

Daily to decadal patterns of precipitation, humidity, and photosynthetic physiology recorded in the spines of the columnar cactus, *Carnegiea gigantea*

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[1] Isotopic analyses of cactus spines grown serially from the apex of long-lived columnar cactuses may be useful for climatological and ecological studies if time series can be reliably determined from spines. To characterize the timescales over which spines may record this information, we measured spine growth in saguaro cactus over days, months, and years with time-lapse photography, periodic marking, and postbomb radiocarbon dating and then analyzed isotopic variability over these same timescales and compared these measurements to local climate. We used daily increments of growth, visible as transverse bands of light and dark tissue in spines, as chronometers to develop diurnally resolved $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ records from three spines grown in series over a 70 day period. We also constructed a 22 year record of $\delta^{13}\text{C}$ variations from spine tips arranged in chronological sequence along the side of a 4 m tall, single-stemmed saguaro. We evaluated two mechanisms potentially responsible for daily, weekly, and annual variability in $\delta^{13}\text{C}$ values of spines; both related to vapor pressure deficit (VPD). Our data suggest that stomatal conductance is unlikely to be the determinant of $\delta^{13}\text{C}$ variation in spines. We suggest that either VPD-induced changes in the balance of nighttime- and daytime-assimilated CO_2 or mesophyll-limited diffusion of CO_2 at night are the most likely determinant of $\delta^{13}\text{C}$ variation in spines. Intra-annual and interannual variability of $\delta^{18}\text{O}$ in spine tissue appears to be controlled by the mass balance of ^{18}O -depleted water taken up after rain events and evaporative enrichment of ^{18}O in tissue water between rains. We were able to estimate the annual growth and areole generation rate of a saguaro cactus from its 22 yearlong isotopic record because VPD, rainfall, and evaporation exhibit strong annual cycles in the Sonoran Desert and these variations are recorded in the oxygen and carbon isotope ratios of spines.

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1. Introduction

[2] Climatic and physiological variations are integrated and recorded in the stable isotope ratios of carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) in the serially produced spines of saguaro cactus (*Carnegiea gigantea* (Engelmann) Britton and Rose) [English *et al.*, 2007]. Further development and broad application of spine isotopic time series (hereafter referred to

as spine series) in cactuses would be useful for documenting physiological responses to environmental variability and reconstructing past climatic events. Such records would be especially valuable where instrumental records are lacking, other land-based climate proxies are sparse (e.g., treeless deserts) and where cactus populations or cactus-dependent species are threatened with extinction [Godinez-Alvarez *et al.*, 2003]. For example, saguaro fruit is an essential food source for birds, bats and insects of the Sonoran Desert [Wolf and McKechnie, 2003], but little is known about the impacts of monsoons, hurricanes or El Niño enhanced winter rains on fruit production. An isotopic record that quantitatively links precipitation and cactus productivity to these climate phenomena could help forecast future ecological responses and improve management strategies for these species. Before spine series can be used in this way, we must: (1) reduce uncertainties in and develop robust techniques for determining when individual spines on a

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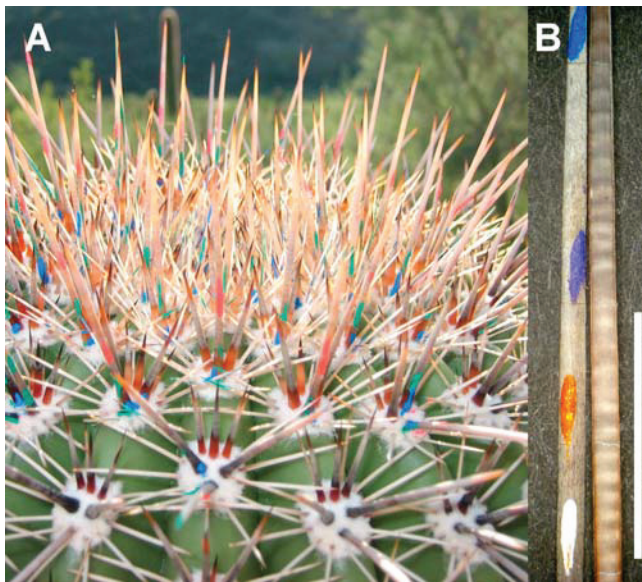


