

Ecological physiology of *Pereskia guamacho*, a cactus with leaves

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ABSTRACT

The specialized physiology of leafless, stem-succulent cacti is relatively well understood. This is not true, however, for *Pereskia* (Cactaceae), the 17 species of leafy trees and shrubs that represent the earliest diverging lineages of the cacti. Here we report on the water relations and photosynthesis of *Pereskia guamacho*, a small tree of the semiarid scrubland of Venezuela's Caribbean coast. Sapwood-specific xylem conductivity (K_{sp}) is low when compared to other vessel-bearing trees of tropical dry systems, but leaf-specific xylem conductivity is relatively high due to the high Huber value afforded by *P. guamacho*'s short shoot architecture. *P. guamacho* xylem is not particularly vulnerable to drought-induced cavitation, especially considering the high leaf water potentials maintained year round. This is confirmed by the lack of significant variation exhibited in K_{sp} between wet and dry seasons. In the rainy season, *P. guamacho* exhibited C₃-like patterns of stomatal conductance, but during a prolonged drought we documented nocturnal stomatal opening with a concomitant accumulation of titratable acid in leaves. This suggests that *P. guamacho* can perform drought-induced crassulacean acid metabolism (CAM photosynthesis), although $\delta^{13}C$ values imply that most carbon is assimilated via the C₃ pathway. *P. guamacho* leaves display very low stomatal densities, and maximum stomatal conductance is low whether stomata open during the day or night. We conclude that leaf performance is not limited by stem hydraulic capacity in this species, and that water use is conservative and tightly regulated at the leaf level.

Key-words: Cactaceae; CAM photosynthesis; Huber value; hydraulic conductivity; physiological evolution; xylem vulnerability curve; water relations.

INTRODUCTION

The Cactaceae are a well-known lineage of stem succulent plants that are widely recognized for their specialized adaptations to drought. These include an extensive and shallow root system with dynamic hydraulic properties that allow

for maximum water uptake following rain, a succulent pith and cortex with high capacitance for cellular water storage, a long-lived stem cortical tissue system that has replaced leaves as the primary photosynthetic organ, and crassulacean acid metabolism (CAM photosynthesis), which minimises transpirational water loss by allowing stomata to open during the night when the vapor pressure deficit is lower (Barcikowski & Nobel 1984; Gibson & Nobel 1986; Kluge & Ting 1978; Lerdau *et al.* 1992; Martre, North & Nobel 2001; Mauseth 1993; Nobel 1977, 1988; North & Nobel 1996; Preston 1900; Szarek & Ting 1975). While cacti inhabit a variety of environments, the lineage has diversified extensively in the New World's desert and semiarid regions.

Pereskia Miller (Cactaceae) comprises 17 species of leafy trees and shrubs that lack many specialized anatomical cactus traits and are thought to represent the 'ancestral' condition of the leafless, stem-succulent cacti (Gibson & Nobel 1986; Leuenberger 1986; Mauseth & Landrum 1997). Recent molecular phylogenetic work indicates that '*Pereskia*' is actually a paraphyletic grade of taxa at the base of the cacti (Edwards, Nyffeler & Donoghue 2005), which improves the potential for *Pereskia* species to inform us about early cactus evolution (Fig. 1). For example, only *Pereskia* species united with the 'core cacti' possess stem stomata and delayed bark formation, two traits that were probably important steps in the early evolution of stem-based photosynthesis. Many previous greenhouse studies have investigated the photosynthetic behaviour of *Pereskia* species (Rayder & Ting 1981; Gibson & Nobel 1986; Nobel & Hartsock 1986, 1987; Martin & Wallace 2000), and small amounts of stem photosynthesis have only been recorded in *P. horrida* Kunth (D.C.) (Martin & Wallace 2000). This suggests that stem photosynthesis did not fully develop in the cacti until further modifications of the cortical tissue evolved (for discussion, see Edwards *et al.* 2005).

The greenhouse studies also suggest that CAM photosynthesis is limited in *Pereskia*. The species chosen for these studies are well sampled across the *Pereskia* phylogeny, and allow us to infer that the CAM-cycling ability was established very early in the cactus lineage, prior to the divergence of the basal *Pereskia* lineage and prior to the evolution of stem stomata and delayed bark formation (Fig. 1). While CAM-cycling and/or drought-induced CAM-idling have been documented in most species used in

