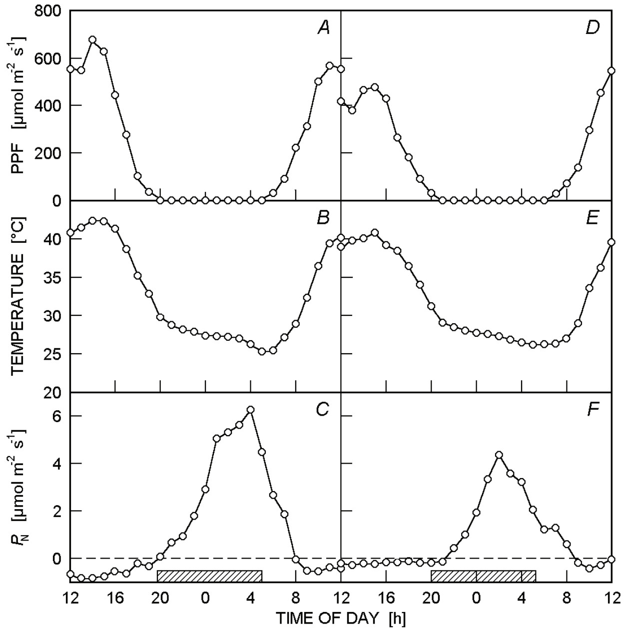

Fig. 1. Daily course of photosynthetic photon flux, PPF (*A, D*), stem temperature (*B, E*), and the net rate of CO_2 uptake, P_N (*C, F*) for *Hylocereus undatus* on 20 July 2004 (*left*) and *Selenicereus megalanthus* on 7 August 2004 (*right*). Data are averaged on an hourly basis. *The shaded area on the bottom of the figure* represents the night time hours.

Withholding water decreased the rates and the daily amounts of net CO_2 uptake (Fig. 2). For *H. undatus*, the maximal P_N was 12 µmol m^{-2} s^{-1} under wet conditions, decreasing about 50 % at 4 d of water stress and 92 % at 8 d. Similarly, the total daily amount of net CO_2 uptake, obtained by integrating the instantaneous rate over 24 h, was 378 mmol m^{-2} d^{-1} under wet conditions, decreasing 53 % at 4 d of water stress and 89 % at 8 d (Fig. 2).

In part because of water stress, considerable variation occurred in the nocturnal P_N (Phase I) at a particular temperature for *H. undatus* in the field. Rates tended to rise as the temperature was raised above 13 °C, achieved a maximum near 27 °C, and then decreased to zero near 33 °C (Fig. 3*A*). The early daytime positive P_N (Phase II) by *H. undatus* also tended to rise above 13 °C, achieved a maximum near 27 °C, and decreased toward zero at 35 °C (Fig. 3*B*). Over the same temperature range, P_N tended to be lower for *S. megalanthus*, whose maxima in Phase I (Fig. 3*C*) and Phase II (Fig. 3*D*) also occurred at about 27 °C.

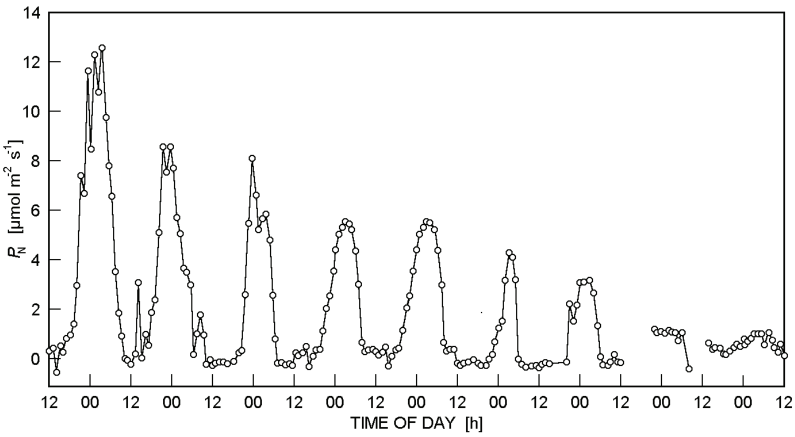

Fig. 2. The net CO_2 uptake rate, P_N for *H. undatus* on the day of watering and the subsequent 8 d without watering. For this period (8–17 July 2004), the daytime stem temperature averaged 38 °C and the night time one averaged 29 °C; the daytime relative humidity averaged 39 % and the night time averaged 61 %.

