

Environmental and Ontogenetic Influences on Growth, Photosynthesis, and Survival for Young Pitayo (*Stenocereus queretaroensis*) Seedlings[♦]

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ABSTRACT

The columnar cactus *Stenocereus queretaroensis* is cultivated for its fruits in southern Jalisco, Mexico. Plantations are established by clonal propagation of individuals that produce fruits with desirable characteristics. Because recruitment of wild individuals originated from sexual reproduction is paramount for the maintenance of genetic diversity for this species (for example, plantations are established near wild populations where gene flow may occur) and because young seedlings are most vulnerable to mortality, their performance was studied in the greenhouse for young *S. queretaroensis* seedlings of different ages (3, 5, 9, or 14 weeks after germination). Initially, seedling height and fresh weight were similar among cohorts, averaging 0.92 cm and 0.03 g. The volume-to-area ratio was higher and the nocturnal acid accumulation was lower for older seedlings. Seedlings were exposed to two watering treatments (Irrigated and Droughted) and to three photosynthetic photon flux (PPF) treatments (2.83, 9.53, or 18.49 mol m⁻² day⁻¹) in a factorial experimental design, and harvested after one month. In general, irrigated seedlings had greater height and fresh weight than droughted seedlings. Volume to area ratio decreased during the experiment for the younger seedlings under the highest PPF treatment and for the oldest seedlings under the lowest PPF. Nocturnal acid accumulation decreased for younger seedlings under the two higher PPF treatments, but it increased for 5-week-old seedlings under the lowest PPF treatment. Seedling survival was also assessed during the experiment. The greatest contrast was observed between the seedlings under the lowest and the highest PPF treatments, for which survival amounted to 67% or 97%, respectively.

Keywords: acclimation, agroecology, cotyledon, deep shade, domestication, establishment, nurse plants, reproductive ecophysiology

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INTRODUCTION

In addition to the various cultivated species of cactus pear (genera *Opuntia* and *Nopalea*), globally covering over 1 Mha, and vine cacti, which are cultivated in over 20 countries, columnar cacti are important cactus crops in Mexico owing to their bright colored, sweet, fleshy fruits (Pimienta-Barrios 1999; Nobel 2000; Nobel and De la Barrera 2004). For instance, in the Tehuacán Valley, States of Oaxaca and Puebla, Mexico, a site of ample cactus diversity that is recognized as one of the centers of origin for agriculture, more than 20 native columnar cacti are managed and consumed (Casas *et al.* 1999; Casas and Barbera 2002; Zeder 2006). Moreover, archaeological evidence suggests that such cacti have been utilized for at least 9,000 years (Casas *et al.* 2007; MacNeish 1967; Callen 1965). Of more recent domestication, a mere 1,000 to 2,500 years, *Stenocereus queretaroensis* (common name is *pitayo*) is probably the most important columnar cactus under cultivation, with approximately 1,500 ha in the southern portion of the west-central State of Jalisco, Mexico (Benz *et al.* 1997; Pimienta-Barrios and Nobel 1994; Nobel *et al.* 2002). In fact, the popularity of *pitayas*, the name for *S. queretaroensis* fruits, has increased over the past 15 years to the point where producers from various locations of southern Jalisco have organized a seasonal farmers' market in the city of Guadalajara (the State's capital with a metropolitan population exceeding 3.5 million; INEGI 2000) where *pitayas* are sold directly to consumers.

Owing to slow growth rates, typical for long lived cacti, commercial *pitayo* plantations are established by clonal propagation of plants that produce fruit with desirable characteristics (Pimienta-Barrios and Nobel 1994; Nobel *et al.* 2002). This way, instead of having to wait various decades until fruit harvest, as it would occur for individuals originated from seed, the establishment of plants from severed branches allows for fruit production within five years (Pimienta-Barrios 1999). Nevertheless, as it occurs for other clonally propagated crops, genetic diversity may be reduced for this species. Contrasting is the case for *Polaskia chende* and *P. chichipe* from Tehuacán. Serving a smaller market, their fruits are still obtained from wild and from *in situ* managed populations, as well as from commercial plantations. Nevertheless, a point needs to be made that for *pitayo*, wild individuals tend to be "tolerated" in agricultural lands which may constitute a way for the conservation of genetic diversity as long as genetic flow actually occurs (Pimienta Barrios 1990, Pimienta-Barrios *et al.* 1997, Arias and Rieseberg 2004).

Fruit production for *S. queretaroensis* occurs during the Spring (the dry season; Pimienta-Barrios *et al.* 1997), allowing germination during the Summer (the rainy season) when, in addition to water, light and temperature are optimal for seed germination (in contrast, during the Winter rains, low air temperatures inhibit germination for this species; De la Barrera and Nobel 2003). While seed germination is rapid and abundant under summer favorable conditions, seedling mortality is very high, especially for very young individuals, limiting the actual recruitment of new cohorts to natural populations (Godínez Álvarez and Valiente-Banuet 1998).

The present work studied young seedlings of *S. queretaroensis* under various environmental conditions, in order to evaluate how age influences their development and ecological performance. In particular, it was expected that the ability to withstand adverse environmental conditions, i.e., drought and high photosynthetic photon fluxes, would increase with age.

MATERIALS AND METHODS

Plant Material

Mature fruits of *Stenocereus queretaroensis* Weber (Buxbaum) were obtained from Techaluta (20°5' N latitude, 103°32' W longitude, 1380 m in elevation), Jalisco, Mexico, in May of 2001. After being

removed from the pulp, their seeds were rinsed in tap water and dipped into a sodium hypochlorite solution (1% v/v) for sterilization. The seeds were air dried and stored in the dark at 25°C and 42% relative humidity until utilized. At different times in March-June 2006 (Table 1), seeds were soaked in tap water for 24 h and then planted in plastic containers (12 cm wide, 12 cm long, and 8 cm tall; with a cover that prevented desiccation) containing washed silicate sand. Seedlings, which emerged at approximately 7 days after sowing, were watered various times each week. Air temperature in the laboratory where the seeds were germinated averaged $26.5 \pm 0.01^\circ\text{C}$ (it ranged from 26.0 to 27.5°C) and the available photosynthetic photon flux (PPF, wavelengths of 400 to 700 nm) was $25 \mu\text{mol m}^{-2} \text{s}^{-1}$, amounting to $1.08 \text{ mol m}^{-2} \text{ day}^{-1}$. Emerged seedlings were transplanted to larger containers (15 cm wide, 45 cm long, and 10 cm tall) with a 5-cm-deep layer of sand on 4 July 2006, two weeks before the start of the experiments. For clarity and consistency, seedling cohorts are identified throughout the manuscript by their age, in weeks, at the start of the experiment (Table 1).

Table 1. Sowing and germination dates, initial and final ages, and initial status for young seedlings of *Stenocereus queretaroensis*. All dates are for 2006. Different letters within a row indicate a statistical difference between cohorts, with $P < 0.05$, for each response variable. Nocturnal acid accumulation (ΔH^+) measurement was not possible for Cohort 2.