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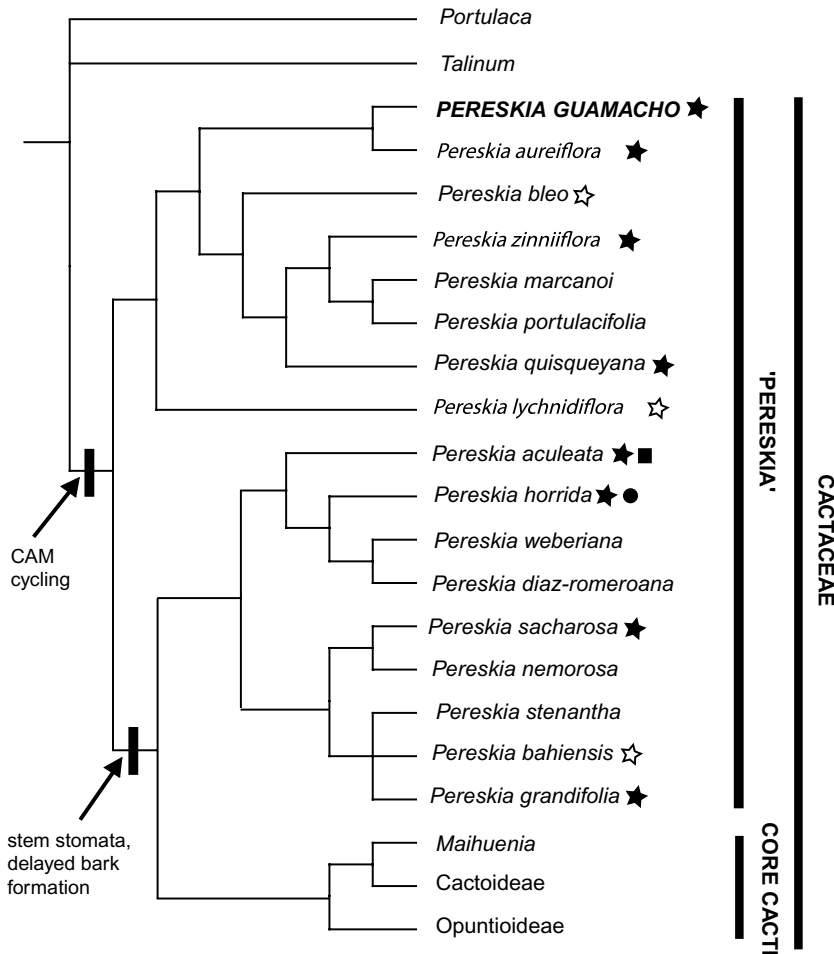


Figure 1. Basal cactus phylogeny inferred from molecular sequences of five gene regions, adapted from Edwards *et al.* (2005). *Pereskia* is paraphyletic, and consists of three major clades, two of which are more closely related to the rest of the cacti. *P. guamacho* is a member of the earliest diverging cactus lineage, and therefore lacks stem stomata and delayed bark formation. Species with stars have previously been investigated for photosynthetic pathway variation: a black star indicates that CAM-cycling has been recorded; a square indicates drought-induced CAM-idling; a circle indicates a limited degree of stem photosynthesis. Ancestral trait reconstruction was inferred using parsimony methods.

the greenhouse studies, none have exhibited any nocturnal CO₂ uptake under any conditions. In two wild populations of *Pereskia guamacho* F.A.C. Weber in northern Venezuela, however, Diaz (Diaz 1984; Diaz & Medina 1984) observed increases in leaf conductance after midnight with a small concomitant increase in titratable acidity during a drought, suggesting weak drought-induced CAM photosynthesis. It was hypothesized that while the CAM photosynthetic pathway may not play a significant role in total carbon assimilation, inducing even small amounts of night fixation during droughts may still be adaptive by maintaining a positive leaf carbon balance and allowing for a longer leaf lifespan.

While *Pereskia* photosynthetic physiology has received considerable attention, little is known regarding any aspect of *Pereskia* water relations. They have typically been described as drought deciduous, 'mesic' plants (Mauseth & Landrum 1997; Mauseth 1999), suggesting that *Pereskia* water relations are different from those of the specialized core cacti and that *Pereskia* regulates water loss in the same way as a typical C₃ woody plant. Whole plant water-use regulation is a complex process, however, and co-occurring 'typical C₃ woody plants' consistently show vast differences in growth phenology, rooting structure, hydraulic efficiency, safety from hydraulic failure, stomatal conductance, oper-

ational tissue water deficits and photosynthetic water-use efficiency (Langan, Ewers & Davis 1997; Comstock 2000; Pockman & Sperry 2000; Brodribb, Holbrook & Gutierrez 2002; Bombelli & Gratani 2003; Brodribb *et al.* 2003; Nardini *et al.* 2003; Sack *et al.* 2003; Ackerly 2004; Cavender-Bares *et al.* 2004).

Pereskia species, when considered as the extant cacti that best represent the 'ancestral cactus', present a unique opportunity to infer how the cacti regulated their water use prior to the evolution of leaflessness and a photosynthetic, succulent stem. As the first part of a broad survey that aims to characterize the physiological ecology of species in each of the main *Pereskia* clades, here we report on the water relations and photosynthetic behaviour of wild populations of *Pereskia guamacho*.

MATERIALS AND METHODS

Study site

Pereskia guamacho grows as a small tree in the semiarid coastal regions of Colombia and Venezuela, and is relatively common from sea level to 800 m. Our two study populations (hereafter referred to as the 'Arenales' and