Parameterization of the upper envelopes (Table 1) from 13 to 27 °C indicated an activation energy (*A*, Eq. 1) of 103 kJ mol^{-1} for P_N in Phase I for *H. undatus* and 25 % less for *S. megalanthus*. For Phase II, *A* was 86 kJ mol^{-1} for *H. undatus* and 43 % less for *S. megalanthus*. Parameter *B* (Eq. 1), which indicates the P_N for the unrealistic case of zero *A*, was also larger for *H. undatus* than for *S. megalanthus* (Table 1). The decline in P_N above the optimal temperature was characterized by parameter *C* (Eqs. 2 and 3), which was highest for Phase I of *H. undatus*, 1.01 K^{-1}, and 26 % less for *S. megalanthus*; *C* was similar for Phase II for both species, averaging 0.36 (Table 1). The empirical parameter *D* (Eq. 3) was higher for Phase I than for Phase II for both species (Table 1).

Discussion

Drought greatly affected P_N for *Hylocereus undatus*, as this shallow–rooted vine cactus responded rapidly to drying under field conditions. Both the maximal rate and the total daily net CO_2 uptake decreased about 50 % in 4 d of withholding water and 90 % in 8 d with day/night average temperatures of 38/29 °C and day/night average relative humidities of 39/61 %. Under more moderate conditions in environmental chambers, withholding water for 12 d (day/night temperatures of 30/20 °C; day/night relative humidities of 40/70 %) reduced the maximal P_N by 57 % for *H. undatus* (Nobel and De la Barrera 2002a) and withholding water for 24 d (day/night temperatures of 25/15 °C; day/night relative humidities of 45/80 %) reduced the total daily net CO_2 uptake by 78 % (Raveh *et al*. 1995). The more rapid decrease in CO_2 uptake ability in the field than in the environmental chambers can be explained based on the differences in temperatures and relative humidities at night, when most stomatal opening occurs for *H. undatus* (Ortiz *et al*. 1996, Nobel and De la Barrera 2004). In particular, the nocturnal water vapour pressure difference (VPD; Allen *et al*. 1998, Nobel 2005) from the stem to the air averaged over three-fold higher under field conditions compared with in environmental chambers. In any case, irrigation every 3 d, as done in the present study, will help maintain maximal P_N for these vine cacti, at least under the relatively extreme conditions that they encountered in the field.

Table 1. Parameters for equations describing the upper envelopes of the responses of net CO_2 uptake to temperature for *Hylocereus undatus* and *Selenicereus megalanthus*. Rising portions (below the optimal temperature) were described by Eq. 1 and falling portions (above the optimal temperature) were described by Eq. 3 for both Phases I and II.

Species	Phase	A [kJ mol^{-1}]	ln B	C [K]	ln D
H. undatus	I	103	29.8	1.01	320
	II	86	36.5	0.32	98
S. megalanthus	I	77	19.2	0.75	225
	II	49	7.1	0.39	116

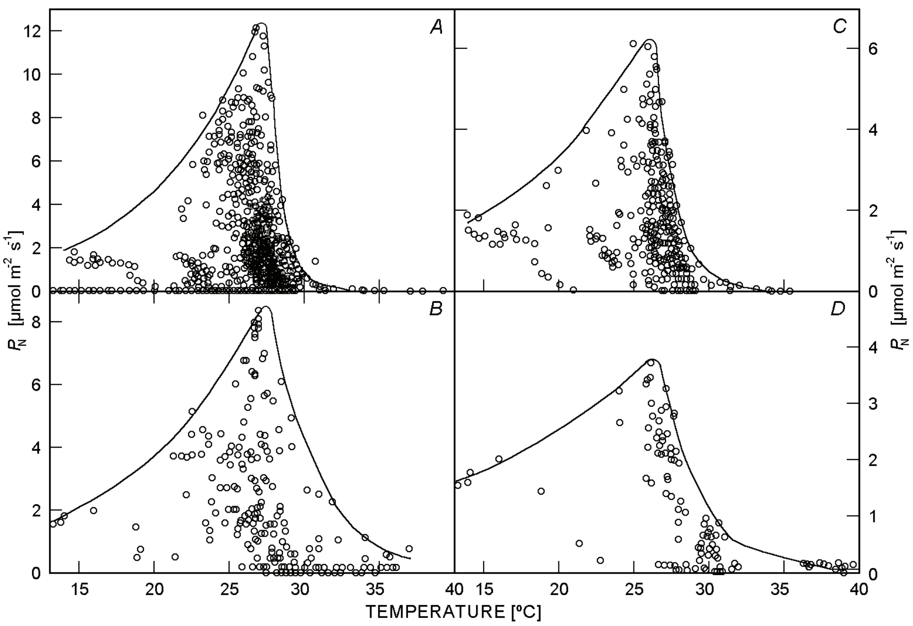

Fig. 3. Temperature dependence of the net CO_2 uptake rate, P_N for *H. undatus* (*left*) *and S. megalanthus* (*right*): (*A*, *C*) Phase I (nocturnal), and (*B, D*) Phase II (early morning). Data were obtained at 30 min intervals under various temperatures and degrees of water stress; 1 581 (984 for *C*) data points are in Phase I and 354 (113 for *D*) in Phase II. The envelopes for the rising portions were analyzed using Eq. 1, and the envelopes for the falling portions were analyzed using Eq. 3; parameter values are summarized in Table 1.