	Cohort			
	1	2	3	4
Sowing date	30 March	9 May	2 June	15 June
Germination date	6 April	16 May	9 June	22 June
Initial age (weeks)	14	9	5	3
Final age (weeks)	18	13	9	7
Height (cm)	1.04 ± 0.06^a	0.92 ± 0.10^a	0.88 ± 0.06^a	0.813 ± 0.02^a
Fresh weight (g)	0.027 ± 0.001^a	0.032 ± 0.003^a	0.034 ± 0.002^a	0.032 ± 0.002^a
V/A (mm^3/mm^2)	0.043 ± 0.001^a	0.049 ± 0.003^a	$0.054 \pm 0.003^{a,b}$	0.055 ± 0.002^b
ΔH^+ ($\mu\text{mol m}^{-2} \text{g}^{-1}$)	117 ± 19^a	—	$78 \pm 12^{a,b}$	59 ± 8^b

Experimental Manipulations

On 18 July 2006, young *Stenocereus queretaroensis* seedlings of various ages were placed in a greenhouse at Centro de Investigaciones en Ecosistemas (CIEco), UNAM, Morelia where a three factor experiment was established to evaluate their responses to drought and PPF (Table 1). In particular, a group of seedlings was watered every other day (“Irrigated”), while watering was withheld for another group (“Droughted”). Also, three light regimes were established that allowed full PPF inside the

greenhouse or one of two attenuation levels created with shade-cloth to reach the seedlings. Air temperature, incident PPF, and volumetric soil water content, were measured with Onset Smart Sensors (Onset Computer Corporation, Bourne, MA), connected to an Onset Weather Station, that recorded the average of instantaneous measurements every 20 seconds.

Photosynthesis, Growth, and Survival

Photosynthesis for young seedlings of *Stenocereus queretaroensis* was assessed by measuring the accumulation of organic acids between the evening, when CO₂ uptake starts for CAM plants, and the following morning, when CO₂ uptake ceases (Andrade *et al.* 2007). In particular, seedlings were collected and their fresh weight was recorded before being stored at -20°C. For each assay, one frozen seedling was macerated with a mortar and pestle and 50 ml of distilled water were added. The resulting fine slurry was titrated to pH 7.0 with a 1 mM NaOH aqueous solution (Nobel and Hartssock 1983).

Seedling growth was measured as changes in seedling length. In addition, assuming a conical geometry for young seedlings, apical diameter was also measured to calculate their volume and surface area. The volume to area ratio was determined for seedlings as an indicator of their capacity of storing water vs. the surface permitting water loss. Finally, seedling survival was determined every week as a percentage of the seedlings planted initially for each condition.

Data analyses

Differences between seedlings of various ages were assessed at experiment onset with a one-way ANOVA. At the end of the experiment, which was a factorial design including four seedling ages, three levels of PPF, and two levels of irrigation, seedlings were evaluated with a three-way ANOVA. Two response variables, fresh weight and volume-to-surface-area ratio (V/A), were not normal, so data were log-transformed before statistical analyses were conducted. *Post hoc* pair-wise comparisons were performed with a Holm-Sidak test with a significance level of 0.05. All statistical analyses were performed with Sigma Stat 3.5 (Systat Software, Point Richmond, CA). Data are presented as mean ± 1 S.E.

RESULTS

The shading treatments created three distinct light environments (Figure 1A). In particular, exposed seedlings (treatment hereafter referred to as “100%”) received 18.49 mol m⁻² day⁻¹, while shaded seedlings received 9.53 (“50%”) or 2.83 (“15%”) mol m⁻² day⁻¹ (Figure 1B). Similarly, the temperature regimes were significantly different between each other (Figure 1B). For seedlings under 15%, air temperatures averaged 23.5 ± 0.2°C, ranging from 14.6 to 34.5°C. Temperature increased for seedlings under 50%, with an average of 23.8 ± 0.2°C, ranging from 14.5 to 35.2°C. Finally, for seedlings under 100%, air temperature averaged 25.1 ± 0.2°C, ranging from 14.7 to 37.8°C. Volumetric water content measured after ten days of experiment onset also was significantly different among shade treatments (Figure 1C). In particular, for Irrigated seedlings it averaged 0.21 ± 0.00 under 15%, decreasing by 28% under 50%, and by 34% under 100%. While for droughted seedlings it averaged 0.06 ± 0.00 under 15%, decreasing by 25% under 50%, and by 62% under 100%.

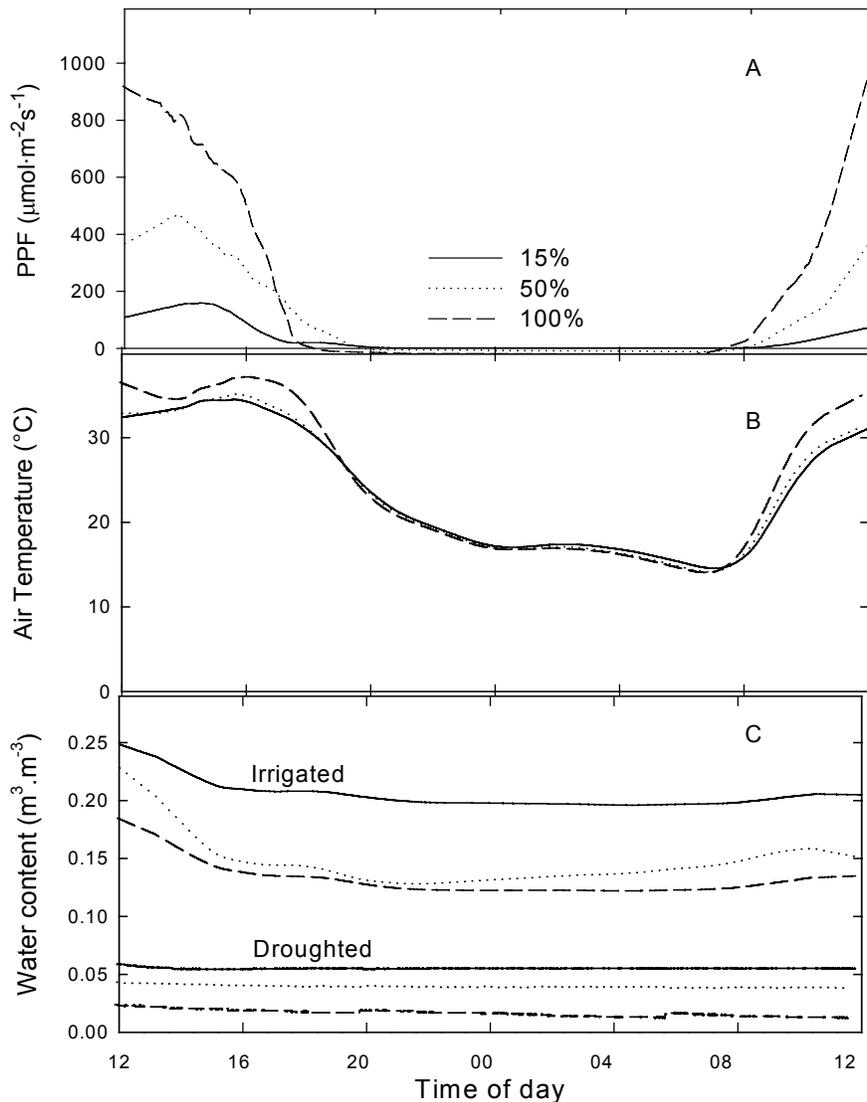


Figure 1. Prevailing environmental conditions in the greenhouse at Centro de Investigaciones en Ecosistemas, UNAM, Morelia, where the experiment was conducted. Data are diel courses for (A) Photosynthetic photon flux, (B) Air temperature, and (C) Volumetric water content for Irrigated and Droughted seedlings, which were measured ten days after experiment onset, under the three experimental shading treatments.