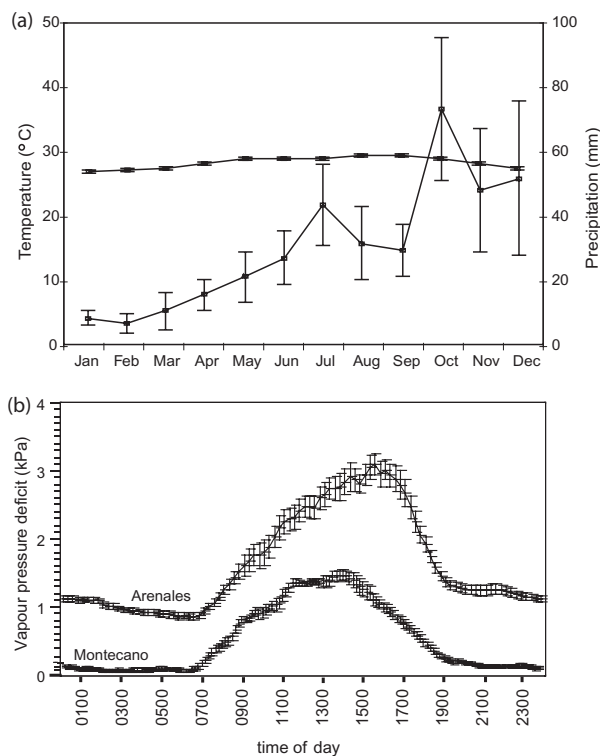


Figure 2. Climate data for the Paraguaná peninsula, Venezuela. Error bars are ± 1 SE. (a) Walter climate diagram for Coro, Venezuela, showing mean monthly temperature and precipitation from 1989 to 2003. (b) Daily flux in vapour pressure deficit (VPD) for Arenales and Montecano field sites in May 2003. VPD was calculated from temperature and relative humidity data. Curves represent the average VPD from 5 May to 13 May 2003.

'Montecano' sites) were located approximately 60 km apart, in the very dry forest of Venezuela's Paraguaná peninsula and adjacent coastal zones. This semiarid region is characterized by localized, infrequent rainfall events that are generally concentrated October through December, with a mean annual rainfall of approximately 500 mm (Fig. 2a).

The plant community in both the Arenales and the Montecano sites is dominated by drought-tolerant vegetation, i.e. *Cereus horrispinus* Backeb., *Opuntia wentiana* Britton & Rose, *Capparis odoratissima* Jacq., *Acacia tortuosa* L. (Willd.), *Prosopis juliflora* (Swartz) D.C., and *Croton heliaster* S.F. Blake. Montecano is located in the centre of the peninsula, and is unique in that atmospheric relative humidity rises to 100% every night due to saturated air moving inland from the Caribbean Sea.

Field measurements at both sites were undertaken from April to May 2003, and again in December 2003. Daily and monthly fluctuations of air temperature and relative humidity (RH) were monitored at both sites using portable HOBO temperature/RH weather stations (Onset Corporation, Bourne, MA). Yearly temperature and precipitation data were obtained from the U.S. National Climate Data Center (<http://www.ncdc.noaa.gov/oa/ncdc.html>). Rainfall usually increases slightly in April–September; in 2003, how-

ever, the region was experiencing a pronounced drought, and April and May were uncharacteristically dry. The general phenological patterns of the plant communities at the two sites were similar, even though the April–May drought was intensified in Arenales by higher daily temperatures and lower RH (Fig. 2b). Herbaceous undergrowth was sparse in both sites at this time, and some deciduous trees in Arenales had begun leaf shedding. Our second round of measurements, which followed heavy rains in November, found understory and canopy foliage abundant and green at both sites.

Water relations

Leaf traits

Minimum and maximum leaf water potential (Ψ_{\min} and Ψ_{\max}) were measured using a Scholander pressure chamber equipped with a compression gland cover (PMS Instruments Inc., Corvallis, Oregon). Due to the very short petiole we typically wrapped a fresh leaf in Parafilm (American National Can, Chicago, USA) and then excised a small amount of mesophyll surrounding the midvein to fit the leaf into the chamber lid. We also estimated leaf water potential by keeping the leaf attached to the stem and reading the endpoint from the distal twig. These readings were consistently about 0.1–0.2 MPa less negative than reading from the leaf alone. Numbers reported here are those for leaves with excised tissue.

At Montecano (in May and December) and Arenales (May, see Results section), multiple leaves were collected from three trees and pooled for estimates of mean leaf area (m^2), specific leaf area ($SLA = \text{fresh leaf area}/\text{dry mass}$, $m^2 \text{ kg}^{-1}$) and leaf water content ($LWC = \text{fresh mass} - \text{dry mass} / \text{dry mass}$, $g_{\text{water}} g_{\text{dry mass}}^{-1}$). Guard cell length and stomatal density were measured on five leaves per population in May and December, and a mean stomatal pore index [$SPI = (\text{stomatal density})(\text{guard cell length})^2$, $mm^{-2} mm^2$] was calculated. A tight correlation between SPI and whole leaf laminar conductance (K_{lamina}) has been demonstrated recently (Sack *et al.* 2003). Measuring K_{lamina} directly in *P. guamacho* was problematic due to succulence and, for all practical purposes, a lack of a petiole, so instead we used SPI as a proxy for K_{lamina} .

Stem hydraulic conductivity

Long shoots more than twice as long as the longest vessel (longest vessel ~ 40 cm) were cut from trees in the early morning and immediately placed in large plastic bags. From these branches, segments longer than 40 cm were recut under water in the laboratory and attached to a steady-state flow meter (Zwieniecki *et al.* 2000). Stem hydraulic conductivity (K_H ; $kg \text{ m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$) was measured by perfusing stems with a 10 mM KCl solution, using a constant driving force generated with hydrostatic pressure (~ 0.01 – 0.015 MPa). Readings were recorded only after steady-state conditions were reached. After each measurement, stems were then injected with safranin dye to estimate the

sapwood area, and the area of stained wood was measured using digital calipers and a stereo microscope. All leaves distal to the stem segment were collected and their outlines were traced while still fresh. Leaf area was later measured by running the traced leaf outlines through a leaf area meter (Li-Cor 3100, Licor Inc., Lincoln, Nebraska, USA). Sapwood-specific xylem conductivity was calculated as ($K_{sp} = K_H/\text{sapwood area}$), and leaf-specific xylem conductivity was calculated as ($K_L = K_H/\text{distal leaf area}$). Huber value was calculated as ($HV = \text{sapwood area}/\text{distal leaf area}$).