Figure 1. (a) Transverse bands visible in situ on growing spines atop a ~1 m tall saguaro cactus. Growing spines at the apex have yellow/orange fleshy bases. These spines bases were marked periodically with fabric paint (visible) to indicate spine growth over several months. (b) Transverse bands visible within a single bisected spine. The left and right half of the bisected spine are aligned, and the blue, purple, orange, and white paints were applied on 30 August and 9, 16, and 21 September 2006, respectively. The tip of the spine (older) is toward the top, and the white bar is 10 mm.

cactus grow (i.e., their “ages”); and (2) establish a clearer understanding of the processes and environmental parameters recorded in the isotopic composition of cactus spines. Our purpose here is to present several chronologies at different temporal scales of saguaro spine growth and to identify and evaluate probable physiological and climatic drivers of isotopic variability in spines. The temporal context provided by a spine series, when coupled with an understanding of isotopic variability in spines, can be used to link climate to plant or ecosystem productivity and to inform climate models in regions with relatively sparse historical climate data. In the work of *English et al.* [2007] we discussed the methods used to construct a chronology of saguaro spines using $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and F^{14}C . Here we will expand our observations to a larger range of timescales, subdaily to decadal, and show that: (1) the regularly spaced light and dark bands of tissue within and transverse to the long axis of spines (Figure 1, hereafter referred to as “transverse bands”) represent daily increments of growth; (2) there is a strong relationship between climate and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variability found in spines over scales of days, weeks and years; (3) $\delta^{13}\text{C}$ variation in spines is most likely not driven by stomatal conductance; and (4) F^{14}C and $\delta^{13}\text{C}$ in spines can be used to determine the growth rates of cactus without the need for repeated direct observation.

1.1. Saguaro Spine Growth and Anatomy

[3] The massive, long-lived (~125–175 years) saguaro cactus occurs throughout the Sonoran Desert of southwestern

Arizona and western Sonora, Mexico [*Turner et al.*, 1995]. This range roughly coincides with the region affected by the North American Monsoon, a period of strong convective storms between July and September. Saguaros use water derived from monsoon and winter precipitation [*Drezner and Balling*, 2002; *Drezner*, 2005], the latter strongly influenced by the El Niño-Southern Oscillation. As in other cactuses, spine tissue in saguaro grows from areolar meristems near the shoot (stem) apex (Figure 1) [*Mauseth*, 2006]. Areoles form on each elongate set of fused tubercles (the “rib” of the stem) and are displaced laterally on the large dome-shaped apex of the cactus stem as new areoles are produced on the upward extending apex [*Mauseth*, 2006]. The anatomical development and growth of cactus stems, areoles and spines over daily and annual cycles have been well studied [e.g., *Robinson*, 1974; *Gibson and Nobel*, 1986; *Gouws et al.*, 2005; *Mauseth*, 2006], but not with a focus on the chronology of spine growth or anatomical patterns over multiple time-scales. And while many studies have linked physiological and demographic changes in saguaro to climate variability over the last century, such as the El Niño-Southern Oscillation [*Pierson and Turner*, 1998; *Drezner and Balling*, 2002; *Drezner*, 2003, 2005], few have made direct links between morphological features of cactus growth and environmental conditions. *Buskirk and Otis* [1994] for example noted waxy, annual bands on the columnar cactus *Lemaireocereus aragonii* (Webb) and used these to accurately infer stem growth rates. More directly, *Nobel* [1988] has documented that water status, temperature, and photosynthetically active radiation (PAR) affect the overall productivity and areole production of many cactus species.

1.2. Saguaro Spines, F^{14}C , $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$

[4] Carbon and oxygen isotopes are incorporated in the molecular structure of the lignin-rich spine tissue as it emerges from the areole [*English et al.*, 2007]. Like isotopes in tree rings [*McCarroll and Loader*, 2004], the carbon and oxygen isotope ratios are retained for decades once fixed in spine tissue. Over the growing season when spines are added (April–October) [*Steenbergh and Lowe*, 1983], changing environmental conditions, plant physiological responses and water sources (i.e., North American Monsoons in summer or El Niño enhanced rains in the winter) alter the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of organic metabolite pools and stem water and these changes are recorded in growing spine tissue [*English et al.*, 2007]. However, unlike secondary wood growth in many trees, no easily observable anatomic patterns of cactus spine growth have been documented.