The large number of data points for positive P_N at various temperatures (over 3 000) together with their inherent great scatter enabled upper envelopes to be created. Such envelopes indicate when factors other than temperature are not limiting P_N. In particular, for both *H. undatus* and *S. megalanthus*, the maximal P_N increased as the temperature increased from 13 to 27 °C in both Phase I and Phase II. The maximal rate of 12 µmol m^{-2} s^{-1} for *H. undatus* in the field and its maximal total daily net CO_2 uptake of 378 mmol m^{-2} (obtained by integrating the instantaneous rate over 24 h) are higher than previously reported for this species (Raveh *et al.* 1995, 1997, 1998, Nobel and De la Barrera 2002a,b, 2004), indicating that the plants were under excellent conditions; such data for *S. megalanthus* have apparently not been previously reported. Also, the optimal temperatures of 27 °C in Phase I for both cacti was higher than usually found for PEPC of CAM species (Nobel 1988) but was consistent with the high temperatures in their native habitats (Benzing 1990, Lüttge 1997). Based on the Arrhenius equation, the increases in P_N with temperature reflect increases in the number of molecules that have sufficient energy to surmount the energy barriers represented by the underlying reactions (Eq. 1; Nobel 2005). Above the optimal temperature, P_N decreased and became effectively zero by 35 °C for Phase I and by 40 °C for Phase II for both species. Such decreases reflect thermal disruption of the catalytic properties of the enzymes involved, or "deactivation" (Bernacchi *et al.* 2002, Sharkey 2005). In the Arava and the Jordan River valleys of Israel average daily temperature that exceeded 37–38 °C caused shoot's tissue liquidation and death (Mizrahi and Nerd 1999, Pelah *et al.* 2003).

From the analysis of the envelope of P_N as the stem temperature increased from 13 °C to the optimal value of 27 °C, the apparent activation energy in Phase I (mediated by PEPC; see Eq. 1) was 103 kJ mol^{-1} for *H. undatus* and 77 kJ mol^{-1} for *S. megalanthus*. These values of *A* are similar to those for PEPC of other CAM plants, *e.g.* 38–96 kJ mol^{-1} for nine species determined by microcalorimetry (Feng *et al.* 1994), and for C_4 plants, *e.g.* 69–194 kJ mol^{-1} for *Flaveria bidentis* at various temperatures (Kubien *et al.* 2003). Analysis of the envelopes for Phase II indicates an *A* for RuBPCO of 86 kJ mol^{-1} for *H. undatus* and 49 kJ mol^{-1} for *S. megalanthus*. These values of *A* are similar to values of 51–69 kJ mol^{-1} for RuBPCO from ten C_3 species, 50–68 kJ mol^{-1} for seven C_4 species (Sage 2002), 59–85 kJ mol^{-1} for leaves of *Spinacia oleracea* with various temperature treatments (Lixiong *et al.* 2002), and 56–101 for *F. bidentis* at various temperatures (Kubien *et al.* 2003). Therefore, the activation energies for PEPC and RuBPCO for *H. undatus* and *S. megalanthus* in the field determined using the upper envelopes of the responses of net CO_2 uptake to temperature are within the range of literature values measured in the laboratory for these enzymes with various methodologies and a diverse group of species.

Temperatures above those optimal for P_N can inactivate enzymes *via* various cellular processes as well as can influence stomatal responses (Nobel 2005, Sharkey 2005). Such inhibition, deduced from the upper envelope of the data points, was empirically represented by an exponential decay with temperature that was quantified by the relative fractional inhibition per K unit or, equivalently, per °C (*C* in Eqs. 2 and 3). Although *C* is empirical, it clearly describes the large inhibition of net CO_2 uptake with increasing temperature for these two vine cacti in a temperature range that they encounter under field conditions. For example, a *C* of 1.00 indicates that P_N will decrease by 63 % per °C beginning at 28 °C, as occurred for Phase I of *H. undatus*, and a *C* of 0.3 indicates a decrease of 26 % per °C also beginning at 28 °C, as occurred for its Phase II. The inhibition with increasing temperature was greater for Phase I, averaging 58 % per °C for *H. undatus* and *S. megalanthus*, than it was for Phase II, for which it averaged 30 % per °C for the two species. Information on the relative proportion of the inhibition due to enzyme inactivation (deactivation) *versus* stomatal and other factors awaits future research. In any case, the steep decline in P_N with temperature above 28 °C for these cacti means that small increases in ambient temperature can then lead to significant decreases in net CO_2 uptake ability.

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