Xylem vulnerability to drought-induced cavitation

We constructed our vulnerability curve using a positive pressure air injection method similar to that described in Melcher, Zwieniecki & Holbrook (2003). Briefly, seven branches approximately 1.0 m in length were cut during the early morning; bagged, brought to the lab and allowed to rehydrate with cut ends in water. Stem segments > 40 cm were recut under water, attached to the flow meter, and the K_H value was recorded. The distal end of the stem was then sealed into a pressure chamber and subjected to 1 min of positive pressure (beginning with 0.5 MPa), with the proximal end of the stem submerged in KCl solution. After the stem had re-equilibrated to ambient pressure with both ends submerged in KCl solution, it was re-attached to the flow meter and the new K_H was recorded. The degree of cavitation caused by pressure injection P was calculated as percent loss conductivity ($PLC = 1 - (K_{\text{original}} - K_P)/K_{\text{original}}$). The entire process was then repeated, incrementally increasing the air-injection pressure by 0.5 MPa, until PLC neared 100%.

Traits related to photosynthesis and stomatal conductance

Stomatal conductance curves

We used a Li-Cor 1600 steady-state porometer to estimate stomatal conductance (g_s) of leaves from multiple trees in both populations. At Arenales, we made rounds of measurements approximately every two hours over a thirty-hour period. At Montecano the nightly 100% RH prevented reliable measurements with the porometer during the night, so our conductance curves at this site are only predawn to dusk. We confirmed our porometer data by creating leaf surface impressions with a quick-hardening dental epoxy (President light, Coltene brand, Net 32, Inc., Cary, North Carolina, USA) to abaxial leaf surfaces in the field. Later, the epoxy casts were coated with clear nail polish to create a positive impression of the leaf surface, and the nail polish was inspected for open stomata using a standard light microscope.

Diel acid fluctuation

We measured C_4 acid accumulation by collecting three leaves from each study tree at each round of stomatal mea-

surements. A known area of tissue was sampled from each leaf, immediately wrapped in aluminium foil and frozen with dry ice. In the laboratory, each leaf sample was boiled in glass-distilled water and ethanol to extract the acids. After all of the ethanol had evaporated from the sample, the acid content was measured by titration to pH 7 with 0.01 M NaOH. We then divided the total added NaOH by two (to account for the two H^+ ions donated by each C_4 acid) to obtain an estimate of the total sample acid content as $\mu\text{mol cm}^{-2}$. Due to the relatively thin leaves of *P. guamacho* (when compared to thickness of photosynthetic cortical tissue of other cacti), a more meaningful comparative number may be $\mu\text{mol g}^{-1}$ fresh weight, thus we estimated a relationship between cm^{-2} and fresh weight (g) separately, and report final numbers in $\mu\text{mol g}^{-1}$ fresh weight.

Photosynthetic water-use efficiency ($\delta^{13}C$)

Stable carbon isotope ratios of leaf tissue were used as a time-integrated measure of photosynthetic water-use efficiency (Farquhar, O'Leary & Berry 1982). Carbon stable isotope ratios of bulk leaf tissue were measured for multiple trees from both study sites and from both wet and dry seasons. Three leaves from each individual were dried, bulk ground with mortar and pestle, and subsampled. The $^{13}C/^{12}C$ ratios were determined for carbon dioxide collected from the samples after combustion, using a Finnigan MAT delta E isotope ratio mass spectrometer. Numbers here are expressed relative to the PDB standard using the equation:

$$\delta^{13}C = 1000(^{13}C/^{12}C_{\text{sample}}/^{13}C/^{12}C_{\text{standard}} - 1)$$

RESULTS

Xylem hydraulics

Stem hydraulic capacity

Table 1 lists stem hydraulic parameters for both populations measured in May and again in December. As the Arenales population was essentially leafless in December (see Results section), K_L and HV were recorded for May only. While there were slight differences in K_{sp} , K_L and HV, both between the two sites and between seasons, no pairs of values were significantly different (Tukey HSD, $P > 0.05$), and all measurements were pooled to estimate a species average.

Stem xylem vulnerability to drought-induced cavitation

High pressure air injection of *P. guamacho* stems resulted in a strong cavitation response (Fig. 3), though sealing problems prevented us from obtaining high enough pressures to induce 100% PLC. The shape of the curve is relatively shallow, with 10% PLC occurring at 0.5 MPa and 50% PLC occurring at 2.0 MPa.