Initially, the seedlings of *Stenocereus queretaroensis* were similar in terms of their height, fresh weight, V/A , and nocturnal acid accumulation, regardless of their age (Table 1; Figs. 2 through 5). An exception being those that were 14 weeks old at the start of the experiment, whose V/A were significantly lower than for 3- and 5-week-old seedlings by about 20%, and whose nocturnal acid accumulation was twice as high than for 3-week-old seedlings.

After four weeks under the experimental conditions, height had increased significantly for irrigated seedlings growing under 15%, with the exception of 5-week-old seedlings, whose height did not change (Figure 2). At the time of harvest, seedling age, PPF treatment, and watering regime acted independently (*i.e.*, neither the three way interaction nor either of the two way interactions were significant), and all of the factors had significant effects on final seedling height and weight (Table 2). With respect to seedling height, 14-week-old seedlings were taller than younger seedlings, and irrigated seedlings tended to be taller than those maintained under drought.

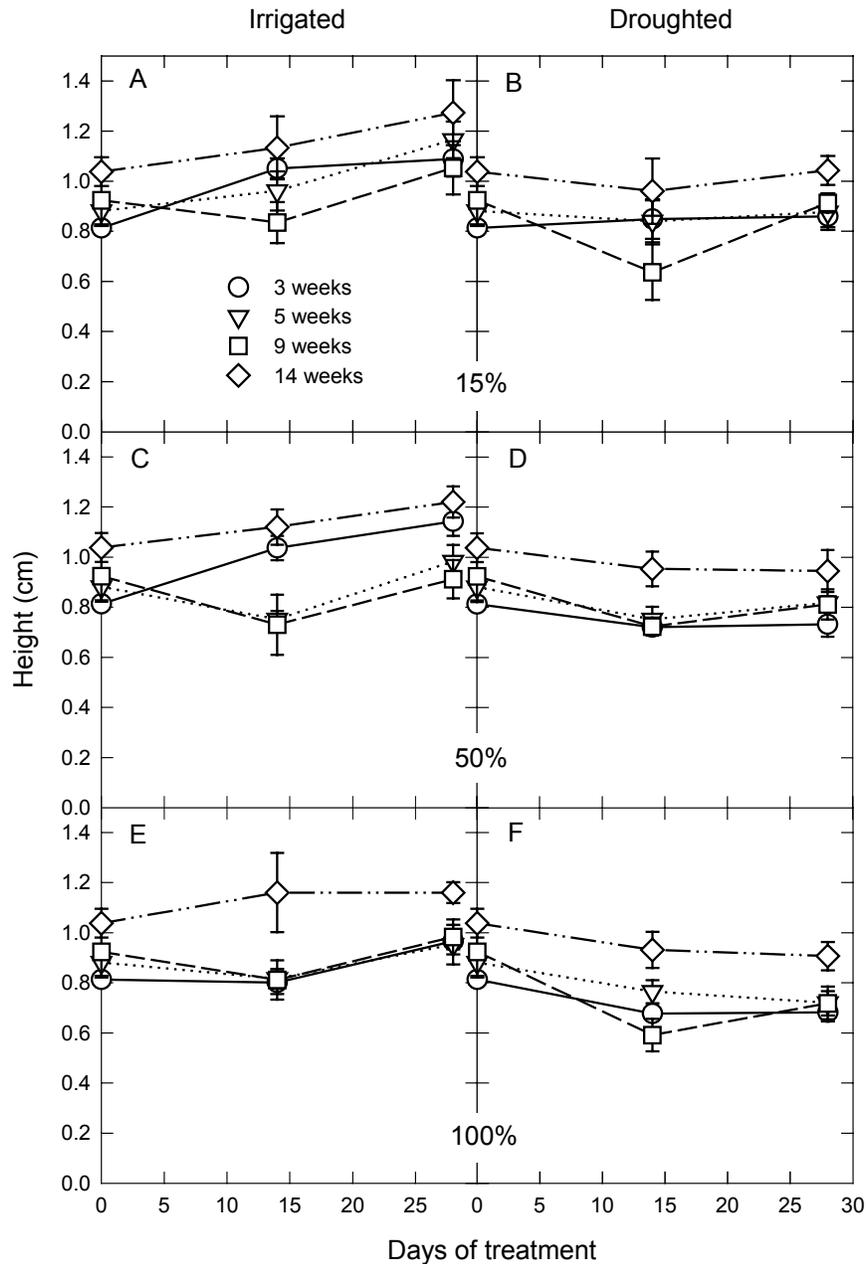


Figure 2. Height for young seedlings of *Stenocereus queretaroensis* of various ages that were either watered every other day (A, C, E) or whose watering was withheld (B, D, F), which were exposed to 15%

(A, B), 50% (C, D), or 100% (E, F) of the PPF available under greenhouse conditions. Data are shown as mean \pm 1 S.E. ($n = 6$ seedlings).

Table 2. Three-way ANOVA at the time of final harvest for young *S. queretaroensis* seedlings. Within a column, F values with one asterisk (*) were significant at $P < 0.01$, while those with two asterisks (**) were significant at $P < 0.001$.

Factors	Variable					
	Height		Fresh Weight	V/A	ΔH^+	
	<i>df</i>	<i>F</i>	<i>F</i>	<i>F</i>	<i>df</i>	<i>F</i>
Age	3	10.52**	4.45*	27.35**	2	22.37**
Light	2	10.33**	23.44**	10.42	2	1.35
Water	1	80.03**	222.78**	56.97**	1	17.45**
Age \times Light	6	0.33	1.73	0.64	4	6.04**
Age \times Water	3	1.17	2.65	0.83	2	8.93**
Light \times Water	2	0.59	2.86	0.69	2	12.31**
Age \times Light \times Water	6	0.79	1.78	1.35	4	16.65**

Fresh weight significantly increased during the experiment for irrigated seedlings of all ages growing under 15%, and for irrigated 3- and 5-week-old seedlings growing under 50% (Figure 3). Opposite was the case for droughted 3- and 5-week-old seedlings under the two higher PPF treatments, whose fresh weight decreased. At the time of harvest, initial seedling age, PPF treatment, and watering regime acted independently (*i.e.*, neither the three-way interaction nor either of the two-way interactions were significant), and all of the factors had significant effects on final seedling height and weight (Table 2). Except for seedling fresh weight, for which the 3-week-old seedlings tended to be heavier than 9- and 14-week-old seedlings. In addition, seedling fresh weight tended to decrease under higher PPF levels and, as could be expected, for seedlings under drought.