[5] The F^{14}C and $\delta^{13}\text{C}$ variability in a spine series can be used to determine the age of spines and the growth of saguaro stems [*English et al.*, 2007]. In addition, $\delta^{13}\text{C}$ variability might provide a useful record of past climate changes. While the F^{14}C is based on the dilution and removal of anthropogenic ^{14}C in the atmosphere produced by atmospheric nuclear testing [*Reimer et al.*, 2004], the $\delta^{13}\text{C}$ chronometer is based on interspine $\delta^{13}\text{C}$ cycles likely associated with variable ^{13}C fractionation during photosynthesis. Saguaro, like other cactuses, employ the Crassulacean acid metabolism (CAM) photosynthetic pathway to acquire atmospheric CO_2 for growth and this has consequences for the $\delta^{13}\text{C}$ of cactus tissue and spines. Thorough reviews of CAM physiology and isotope systematics can be found in the works of *Dodd*

et al. [2002], Black and Osmond [2003], and Griffiths et al. [2007]. In CAM plants, it is common for stomata to open at night (Phase I of the diurnal CAM cycle) during which CO₂ is fixed by phosphoenolpyruvate carboxylase (PEPC). Stomata close during the day when stored organic acids are decarboxylated (Phase III). This prevents water loss and maintains high CO₂ concentrations within photosynthetic tissues, leading to more efficient carboxylation by Ribulose-bisphosphate carboxylase (Rubisco).

[6] Aside from long-term changes in atmospheric $\delta^{13}\text{C}$ [Francey et al., 1999], mesophyll conductance [Griffiths et al., 2007] and post-CO₂ assimilation processes [Badeck et al., 2005], there are two possible mechanisms that may influence $\delta^{13}\text{C}$ values in CAM plant biomass. One is variation in nighttime (Phase I) stomatal conductance associated with changes in vapor pressure deficit (VPD) and the ratio of CO₂ partial pressures between the photosynthetic tissue and ambient air. Osmond et al. [1979a] have shown that nighttime stomatal opening in the cactus *Opuntia stricta* (Haworth) responded strongly and within hours to VPD. Other CAM species have also shown this response [Lange and Medina, 1979; Griffiths et al., 1986]. If stomata are closed during the morning and daylight hours (Phases II, III and IV), then only CO₂ derived from fixation by PEPC during Phase I is available for photosynthate production and carbon allocation to spine tissue growth. In this case, carbon isotope discrimination (Δ) during Phase I would be equivalent to that in a C₄ plant with no CO₂ leakage, represented by the model [after Farquhar et al., 1989]:

$$\Delta = a + (b_4 - a)p_i/p_a \quad (1)$$

where a is discrimination due to diffusion of CO₂ through stomata (4.4‰), b_4 is the net discrimination associated with fixation by PEPC (−5.7‰ in equilibrium with HCO₃[−] at 25°C), and p_i/p_a is the ratio of internal tissue to ambient partial pressures of CO₂. The balance of atmospheric CO₂ supply through stomata and CO₂ demand by PEPC reactions is reflected in the p_i/p_a value. This and the temperature dependence of b_4 are the only parameters that vary significantly in the above model. If daytime CO₂ exchange is negligible, then nighttime changes in stomatal opening, and thus p_i/p_a would account for measurable variation in $\delta^{13}\text{C}$ of the photosynthate pool [Roberts et al., 1997]. Indeed, drought and high nighttime atmospheric VPD have been previously shown to reduce stomatal conductance, p_i/p_a and Δ (increase in $\delta^{13}\text{C}$) in CAM plants [Lange and Medina, 1979; Osmond et al., 1979b; Griffiths et al., 1986; Roberts et al., 1997] and saguaro [Lajtha et al., 1997].

[7] The second possible mechanism of $\delta^{13}\text{C}$ variation in CAM plants is the time of day during which atmospheric CO₂ is fixed [Osmond et al., 1979b; Farquhar et al., 1989; Dodd et al., 2002; Winter and Holtum, 2002; Griffiths et al., 2007]. In saguaro, MacDougal and Working [1933] observed that stomata remained open well into midmorning in March, while Lajtha et al. [1997] demonstrated that stomata are closed all day in the dry premonsoon months. English et al. [2007] hypothesized that if stomata are open after dawn (Phase II) or before sunset (Phase IV) then carbohydrates in the photosynthate pool will be partially derived from Rubisco fixation of atmospheric CO₂ (leading to tissues with $\delta^{13}\text{C}$ approaching −27‰ [Winter and Holtum, 2002]). These