Table 1. Stem hydraulic parameters of two populations of *Pereskia guamacho* during a drought (May) and rainy season (December) in 2003. Dashes indicate that leaf drop had occurred at this time, making it impossible to estimate K_L and Huber value

	May 2003		December 2003		
	Arenales <i>n</i> = 4	Montecano <i>n</i> = 5	Arenales <i>n</i> = 7	Montecano <i>n</i> = 4	Pooled <i>n</i> = 11, 20
K_{sp} (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	0.97 ± 0.25 SE	0.68 ± 0.16 SE	0.76 ± 0.18 SE	1.09 ± 0.51 SE	0.85 ± 0.12 SE
K_L (kg m ⁻¹ s ⁻¹ MPa ⁻¹ 10 ⁻⁴)	6.71 ± 1.96 SE	4.46 ± 0.95 SE	–	5.11 ± 2.3 SE	5.35 ± 0.95 SE
Huber value (10 ⁻⁴)	6.76 ± 0.55 SE	6.79 ± 0.53 SE	–	4.82 ± 1.0 SE	6.19 ± 0.46 SE

Leaf characteristics and gas exchange

P. guamacho leaves exhibited drastic functional differences during the study period, and plants from the two study sites did not show any phenological coordination either with each other or with their respective plant communities.

Montecano

When our study began in mid-April 2003, the Montecano population of *P. guamacho* had recently flushed a new set of leaves following several days of rain in late March (M. Diaz, unpublished observation). After the brief rains in March, there was no substantial rainfall in April and May, and we did not record any daytime stomatal opening or significant C₄ acid accumulation for the Montecano population during this time (data not shown). Ψ_{leaf} did not show any significant diurnal variation either, with a mean of 0.48 MPa (± 0.02 SE, *n* = 9 for each measurement round). Upon returning in December, the same population of leaves (determined by locating previously marked leaves) had expanded considerably in area, decreased in SLA and stomatal density, increased in guard cell length, mean epidermal cell surface area, and total water content (Fig. 4), and was exhibiting C₃-like patterns of stomatal conductance (Fig. 5a). $\delta^{13}C$ shifted significantly from -24.2‰ in May to -26.3‰ in December (Tukey-Hamer, *P* < 0.05, *n* = 3 trees, 3 leaves per tree bulk ground). Ψ_{leaf} showed diurnal variation in December, with predawn Ψ_{max} averaging -0.43 MPa (± 0.04 SE, *n* = 6), while Ψ_{min} , occurring in late afternoon, averaged -0.95 MPa (± 0.02 SE, *n* = 6).

Arenales

In May 2003, the Arenales population of *P. guamacho* had leaves that were at least five months old (M. Diaz, unpublished observation), and exhibited CAM-like patterns of stomatal conductance (Fig. 5a). Our stomatal curves correspond with a small but significant increase in C₄ acid accumulation throughout the evening, with a maximum value of 29.8 $\mu\text{mol g}^{-1}$ at 0600 h, and a minimum of 9.9 $\mu\text{mol g}^{-1}$ at 1830 h (Fig. 5b). The greatest rate of C₄ acid accumulation occurred between 0400 and 0600 h, which coincides with the period of maximal stomatal conductance. Ψ_{leaf} did not show any large diurnal fluctuations during this time, though a mean Ψ_{min} of -1.0 MPa (± 0.04 SE, *n* = 9) was recorded at

dawn. Arenales leaves had significantly higher water content, SPI and lower stomatal densities than Montecano leaves in May (Fig. 4). By December, the Arenales population of *P. guamacho* had shed nearly all of its leaves, standing in stark contrast to the surrounding vegetation.

DISCUSSION

The unusual weather patterns that coincided with the timing of our study allowed us to observe *P. guamacho* during a typical growing season (December 2003), while also providing the opportunity to examine how *P. guamacho* responds to an extended drought in the field (May 2003).

Xylem hydraulics

K_{sp} , K_L and HV

A mean K_{sp} of 0.85 kg m⁻¹ s⁻¹ MPa⁻¹ is quite low when compared with other studies of vessel bearing tropical dry for-

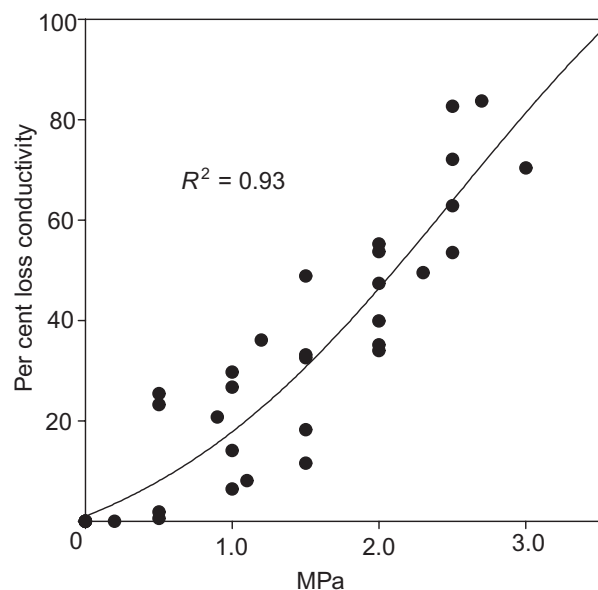


Figure 3. Stem xylem cavitation response. This vulnerability curve indicates the per cent loss of stem hydraulic conductivity due to embolism caused by applying various positive pressures to stem xylem. Data from both populations are pooled.