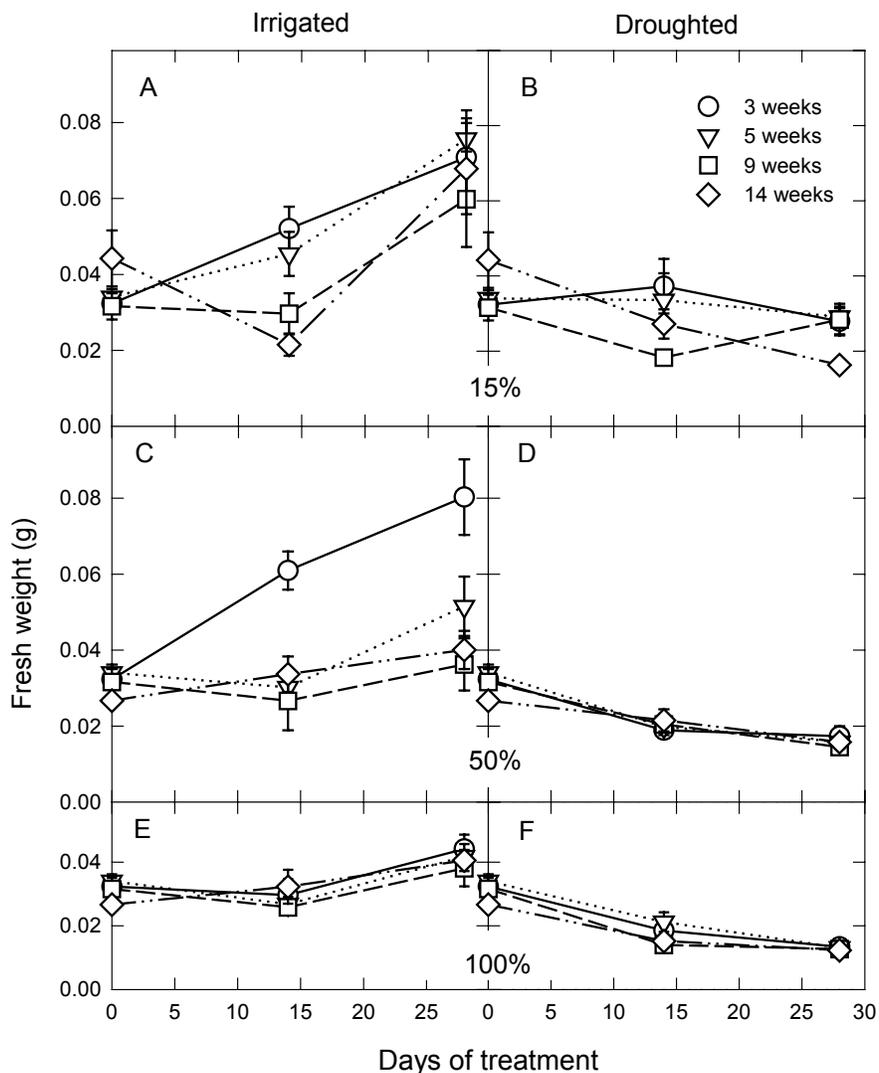


Figure 3. Fresh mass for young seedlings of *S. queretaroensis* of various ages that were either watered every other day (A, C, E) or whose watering was withheld (B, D, F), which were exposed to 15% (A, B), 50% (C, D), or 100% (E, F) of the PPF available under greenhouse conditions. Data are shown as mean \pm 1 S.E. ($n = 6$ seedlings).

With respect to the volume-to-surface-area ratio (V/A), significant decreases were observed only under droughted conditions (Figure 4). In particular, V/A was lower for 3-week-old seedlings growing under 100%, for 5-week-old seedlings under 50%, and for 14-week-old seedlings under 15%. At the end of the experiment, the environmental factors considered also acted independently on V/A , but PPF treatment had no effect on this morphological attribute (Table 2). The volume to surface area ratio at the end of the experiment was lower for the 14-week-old seedlings than for the younger ones (Figure 4). In addition, V/A tended to be lower for droughted seedlings than for those that were irrigated every other day (Figure 4).

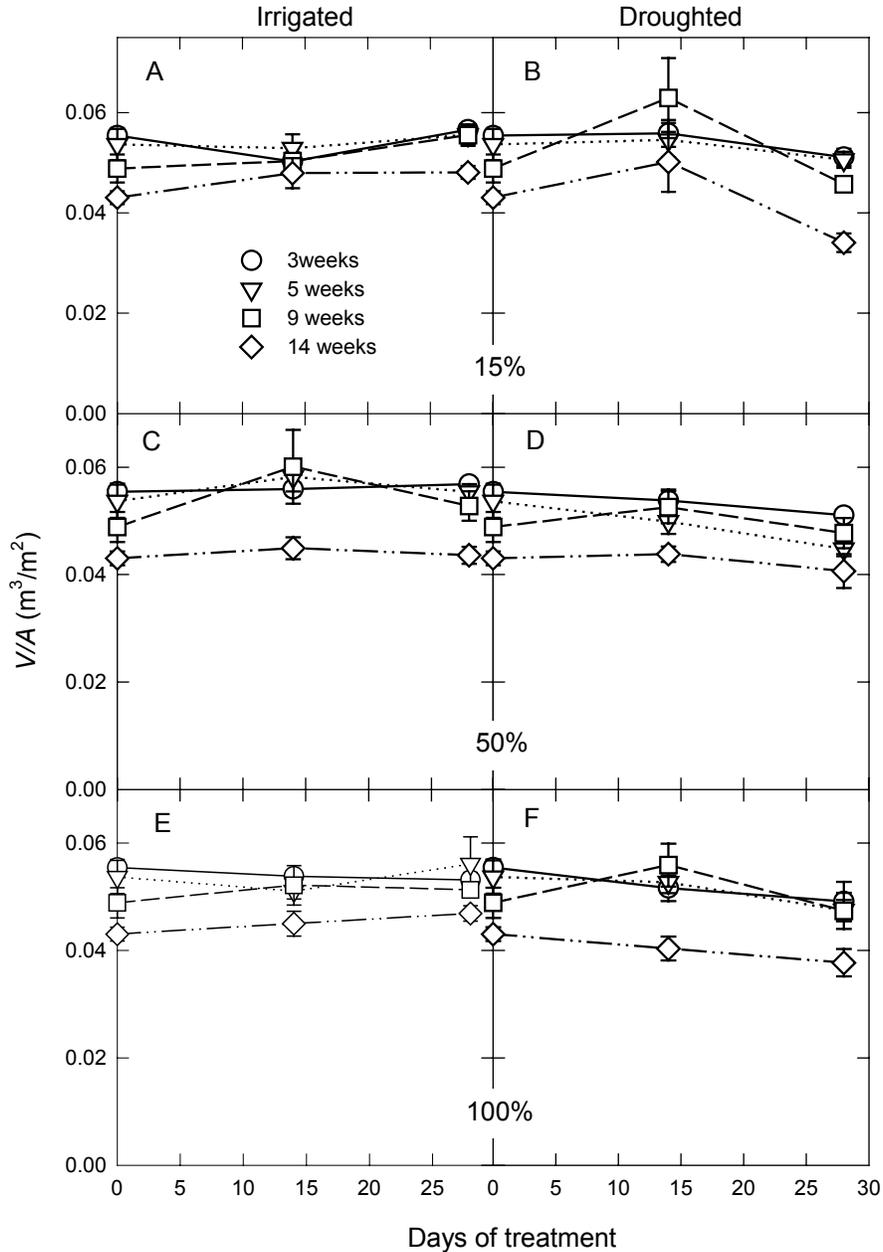


Figure 4. Response of the volume to surface area ratio (V/A) for young seedlings of *S. queretaroensis* of various ages that were either watered every other day (A, C, E) or whose watering was withheld (B, D, F), which were exposed to 15% (A, B), 50% (C, D), or 100% (E, F) of the PPF available under greenhouse conditions. Data are shown as mean \pm 1 S.E. ($n = 6$ seedlings).