carbohydrates can be expected to mix later in the day and overnight with carbohydrates derived from CO₂ originally fixed by PEPC during Phase I [Winter and Holtum, 2002; Griffiths et al., 2007]. Only a small amount (~10%) of CO₂ fixed by Rubisco during Phase II or IV would be required to shift $\delta^{13}\text{C}$ in photosynthate by −1.8‰ [Winter and Holtum, 2002], a plausible amount given that Osmond et al. [1979b] observed atmospheric CO₂ fixation by Rubisco during Phase IV of CAM in *O. stricta* following rainfall accounting for 25% of the carbon in newly grown tissue. Irrigation of *O. stricta* also induced a burst of Phase II CO₂ fixation. Interestingly, Osmond et al. [1979b] did not observe a concurrent shift in $\delta^{13}\text{C}$ of whole stem tissues, probably because the contribution of carbon assimilated during the day was very small compared to the overall growth of the stem during the year. However, spine tissue may develop from more labile and immediate photosynthate pools isolating it from long-term, integrated carbohydrate reservoirs. As such, we predict that short-term changes in $\Delta^{13}\text{C}$ are most likely recorded permanently in spine tissue $\delta^{13}\text{C}$.

[8] Alternatively, Griffiths et al. [2007] finds that during CO₂ uptake at night (Phase I), diffusion limitation in the mesophyll of succulent CAM plants has a strong effect on ^{13}C discrimination. As conductance for CO₂ diffusion from intercellular airspaces to the cytosol decreases, Δ increases. Griffiths et al. [2007] found that the magnitude of this effect is strongly dependent on the assimilation rate, with higher assimilation rates leading to greater discrimination (higher Δ). In saguaro's, the result of this would be to decrease $\delta^{13}\text{C}$ during periods of high assimilation rates or during periods of high temperature [Bernacchi et al., 2002]. Very little is known about the variability of saguaro assimilation rates at any timescale, and so our current ability to evaluate this hypothesis is limited; however, it is very likely that future mechanistic models of carbon isotope variability in saguaros will incorporate mesophyll conductance.

[9] Variability in the $\delta^{18}\text{O}$ of spines is strongly influenced by isotopic changes of water in the stem of the cactus, especially at the apex, associated with plant transpiration and uptake of rainfall [English et al., 2007]. Evaporation from cactus tissue causes an enrichment of ^{18}O in stem water while water uptake after rainfall enriches ^{16}O in stem water [English et al., 2007]. There are strong vertical and lateral gradients in the $\delta^{18}\text{O}$ of water in the stem that add complexity to our interpretation of what determines $\delta^{18}\text{O}$ variation in spines. Gradients steepen during the premonsoon drought, and lessen during the monsoon and winter [English et al., 2007], but we do not know how they change over daily, weekly or interannual timescales.

2. Sampling and Analytical Methods

2.1. Transverse Bands Within Single Spines

[10] To determine how fast a saguaro spine may grow and at what time of day, we measured the incremental elongation of a single spine growing on a naturally established ~30 cm-tall saguaro cactus at the University of Arizona Desert Laboratory in Tucson, AZ (32.22°N, 111.00°W, 800 m elevation). We assume that spine elongation corresponds to spine growth (the addition of tissue mass). The target spine was marked with nontoxic white fabric paint at the position where the spine emerged from the areole (Figure 2). A scale