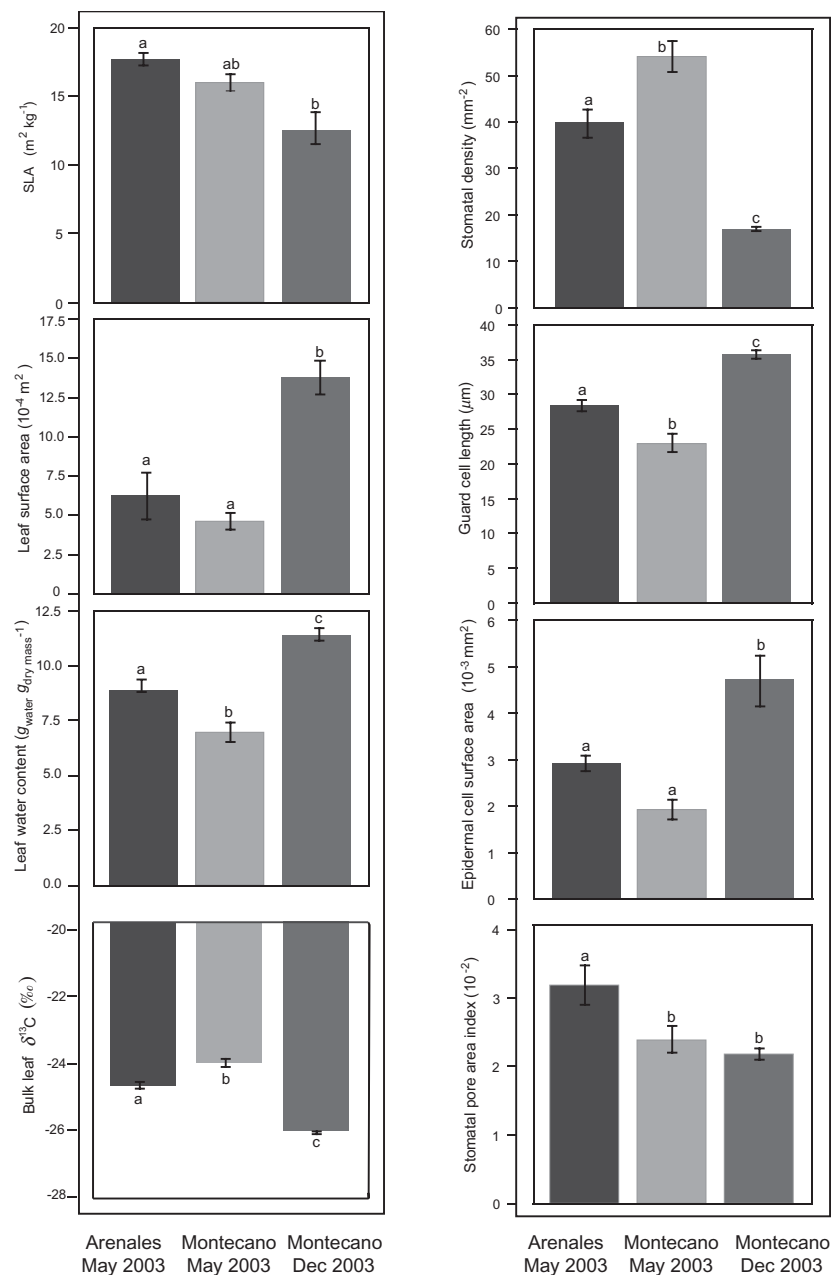


Figure 4. Variation in measured leaf traits between seasons and field sites. Error bars are ± 1 SE.

est trees, while a K_L of $5.35 \times 10^{-4} \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ is alternatively quite high (Brodrribb *et al.* 2002). This is due to a similarly high HV of 6.18×10^{-4} , indicating a high wood to leaf allocation pattern. The HV of *P. guamacho* is easily explained by its long shoot–short shoot architecture, which is reminiscent of many woody desert plants. *P. guamacho*, like many other *Pereskia* species, bears leaves directly on its areoles (the modified short shoot in the cacti that produces spines, flowers, and in the case of some *Pereskia* species, leaves). This allows leaves to be directly connected to older wood, alleviating the need for the new stem growth that generally accompanies leaf flush. Assuming that stem xylem is utilized in water transport for multiple

seasons, this short shoot system bypasses the hydraulic bottleneck presented by the limited secondary growth of current year xylem. Thus, for a given K_{sp} , K_L will be elevated, allowing for a greater supply of water to the leaves. The prevalence of long shoot–short shoot architecture in woody desert plants is often attributed to the increased ability to rapidly produce new leaves in response to rains. The relationship between K_{sp} , K_L and HV seen here, however, suggests that the evolution of a short shoot system may also reflect a mechanism for lineages with low K_{sp} to increase water supply to transpiring leaves without evolving wood that is inherently more efficient in transporting water.