Nocturnal acid accumulation also changed for seedlings of all ages during the experiment (Figure 5). It increased for 3-week-old seedlings under the two higher PPFs. For 5-week-old seedlings, nocturnal acid accumulation increased for droughted seedlings under 15%, but it decreased for irrigated seedling under the same PPF. Finally, nocturnal acid accumulation decreased under all PPF levels for 14-week-old seedlings, regardless of their water treatment. Nocturnal acid accumulation was the only response variable

for which the three way interaction (seedling age \times PPF treatment \times watering regime) was significant (Table 2), which was caused by the significant seedling age \times PPF interaction that occurred for droughted seedlings. For the case of irrigated seedlings, PPF level did not have a significant effect on nocturnal acid accumulation, which was lower for 3-week-old than for 5-week-old irrigated seedlings.

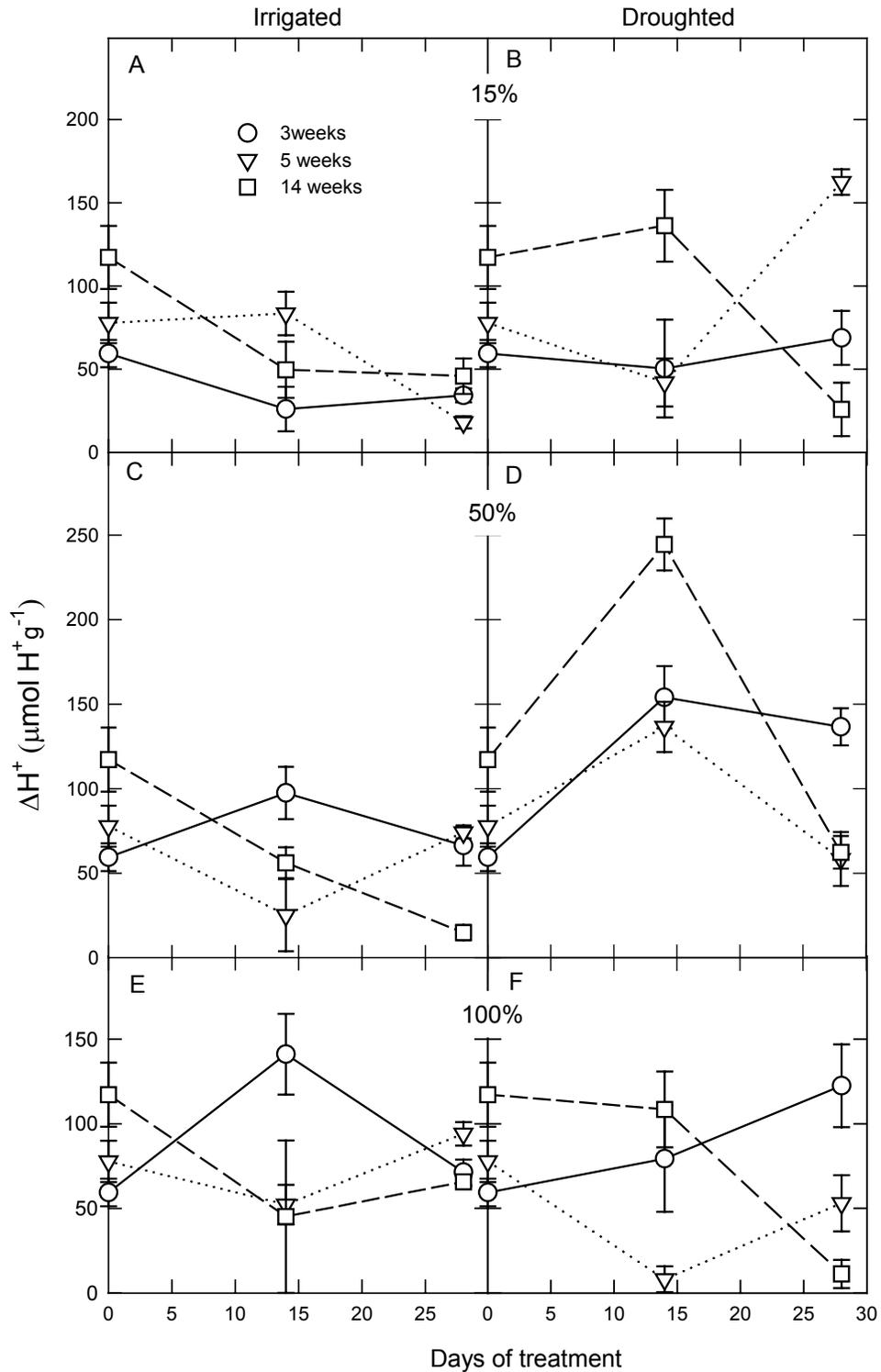


Figure 5. Nocturnal accumulation of titratable acidity for young seedlings of *S. queretaroensis* of various ages that were either watered every other day (A, C, E) or whose watering was withheld (B, D, F), which were exposed to 15% (A, B), 50% (C, D), or 100% (E, F) of the PPF available under greenhouse conditions. Data are shown as mean \pm 1 S.E. ($n = 3$ seedlings).

Seedling survival tended to be higher for younger seedlings than for older ones, for those that were exposed to lower PPF than for those at higher PPF levels, and for irrigated seedlings compared with those that were droughted (Figure 6). The greatest contrast can be observed between irrigated seedlings under 15% and droughted seedlings under 100% (Figure 6A,F). In the first case, survival after 28 days of treatment averaged $96.9 \pm 1.8\%$, while it only averaged $67.4 \pm 15.6\%$ in the latter. Indeed, survival for the older seedlings was less than 50% at 28 days of treatment (Figure 6).