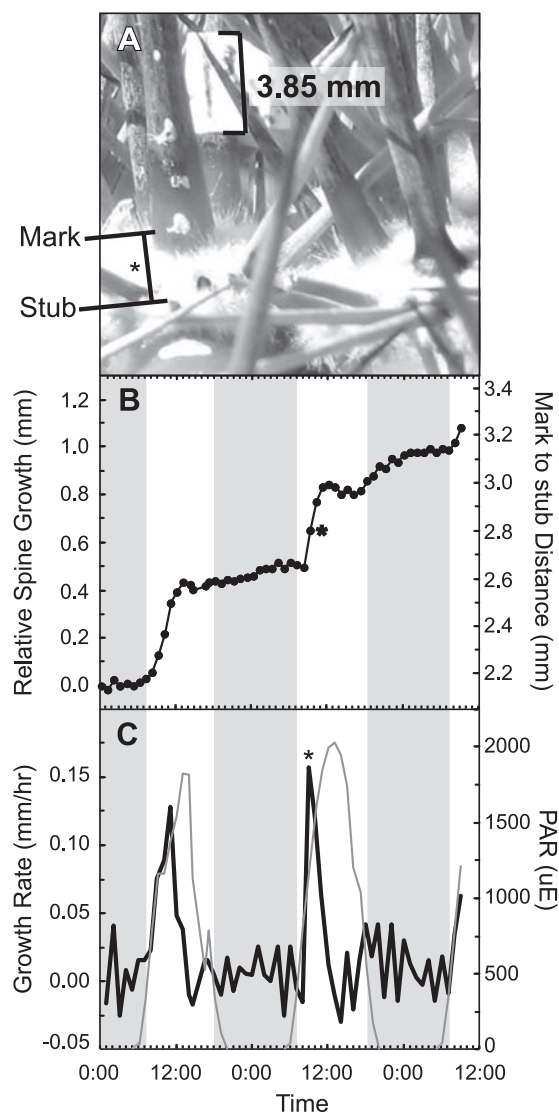


Figure 2. Linear spine growth and PAR over 2 days (16 and 17 May 2007). (a) Photograph of the spine, the fixed scale, and the reference marks as they appeared on 17 May at 8 A.M., the time of the maximum linear growth rate (asterisk). (b) The relative and actual linear distance between reference marks over time (i.e., spine growth). (c) The spine's linear growth rate (black) compared to hourly PAR measurements (dark gray). Grey boxes correspond to hours with less than 500 μE of light (i.e., nighttime).

bar was affixed to an adjacent spine that was no longer growing. The growing spine was photographed every hour over a 2 day period (16–17 May 2007) with a tripod-mounted, 10 megapixel camera with an automatic flash (Canon Power-shot A640, Canon Inc., Tokyo, Japan) connected to a remote computer running RemoteCapture Task software (version 1.7.0.4, Canon Inc.). For each image, the pixel length of the scale bar and the pixel length between the base of the cut spine and the white paint on the target spine were measured. To account for the angle of the camera and for changes in the focal length of the lens over the 24 h cycle, the scale bar was used to determine the pixel-to-millimeter conversion factor in each image. iMovie (Apple Corp., Cupertino, CA) was used

to build a time-lapse film of the growth from the images (Movie S1, available as auxiliary material).¹ A Hobo micro-meteorological station (Onset Computer Corp., Pocasset, MA), positioned approximately 10 m from the cactus, collected average hourly measurements of photosynthetically active radiation (PAR) and total rainfall over the same 2 day period. The cactus was shaded by a building after 3 P.M. during the 48 h observation period.

[11] To determine if transverse bands on spines represent increments of daily growth, fabric paint was used to periodically mark the apical spines of 13 randomly selected saguaro (0.4–1.8 m tall) within ~1 km of the University of Arizona Desert Laboratory. The base of growing and newly emerging spines was marked (Figure 1; visible as a soft, yellow to red tissue at the base of the spine) with different colored fabric paint six times between 13 August and 8 October 2006. On 2 November 2006, up to 10 randomly selected spines from each plant were collected by clipping them at the base. The collected spines were bisected longitudinally with a razor under a reflected-light microscope and the number of transverse bands (one light/dark couplet) between each marked period was counted starting with the most recent mark (closest to the base) as the known reference point (2 November). Possible counting errors on the marked spines resulted from: (1) difficulty in marking the base of a spine due to other spines nearby and a tuft of ~1 mm-tall trichomes that obscure the area of cell generation at the spine base; and (2) the paint occasionally sticking to the trichomes and then stretching while the spine grew, leaving a paint “flap” that obscured the actual placement of the original mark on the spine. The resulting band counts from 122 spines were analyzed with the statistical software package JMP 5.1.2 (SAS Institute, Cary, NC). A thin section of one spine was examined with both epifluorescent and normal transmitted-light microscopes to examine banding patterns.

2.2. Subdaily, Daily, and Annually Resolved Stable Isotope Records

2.2.1. Subdaily Records

[12] We investigated whether changes in $\delta^{13}\text{C}$ are present within spines and transverse bands. One spine, from the spines collected for the transverse bands experiment above, was sampled over five transverse bands (5 days growth) for $\delta^{13}\text{C}$ analysis ($n = 29$). Each sample represented approximately 1/4 to 1/6 of a day's growth (Figure 3). Sample preparation and stable carbon isotope analyses were identical to those described by English *et al.* [2007]. Reported values of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are in per mil (‰) relative to V-PDB and V-SMOW, respectively. The precision for our method, based on repeated analysis of working standards, was 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{18}\text{O}$.