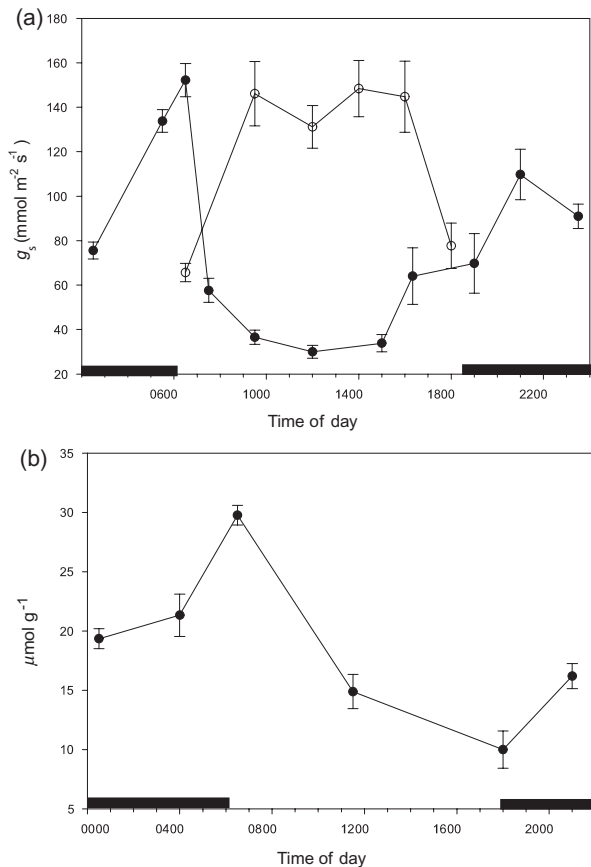


Figure 5. Variation in stomatal behaviour. Error bars are ± 1 SE. (a) Stomatal conductance curves. Dark circles are Arenales in May, open circles are Montecano in December, dark bars along the x-axis indicate night time. (b) C_4 acid accumulation in Arenales leaves in May.

Stem hydraulic capacity, drought-induced embolism and leaf function

Many recent studies have examined the potential coordination between stem hydraulic properties and leaf function (Cochard & Tyree 1990; Tyree & Ewers 1991; Brodribb & Hill 1999; Nardini & Pitt 1999; Brodribb & Feild 2000; Nardini & Salleo 2000; Pockman & Sperry 2000; Sperry 2000; Cavender-Bares & Holbrook 2001; Brodribb *et al.* 2002, 2003; Sperry *et al.* 2002; Preston & Ackerly 2003; Sack *et al.* 2003). The conclusions gathered from most of this work are: (1) stem hydraulic capacity and leaf photosynthetic capacity are closely linked; (2) stem hydraulic capacity and xylem cavitation resistance are often negatively correlated, such that plants with higher K_{sp} are also more vulnerable to embolism; (3) plants often operate at a Ψ_{leaf} that is close to a Ψ that would induce substantial xylem cavitation in the stem; and (4) in seasonal environments, K_{sp} is dynamic due to the formation of drought or freezing induced emboli, and that seasonal reductions in K_{sp} are often correlated with leaf shedding.

Our populations of *Pereskia guamacho* do not fit neatly into this conceptual framework. As mentioned above, K_L

in *P. guamacho* is relatively high in comparison to other investigated trees living in dry tropical systems, but maximum stomatal conductance (g_s , our proxy for photosynthetic rates) peaked at $\sim 150 \text{ mmol m}^{-2} \text{ s}^{-1}$, which is low when compared either to species in other studies of tropical dry forest trees (Brodribb *et al.* 2003) or to co-occurring species in Arenales and Montecano ($250\text{--}400 \text{ mmol m}^{-2} \text{ s}^{-1}$, data not shown). Similarly, K_{lamina} (using SPI as a proxy) is among the lowest recorded for any broad-leaved plant (Sack & Tyree 2005); unfortunately, to the best of our knowledge, this is also the first documentation of SPI of a succulent leaf. More work is needed to determine both whether the $\text{SPI}/K_{\text{lamina}}$ relationship holds for succulent tissues, as well as whether low SPI values are typical for succulent plants. The lowest Ψ_{min} recorded throughout our study was -1.2 MPa ; placed in the context of our constructed vulnerability curve (Fig. 3), this Ψ_{min} would induce less than a 20% loss of stem xylem conductivity. The slight and statistically insignificant decline in K_{sp} during leaf shedding supports the conjecture that leaves are not operating near a cavitation threshold, and stem xylem embolism formation does not play a major role in *P. guamacho* water relations.

These data present an apparently uncommon scenario of coordination between stem hydraulic properties and leaf function: high K_L , low g_s , low K_{lamina} , high Ψ_{min} and relatively cavitation resistant xylem lead to a consistent and reliable water delivery system to leaves. Conservative stomatal behaviour avoids the development of excessive tensions in stem xylem, and the maintenance of an intact water column in the stem allows for the continued absorption of soil water in response to rainfall events. The maintenance of high plant Ψ_{min} in the face of drought requires that the plant become decoupled from the soil, as soil Ψ_{min} in Arenales in May exceeded -2.0 MPa (estimated from Kelmhorst KS-D1 soil moisture meter and predawn Ψ_{max} of an assortment of co-occurring tree species). The maintenance of Ψ_{min} higher than Ψ_{soil} is a trait that is commonly observed in the core cacti, and is an integral component of their ability to utilize stored water in times of drought. Research on several species of core cacti has documented large reductions in root radial and axial hydraulic conductivity in response to soil drying that are partly reversible with rewetting of the roots (North & Nobel 1992; North & Nobel 1996; Martre *et al.* 2001). Given the high Ψ_{min} maintained year round in these *P. guamacho* populations, it seems plausible that their roots are responding to soil moisture in a similar way, suggesting that these mechanisms were present in the cactus lineage prior to the evolution of the massively succulent stems of the core cacti.