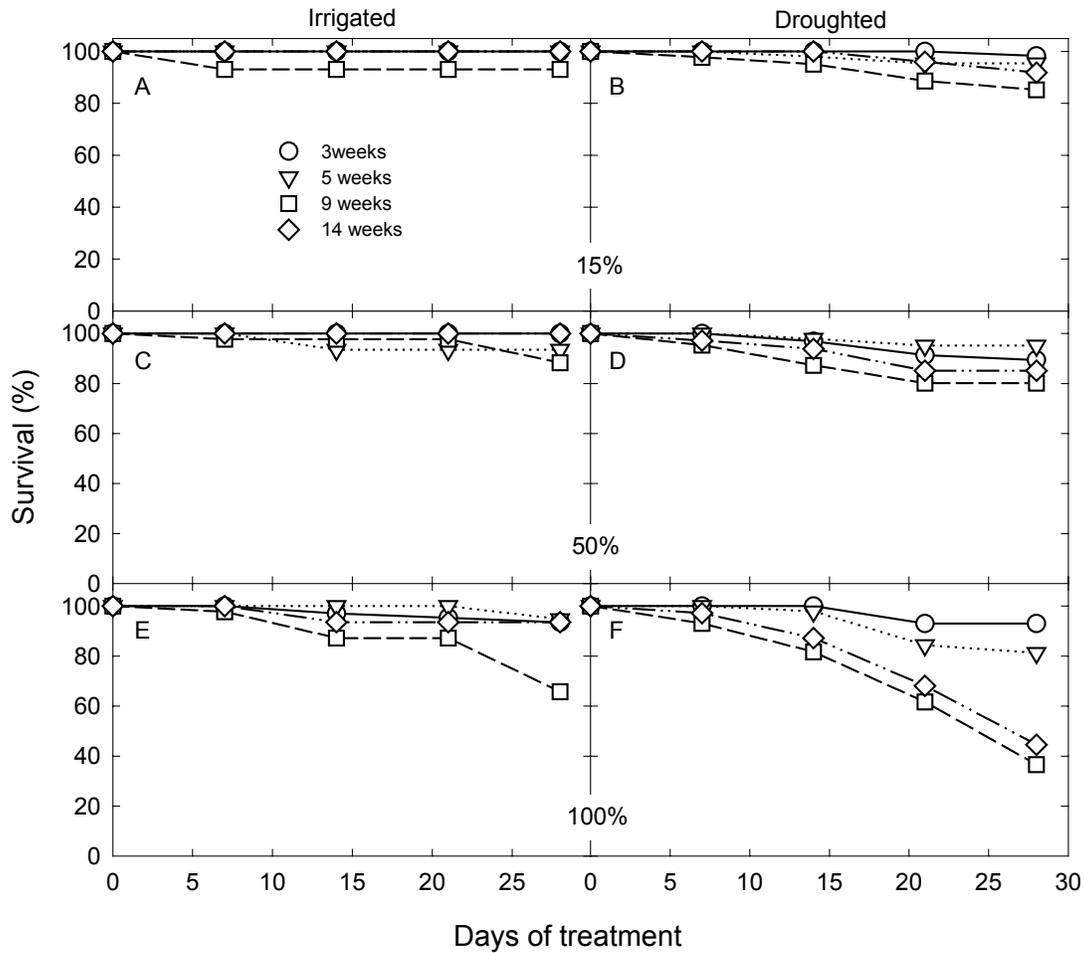


Figure 6. Survival for young seedlings of *S. queretaroensis* of various ages that were either watered every other day (A, C, E) or whose watering was withheld (B, D, F), which were exposed to 15% (A, B), 50% (C, D), or 100% (E, F) of the PPF available under greenhouse conditions. Living seedlings were counted weekly and data are reported as a percentage of the 60 seedlings that were initially planted under each experimental condition.

DISCUSSION

Traits that were measured for young seedlings of *Stenocereus queretaroensis* of various ages responded differently to the microenvironments created by the shading and watering treatments. In general, irrigated and shaded seedlings had better performance than did those that were droughted and not shaded. However, it was unexpected that younger seedlings tended to perform better than older seedlings.

Abundant seed germination for *Stenocereus queretaroensis* occurs during the summer rainy season, when air temperature and soil water potential are optimal (De la Barrera and Nobel 2003). In fact, germination can be reduced for this species under soil water potentials comparable to those measured after only one week of drought. Also, during the winter, low air temperatures inhibit germination despite that soil water potential is high due to rain. However, young seedlings are not as abundant in the field as could be expected when their massive germination is considered (De la Barrera, unpublished observations). In this respect, while seeds of *S. queretaroensis* are viable for several years, seeds and emerged seedlings are very susceptible to predation as is the case for other cacti (Valiente-Banuet and Ezcurra 1991; Godínez-Álvarez and Valiente-Banuet 1998; De la Barrera and Nobel 2003).

In general, performance and survival for cactus seedlings tends to be favored by shade, as was the case for *S. queretaroensis* (Valiente-Banuet and Ezcurra 1991; Godínez-Álvarez and Valiente Banuet 1998; Godínez-Álvarez *et al.* 2005; Cervera *et al.* 2006). However, for older seedlings dry-mass accumulation can be reduced under shade as a result of an inhibition of CO₂ uptake by the prevailing low light (Nobel 1988; Godínez-Álvarez and Valiente-Banuet 1998). Such an inhibition of plant productivity suggests that the beneficial effects of shade for seedling performance and survival are not a mere amelioration of the light environment, as could be suggested by photoinhibition for plants growing in open spaces (e.g., Cervera *et al.* 2007), but that other environmental factors related with solar radiation are also involved. Indeed, cactus seedling survival under shade, which is usually provided by so called nurse plants, seems to be greatly enhanced by a stabilization of the prevailing temperature microenvironment (Gibson and Nobel 1986; Franco and Nobel 1989; Valiente-Banuet and Ezcurra 1991). For instance, maximum air temperatures in the greenhouse were about 3°C higher for the 100% treatment than for the 15% treatment. While air temperatures can be high in arid and semiarid environments, where cacti are often found, diurnal plant surface temperatures are substantially higher, thus plants have developed a tolerance for such extremely high temperatures (Nobel 1988; Nobel and De la Barrera 2003). However, given the small size of seedlings, soil surface temperatures are of paramount importance for their survival because this factor can exceed air temperature by 20°C or more, easily reaching 40 to 70°C (Nobel 1984; Nobel 1988; Nobel and De la Barrera 2003). Tolerance to high temperatures and the ability of plants to acclimate is reduced for developing organs compared with mature organs, as can be illustrated by *Opuntia ficus-indica*. For such a species, young cladodes, fruits and roots of plants acclimated to a diurnal air temperature of 30°C can withstand temperatures of approximately 56°C, while 1-year-old cladodes are able to withstand temperatures higher than 59°C (Nobel and De la Barrera 2003). Apparently, the case for seedlings *vs.* adult plants of desert succulents is similar. In particular, high-temperature tolerance for *Agave deserti* and *Ferocactus acanthodes* from the Sonoran Desert is nearly 10 °C greater for adults than for seedlings (Nobel 1984; 1988). Yet, tolerance to extremely high temperatures by *S. queretaroensis* is not known for seedlings nor adults.

Annual precipitation in the semiarid lands of southern Jalisco, where *S. queretaroensis* is cultivated, barely reaches 700 mm, most of which occurs during the summer (Pimienta-Barrios *et al.* 1997). The dry season can last up to five months so that, in order to survive, plants need to be able to resist such a drought. While adult plants, with their massive succulent stems, can survive and even maintain their physiological functions during many months of drought (Pimienta-Barrios *et al.* 2000), substantial differences were found here after one month between irrigated and droughted seedlings of *S. queretaroensis*. In particular, droughted seedlings were shorter than irrigated seedlings. Moreover, fresh