2.2.2. Daily Records

[13] Three marked spines that grew consecutively (in series) were collected between 13 August and 8 October 2006 from the apex of a 1.12 m tall saguaro to examine the correlation between spine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and rainfall, VPD and modeled p_i/p_a (Data Sets S1–S4 and Table S1). Single marked spines from four other randomly selected cactuses

¹Auxiliary material data sets are available at <ftp://ftp.agu.org/apend/jg/2009/jg001008>. Other auxiliary material files are in the HTML.

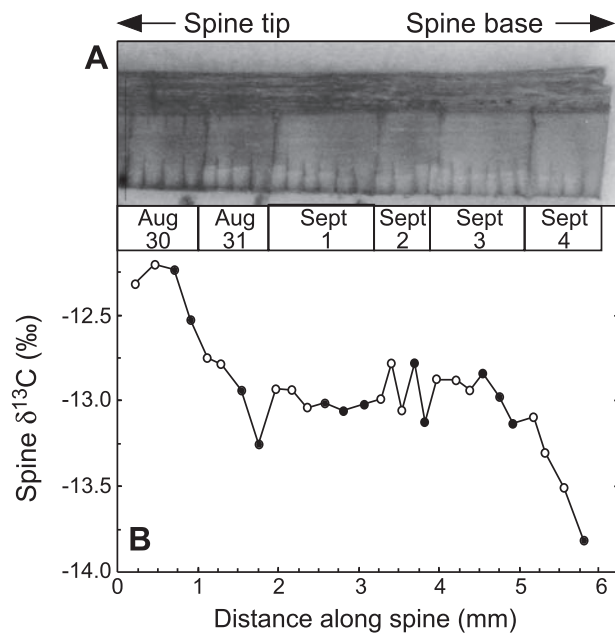


Figure 3. High-resolution $\delta^{13}\text{C}$ record from a saguaro spine grown in late August and early September. (a) Reflected light micrograph of the sampled spine with sample divisions (short dark lines on spine); 2 September was divided into one more sample than shown. (b) The $\delta^{13}\text{C}$ of sampled spine tissue (horizontal scale is the same as in Figure 3a). Solid circles are from dark banded tissue, and open circles are from light banded tissue.

were also collected to see if the isotopic variability expressed in spines was common across individual plants. All collected spines were bisected and digitally photographed using a reflected light microscope. Digital imaging and spectral analysis were used to enhance the contrast and to objectively determine banding, respectively. The surface brightness profile of each spine and the white background (as a reference) were measured using Image J 1.37v [Abramoff *et al.*, 2004; Rasband, 2009]. The surface brightness profile of the spine was corrected with the background brightness profile to account for vignetting near the edges of the image. The corrected surface brightness profile of the spine was spectrally analyzed and the peaks of the second and third components of the reconstructed singular spectrum analysis (RCSSA, K-Spectra 1.0.15A) [Ghil *et al.*, 2000] were used to demarcate individual bands (days) along each spine. Each spine was then laid atop an exact scale printout of the RCSSA and, using the printout as a guide, each band (day) was cut from the spine in series using a razor blade. Careful note was taken as to the location of the painted marks (date) in relation to the sampled bands (days). Each sample was then crushed or cut lengthwise (parallel to the axis of the spine) to provide whole-band subsamples for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis.

[14] In 2006, there was no on-site micrometeorological station at Tumamoc Hill. However, a local meteorological station (KOLD) at the Tucson International Airport (TIA) and Tumamoc Hill are both within city limits, although 12 km apart. To evaluate the usefulness of the TIA meteorological data as a proxy for Tumamoc Hill climate, an on-site

micrometeorological station was installed at Tumamoc Hill in 2008. Daily minimum temperature and maximum relative humidity from TIA (VPD calculated as by Wang *et al.* [2004]) generally reflect atmospheric conditions just before dawn and not throughout the entire night when gas exchange in cactus occurs [Lajtha *et al.*, 1997]. However, there is a significant relationship between VPD averaged hourly for each night ($\text{PAR} < 500\mu\text{E}$) at Tumamoc Hill and daily minimum VPD at TIA in 2008 (simple linear regression, t_{208} , $P < 0.0001$, Data Sets S1–S4 and Table S1). This relationship was used to estimate the hourly averaged nighttime VPD at Tumamoc Hill between August and October 2006 using the daily minimum VPD at TIA. Daily precipitation records are from an accumulating rain gage on Tumamoc Hill ~150 m from the sampled cactuses.