Leaf plasticity and phenology in *Pereskia guamacho*

The leaves of *P. guamacho* exhibited a striking diversity both in their structure and function during the timeframe of our study. The nocturnal stomatal opening exhibited by the Arenales population in May (Fig. 5a) confirms the

results obtained by Diaz (1984), and provides important evidence that *P. guamacho* has the ability to perform CAM-like patterns of gas exchange. While nocturnal stomatal opening has been documented in non-CAM plants (Donovan *et al.* 1999; Snyder, Richards & Donovan 2003) the small but concomitant accumulation of C₄ acids observed in our study (Fig. 5b) leads us to conclude that *P. guamacho* was weakly performing drought-induced CAM photosynthesis. It is not surprising that *P. guamacho*, previously known to be a CAM-cycling plant, may also occasionally fix atmospheric carbon at night. Extensive research of CAM plants over the past several decades has revealed a remarkable level of plasticity in the deployment of particular components of the CAM pathway (Borland *et al.* 1992; Cushman 2001; Winter & Holtum 2002). It also makes sense in light of recent phylogenetic work that clearly places the Cactaceae within the Portulacaceae (Hershkovitz & Zimmer 1997; Applequist & Wallace 2001; Edwards, Nyffeler & Donoghue, 2005), a lineage that contains many of the known facultative CAM species (Martin & Zee 1983; Herrera, Delgado & Paragatay 1991; Martin & Harris 1993; Smith & Winter 1996; Guralnick & Jackson 2001). Carbon isotopic discrimination values (Fig. 4) suggest that the C₃ pathway is the primary mode of photosynthesis for *P. guamacho*, however (Kluge & Ting 1978; Griffiths 1992; Winter & Holtum 2002). While drought-induced CAM may not contribute substantially to carbon assimilation in this species, it may still be beneficial in prolonging leaf lifespan in an environment with highly unpredictable precipitation patterns. The significant differences in $\delta^{13}\text{C}$ exhibited between sites and seasons could result either from associated changes in photosynthetic water-use efficiency, or from possible shifts between C₃ and CAM pathways. More work is needed to differentiate between these two possibilities.

P. guamacho stomatal densities are remarkably low when compared to other leafy arid plants (Fahmy 1997), and are actually more similar to stem epidermal stomatal densities reported from a handful of core cacti species (Mauseth & Sajeve 1992) and other succulents (Kluge & Ting 1978). Neither mean stomatal densities nor SPI appeared to significantly affect the maximum g_s values that we recorded (Figs 4 & 5A), suggesting either that maximal stomatal aperture was reduced in the Arenales population, or that a percentage of stomata were no longer functional. While guard cell length was significantly longer in Montecano leaves (Fig. 4), we still favour the latter explanation, due to the inability of some guard cells from Arenales leaves to become turgid after the epidermis had been removed and soaked in distilled water (E. Edwards, unpublished observation).

Perhaps the most interesting aspect of *P. guamacho* leaf function lies in its unusual phenological behaviour. Conventional wisdom holds that *Pereskia* species are drought deciduous (Leuenberger 1986; Luttge *et al.* 1989; Mauseth 1999), but here we have documented *P. guamacho* responding to drought either by employing low levels of CAM photosynthesis (Arenales in May), or, apparently, by arresting leaf activity and establishing a 'holding pattern' of sorts

(Montecano in May). Ironically, the only leaf shedding event we witnessed occurred during the rainy season. It is currently unclear as to what triggers either leaf drop or leaf flush in this species, as both populations were asynchronous with one another and their respective plant communities. Leaf lifespan is similarly unknown, although observations both at Arenales (Diaz 1984) and Montecano (M. Diaz, unpublished observation) suggest that leaves can persist for more than one year. A detailed study of phenology and leaf survival in *P. guamacho* would help to not only clarify what triggers shedding and flushing in this species, but would also document more closely the highly unusual patterns of leaf expansion (Montecano, May–December) observed in this study.

In summary, we feel that *Pereskia guamacho* is in many ways behaving similarly to its leafless, stem-succulent relatives. It maintains high Ψ_{min} year round in the face of drought, it shows a high capacitance for acquiring and storing water, and stomata exhibit tight regulation of water loss. Drought-induced CAM photosynthesis can occur, although we do not know to what degree this usually happens, and suspect that CAM does not contribute significantly to carbon assimilation in this species. It is currently unclear if *P. guamacho* ecophysiology is representative of all *Pereskia* species, but if so, it follows that these physiological traits were present in the cactus lineage before the evolution of leaflessness and stem succulence. A careful reconstruction of the evolution of these traits among the basal cactus lineages will provide for a closer look at the interplay of anatomy and physiology during this major morphological transition.

ACKNOWLEDGMENTS

This research was funded in part by the Universidad Experimental Francisco de Miranda (Coro, Venezuela), a Deland Award for Student Research (Arnold Arboretum, Harvard University) to E.J.E., a Field Ecology Award (YIBS, Yale University) to E.J.E., and an NSF Graduate Student Fellowship to E.J.E. The authors wish to thank N.M. Holbrook for all of her generous logistical support and invaluable advice. We also are grateful to the C.I.E.Z.A. community and staff at the Montecano Biological Reserve (particularly to Srs. Esteban and Cedeño) for their contributions in the field and laboratory, to the Earth System Center for Stable Isotopic Studies (Yale University), to M.J. Donoghue for providing significant support and critical discussion, and to C.W. Dunn for his help with fieldwork. Comments from two anonymous reviewers, K. Mott and U. Luttge greatly improved our manuscript.

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Received 19 April 2005; received in revised form 13 June 2005; accepted for publication 16 June 2005