weight for droughted individuals was not only much lower than for irrigated seedlings, but it also tended to be lower than at experiment onset. In part, ecological success of stem succulents is due to their ability to store water while reducing the surface available for transpirational water loss, quantified in the volume to surface area ratio (Gibson and Nobel 1986; Nobel 2005). In this case, a higher value of V/A indicates that more water is stored per unit surface area, so water loss would be reduced. For instance, V/A equals about 0.15 cm for seedlings of *Ferocactus acanthodes* older than 50 d after germination (Jordan and Nobel 1981). For 30-day-old seedlings of *Mammillaria gaumeri* a V/A of 0.43 cm is 25% lower for seedlings growing under 50% of the total PPF than for seedlings growing under 20%; and, for the latter species V/A increases by 15% for 60-day-old seedlings (Cervera *et al.* 2007). Here, V/A was about 16% lower for 9- and 14-week-old seedlings than for 3-week-old seedlings at the start of the experiment. This trend of lower V/A for older seedlings tended to be maintained at the end of the experiment, especially under droughted conditions. This could be one reason explaining the higher mortality for older seedlings than for younger ones. A question arises regarding an apparent contradiction between our results and the hypothesis of better performance by older seedlings, when compared with examples for other species. However, while the summer rains amount to 64% of the annual precipitation, occasional autumn rains and a short winter rainy season can supply an additional 170 mm (Pimienta-Barrios *et al.* 1997). The result is a 6-month-long period with moist soils in the tropical dry forests where *S. queretaroensis* is native. The oldest seedlings utilized here germinated just over three months before the start of the experiment. Thus our results suggest that a longer period of development under favorable environmental conditions is required for this species to attain a V/A large enough to survive the Spring dry season. Indeed, V/A increases 0.27 ± 0.01 cm ($n = 5$) for 15-month-old irrigated seedlings of *Stenocereus queretaroensis* (Unpublished observations for plants kept in the greenhouse under the 50% treatment).

Seedlings of *S. queretaroensis* exhibited CAM photosynthesis as indicated by their nocturnal acid accumulation. Similarly, seedlings of five other columnar cacti and two globose cacti exhibit CAM since germination, although the magnitude of such nocturnal acid accumulation does increase with age (Cervera 2006; Hernández-González and Briones-Villarreal 2007). However, no clear trend was observed here with increasing age nor for droughted *vs.* irrigated seedlings. For *S. queretaroensis*, diel courses of tissular acidity for seedlings ranging in age from 2 to 56 weeks tend to be irregular, i.e., no clear pattern of nocturnal acid accumulation can be observed, except for one-year-old seedlings, although tissue acidity does tend to increase with age (Loza-Cornejo *et al.* 2003). In contrast, drought seems to enhance CAM for 9-month old seedlings (Ayala-Cordero *et al.* 2006). Here, a point can be raised about methodology. Measurements of titratable acidity provide valuable information about CAM photosynthesis, and most studies with adult plants focus on the acid content of chlorenchyma, the green photosynthetic tissue (Andrade *et al.* 2007). However, performing such measurements for seedlings requires whole individuals. In this regard, chlorenchyma thickness for mature stems of cacti can be of several millimeters and, for *O. ficus-indica*, nocturnal acid accumulation is substantially lower (22 to 47%) in deeper tissue layers (Nobel *et al.* 1994). In addition, carbohydrates resulting from photosynthesis tend to be accumulated in the underlying water-storing parenchyma (Goldstein *et al.* 1991). For *S. queretaroensis*, seedlings do not have a clearly differentiated chlorenchyma and abundant chloroplasts can be found in parenchyma cells (Loza-Cornejo *et al.* 2003). As a result, acid accumulation could be diluted, probably resulting in the unclear results that have been found for young cactus seedlings.

An unexpected result of this experiment was that the younger seedlings tended to perform better than their older counterparts. Their higher volume to surface area ratios, discussed above, probably contributed to this result, in the sense that their relatively smaller surface areas enabled them to better resist the month-long experimental drought to which they were exposed. A similar trend of decreasing performance with increasing age for seedlings is reported regarding resistance to herbivory, apparently due to maternal effects from the cotyledons (Del-Val and Crawley 2005). Indeed, maternal reserves supply the energy that allows seedlings to emerge from beneath the soil surface after germination and that maintains plant metabolism until autotrophy is reached (Taiz and Zeiger 2002). Also, various studies underscore the

importance of cotyledons in seedling establishment, development, and even flowering (Green and Juniper 2004; Hanley and May 2006; Hanley and Fegan 2007). For instance, seedlings of *Stenocereus beneckeii* originated from smaller seeds are less tolerant of water stress than those from larger seeds (Ayala-Cordero *et al.* 2006). However, most of the research regarding maternal reserves on seedling performance report morphological or functional correlates with either seed size or cotyledon integrity, but further investigation is needed to unravel the actual physiological mechanisms that are involved in such observations.

The seedling stage constitutes the bottleneck for the recruitment of most long-lived plants and mortality is especially high for very young individuals. Yet, recruitment of sexually reproduced individuals is crucial for the maintenance of the genetic diversity of plant populations. This is an issue of special concern for clonally reproduced species of economic importance, such as *Stenocereus queretaroensis*, because genetic variation in wild populations is the “stock” for eventual plant-improvement initiatives. Here, both seedling performance and survival tended to decrease with age, especially under drought and high solar radiation. Thus suggesting that 1) maternal reserves from cotyledons are especially important for very young seedlings of up to 5 weeks in age and 2) it is possible that a long period of favorable temperature and soil moisture conditions are needed for seedlings to be able to withstand the long periods of drought and high air and soil temperatures prevalent during the dry season at places where *S. queretaroensis* is native in western Mexico. Further research is needed to elucidate the causes and possible advantages of the reduction in the volume to surface area ratio observed here for the older seedlings and to explore physiological mechanisms in which maternal reserves from cotyledons influence seedling function.

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LITERATURE CITED

- Andrade JL, De la Barrera E, Reyes-García C, Ricalde MF, Vargas-Soto G, and Cervera JC. 2007. El metabolismo ácido de las crasuláceas: diversidad, fisiología ambiental y productividad. *Boletín de la Sociedad Botánica de México* **81**: 37–51.
- Arias DM and Rieseberg LH. 2004. Gene flow between cultivated and wild sunflowers. *Theoretical and Applied Genetics* **89**: 655–660.
- Ayala-Cordero G, Terrazas T, López-Mata L, and Trejo C. 2006. Morpho-anatomical changes and photosynthetic metabolism of *Stenocereus beneckeii* seedlings under soil water deficit. *Journal of Experimental Botany* **57**: 3165–3174.
- Benz BF, Santana MF, Cevallos EJ, Muñoz ME, Rosales AJ, and Rosales AM. 1997. The structure and productivity of relict stands of pitaya (*Stenocereus queretaroensis*; Cactacea), Jalisco, Mexico. *Economic Botany* **51**: 134–143.
- Callen EO. 1965. Food habits of some Pre-Columbian Mexican Indians. *Economic Botany* **19**: 335–343.