2.2.3. Annual Records

[15] A spine series from a 4 m-tall saguaro (hereafter referred to as SNPE A) in Saguaro National Park was collected on 27 June 2004 (32.21°N, 110.73°W, 870 m elevation). We sampled and recorded the height of one spine for $\delta^{13}\text{C}$ analysis from every areole along the length of one rib over the entire height of the cactus using the methods described in the work of English *et al.* [2007]. After cleaning the spines [see English *et al.*, 2007], their F^{14}C contents were measured at the Keck-Carbon Cycle Accelerated Mass Spectrometry facility at the University of California, Irvine. The software program Calibomb [Reimer *et al.*, 2004] was used to estimate a number of possible ages from spine F^{14}C values, the 95% confidence interval for each possible age, and a probability that each possible age is the correct age [English *et al.*, 2007]. A single finite age for a sampled spine was calculated using the average of all the possible ages weighted by the probability of their being correct. The error of that value was conservatively determined to be the youngest and oldest age from the 95% confidence intervals of all the possible post-1960s possible ages for each sample (2σ age range) [English *et al.*, 2007]. For the $\delta^{13}\text{C}$ spine series, years (1 January to 31 December) were demarcated using minima of the second and third components of the RCSSA (K-Spectra 1.0.15A) [Ghil *et al.*, 2000]. The number of sampled spines (one spine from each areole) within each year as determined by the spectral analysis was used to determine the number of areoles generated each year.

[16] A composite record of total monthly precipitation was constructed for 1980–2004 using data from the National Climate Data Center. Precipitation data for January 1980 through January 1992 are from the Tucson Magnetic Observatory (NCDC Coop ID 028800) and February 1992 to 2004 are from the Vail 7 North station (NCDC Coop ID 28998). Both stations are several kilometers from and bracket the sampled cactus. VPD was calculated using reconstructed monthly mean minimum temperature and mean dew point temperature from PRISM for this location (PRISM Group, <http://www.prismclimate.org>, 2008).

3. Results

3.1. Transverse Bands Within Single Spines

[17] The majority of spine growth and maximum spine growth rate ($\sim 0.16 \text{ mm h}^{-1}$) in a single spine measured occurred in the hours just after sunrise (Figure 2 and

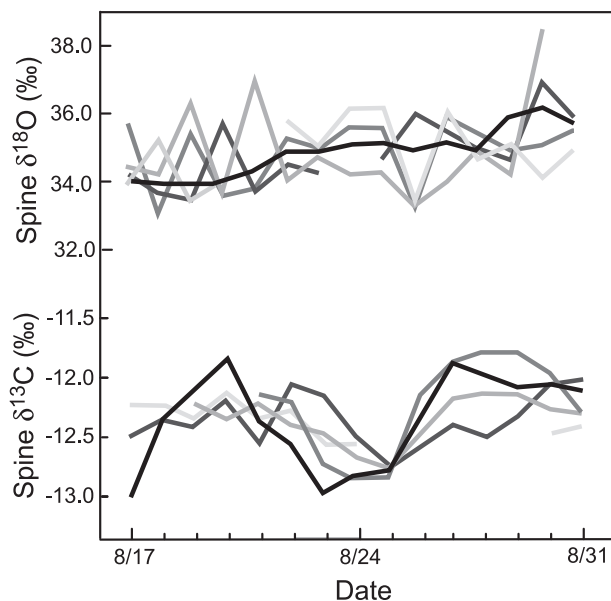


Figure 4. Daily resolution $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ records from five saguaro spines each grown on a randomly selected cactus between August and October 2006. Each line represents the daily isotopic record of each spine, adjusted so that its mean isotopic value matches the mean isotopic value of the dark black line. The dark black line is a spine from the composite spine series shown in Figure 5, specifically the spine taken from the closest areole to the apex.

Movie S1). This burst of morning growth was followed by a short period of negative growth (retraction), but by early evening spine length had recovered to the length achieved at noon. Continued growth occurred overnight and added 16% (Day 1) and 33% (Day 2) to the total daily growth of 0.44 mm and 0.52 mm, respectively. A late-morning thunderstorm on Day 1 reduced total daily PAR by 22% compared to Day 2, which was cloudless (Figure 2c).