- Casas A, Otero-Arnaiz A, Pérez-Negrón E, and Valiente-Banuet A. 2007. In situ management and domestication of plants in Mesoamerica. *Annals of Botany* **100**: 1101-1115. ("in situ" is cursive in the original paper: <http://dx.doi.org/10.1093/aob/mcm126>).
- Casas A and Barbera G. 2002. Mesoamerican domestication and diffusion. In: Nobel PS (ed) *Cacti: Biology and Uses*. University of California Press. pp. 143–162.
- Casas A, Caballero J, Valiente-Banuet A, Soriano JA, and Dávila P. 1999. Morphological variation and the process of domestication of *Stenocereus stellatus* (Cactaceae) in central Mexico. *American Journal of Botany* **86**: 522–533.
- Cervera JC. 2006. Microambiente, asimilación de CO₂, crecimiento, germinación y establecimiento de *Mammillaria gaumeri* (Cactaceae), especie rara y endémica de la costa norte de Yucatán. Ph. D. Dissertation. Centro de Investigación Científica de Yucatán, Mexico.
- Cervera JC, Andrade JL, Simá JL, and Graham EA. 2006. Microhabitats, germination, and establishment for *Mammillaria gaumeri* (Cactaceae), a rare species from Yucatan. *International Journal of Plant Sciences* **167**: 310–318.
- Cervera JC, Andrade JL, Graham EA, Durán R, Jackson PC, and Simá JL. 2007. Photosynthesis and optimal light microhabitats for a rare cactus, *Mammillaria gaumeri*, in two tropical ecosystems. *Biotropica* **39**: 620–627.
- De la Barrera E and Nobel PS. 2003. Physiological ecology of seed germination for the columnar cactus *Stenocereus queretaroensis*. *Journal of Arid Environments* **53**: 297–306.
- Del-Val E and Crawley MJ. 2005. Are grazing increaser species better tolerators than decreaseers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology* **93**: 1005–1016.
- Franco AC and Nobel PS. 1989. Effect of nurse plants on the microhabitat and growth of cacti. *Journal of Ecology* **77**: 870–886.
- Gibson AC and Nobel PS. 1986. *The Cactus Primer*. Harvard University Press.
- Godínez-Álvarez H and Valiente-Banuet A. 1998. Germination and early seedling growth of Tehuacan Valley cacti species: the role of soils and seed ingestion by dispersers on seedling growth. *Journal of Arid Environments* **39**: 21–31.
- Godínez-Álvarez H, Ríos-Casanova L and Pérez F. 2005. Characteristics of seedling establishment of *Stenocereus stellatus* (Cactaceae) in the Tehuacán Valley, Mexico. *The Southwestern Naturalist* **50**: 375–407.
- Goldstein G, Andrade JL, and Nobel PS. 1991. Differences in water relations parameters for the chlorenchyma and the parenchyma of *Opuntia ficus-indica* under wet versus dry conditions. *Australian Journal of Plant Physiology* **18**: 95–107.
- Green PT and Juniper PA. 2004. Seed mass, seedling herbivory and the reserve effect in tropical rainforest seedlings. *Functional Ecology* **18**: 539–547.

- Hanley ME and May OC. 2006. Cotyledon damage at the seedling stage affects growth and flowering potential in mature plants. *New Phytologist* **169**: 243-250.
- Hanley ME and Fegan EL. 2007. Timing of cotyledon damage affects growth and flowering in mature plants. *Plant, Cell and Environment* **30**: 812-819.
- Hernández-González O and Briones-Villarreal O. 2007. Crassulacean acid metabolism photosynthesis in columnar cactus seedlings during ontogeny: the effect of light on nocturnal acidity accumulation and chlorophyll fluorescence. *American Journal of Botany* **94**: 1344-1351.
- Jordan PW and Nobel PS. 1981. Seedling establishment of *Ferocactus acanthodes* in relation to drought. *Ecology* **62**: 901-906.
- Loza-Cornejo S, Terrazas T, López-Mata L, and Trejo C. 2003. Características morfo-anatómicas y metabolismo fotosintético en plántulas de *Stenocereus queretaroensis* (Cactaceae): su significado adaptativo. *Interciencia* **28**: 83-89.
- MacNeish RS. 1967. A summary of the subsistence. In: Byers DS (ed.), *The Prehistory of the Tehuacán Valley. Vol. I: Environment and subsistence*. University of Texas Press, pp. 290-331.
- Nobel PS. 1984. Extreme temperatures and thermal tolerances for seedlings of desert succulents. *Oecologia* **62**: 310-317.
- Nobel PS. 1988. *Environmental Biology of Agaves and Cacti*. Cambridge University Press.
- Nobel PS. 2000. Crop ecosystem responses to climatic change: Crassulacean Acid Metabolism crops. In: Reddy KR and Hodges HF (eds.), *Climate Change and Global Crop Productivity*. CAB International. pp. 315-331.
- Nobel PS. 2005. *Physicochemical and Environmental Plant Physiology*, 3rd. edn. Academic Press/Elsevier.
- Nobel PS and De la Barrera E. 2003. Tolerances and acclimation to low and high temperatures for cladodes, fruits, and roots of a widely cultivated cactus, *Opuntia ficus-indica*. *New Phytologist* **157**: 271-279.
- Nobel PS and De la Barrera E. 2004. CO₂ uptake by the cultivated hemiepiphytic cactus, *Hylocereus undatus*. *Annals of Applied Biology* **144**: 1-8.
- Nobel PS and Hartsock TL. 1983. Relationships between photosynthetic active radiation, nocturnal acid accumulation, and CO₂ uptake for a Crassulacean acid metabolism plant, *Opuntia ficus-indica*. *Plant Physiology* **71**: 71-75.
- Nobel PS, Cui M, and Israel AA. 1994. Light, chlorophyll, carboxylase activity and CO₂ fixation at various depths in the chlorenchyma of *Opuntia ficus-indica* (L.) Miller under current and elevated CO₂. *New Phytologist* **128**: 315-322.
- Nobel PS, Pimienta-Barrios E, Zañudo-Hernández J, and Ramírez-Hernández B. 2002. Historical aspects and net CO₂ uptake for cultivated Crassulacean acid metabolism plants in Mexico. *Annals of Applied Biology* **140**: 133-142.

- Pimienta Barrios E. 1990. *El nopal tunero*. Universidad de Guadalajara.
- Pimienta-Barrios E. 1999. *El Pitayo en Jalisco y Especies Afines en México*. Universidad de Guadalajara.
- Pimienta-Barrios E and Nobel PS. 1994. Pitaya (*Stenocereus* spp., Cactaceae): an ancient and modern fruit crop of Mexico. *Economic Botany* **48**: 76–83.
- Pimienta-Barrios Eu, Nobel PS, Robles-Murguía C, Méndez-Morán L, Pimienta-Barrios En, and Yepez-González E. 1997. Ethnobotany, productivity, and ecophysiology of pitaya (*Stenocereus queretaroensis*). *Journal of the Professional Association for Cactus Development* **2**: 29–47.
- Pimienta-Barrios Eu, Zañudo J, Yepez E, Pimienta-Barrios En, and Nobel PS. 2000. Seasonal variation of net CO₂ uptake for cactus pear (*Opuntia ficus-indica*) and pitayo (*Stenocereus queretaroensis*) in a semi-arid environment. *Journal of Arid Environments* **44**: 73–83.
- Taiz L and Zeiger E. 2002. *Plant Physiology*, 3rd. edn. Sinauer Press.
- Valiente-Banuet A and Ezcurra E. 1991. Shade as a cause of the association between the cactus *Neobuxbaumia tetetzo* and the nurse plant *Mimosa luisana* in the Tehuacán Valley, Mexico. *Journal of Ecology* **79**: 961–971.
- Zeder MA. 2005. Central questions in the domestication of plants and animals. *Evolutionary Anthropology* **15**: 105–117.