[18] The analysis of the 122 marked spines suggests that spines produce a single transverse band for each day of growth, visibly seen as a couplet of dark and light spine tissue in cross section 0.3–0.7 mm wide (Figure 1). In a Model II nested ANOVA the main effect on the number of bands within an interval of time was the number of days within that same interval ($F_{12,109} = 36,687$, $p < 0.0001$). A mean of 0.98 bands were produced each day (95% confidence intervals from 0.97 to 0.99 bands d^{-1}) in spines from the natural population of saguaro on Tumamoc Hill. The plant on which spines grew had a significant, but smaller, effect on band production per day ($F_{12,109} = 5.6$, $p < 0.0001$). We suspect this minor effect is related to either plant microclimate or differences in the ability to mark spines accurately on different plants. In addition to ANOVA, a simple linear regression of one randomly selected spine from each plant yielded a strong 1:1 relationship ($r^2 = 0.98$, $p < 0.0001$) between spine bands and number of days grown ($n = 13$, 95% confidence interval from 0.96 to 1.04). Repeated random analysis in this manner yielded similar results. Microanatomical analysis of a saguaro spine thin section revealed that segments of long and short fibers created the distinctive banding visible to the naked eye.

When viewed from the side with light behind the spine, areas of the band with longer cells and a lower cross-sectional density are lighter in appearance.

3.2. Subdaily, Daily, and Annually Resolved Stable Isotope Records

3.2.1. Subdaily Records

[19] The high-resolution subsamples taken from one of the periodically marked cactuses failed to reveal significant subdaily cycles in $\delta^{13}\text{C}$ (Figure 3). However, we did observe a trend of decreasing $\delta^{13}\text{C}$ values, from -12.2‰ to -13.8‰ , over the sampled length of the spine (a length corresponding to 6 days of growth). Darker areas of the daily transverse bands tended to have more negative $\delta^{13}\text{C}$ values than the lighter portion, potentially because of differences in the degree of tissue lignification or the time of day the tissue was synthesized. The resulting isotopic variability we would expect from PEPC and Rubisco assimilation of atmospheric CO_2 during daylight hours (Phases II and IV) is either not present in this spine, is unobservable at the sampling resolution we chose, or was muted by mixing with previously assimilated carbon used as a substrate for PEP in Phase I.

3.2.2. Daily Records

[20] Interdaily changes in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were observed in spines from multiple, randomly selected plants (Figure 4) and consecutively grown spines from one plant that spanned ~ 70 days of growth (Figure 5). There was visible coherence in the day-to-day $\delta^{13}\text{C}$ variation between spines from different plants between 17 and 31 August (Figure 4). The variability in $\delta^{18}\text{O}$ is less coherent (Figure 4); however, all show an increasing trend over the 2 week period that, when averaged, compares well with the composite record (Figure 5). The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values over the consecutively grown spines were visibly coherent (Figure 5) where they overlapped and ranged from -11.0‰ to -13.6‰ and 33.1‰ to 38.6‰ , respectively. When averaged daily over the 70 day period (Figure 6), $\delta^{13}\text{C}$ variations in the consecutively grown spines were positively correlated (simple linear regression, $r^2 = 0.14$, $p < 0.002$) with daily changes in VPD. The positive correlation was contrary to that predicted by a $\delta^{13}\text{C}$ model solely dependent on nighttime stomatal conductance altered by VPD (Figure 6). Notable increases in both $\delta^{13}\text{C}$ and VPD occurred on 21 and 30 August, while significant decreases in both occurred near 16 and 25 August and 7 September.

[21] Spine $\delta^{18}\text{O}$ decreased rapidly after periods of heavy rain ($\sim > 10$ mm, on 31 July and 7 September), and increased during periods with little or no rainfall (Figure 6). There is a notable positive $\delta^{18}\text{O}$ spike in early September that coincided with a decrease in $\delta^{13}\text{C}$, a reduction in VPD, and a large storm on 7 September. We have observed [English et al., 2007] that plant transpiration and desiccation during dry periods enriches stem water with ^{18}O . The spike on 7 September is possibly an analytical error, or the result of high $\delta^{18}\text{O}$ waters being pushed to the apex of the plant as freshwater is taken up in the roots and recharged to the base of the plant. Conversely, large inputs of fresh water during storms relatively enriched in ^{16}O mixed with stem waters and decreased $\delta^{18}\text{O}$ of spines grown at the apex. To some degree, the effect of precipitation and drought on the $\delta^{18}\text{O}$ of apical stem water, and thus on spine $\delta^{18}\text{O}$, is confounded

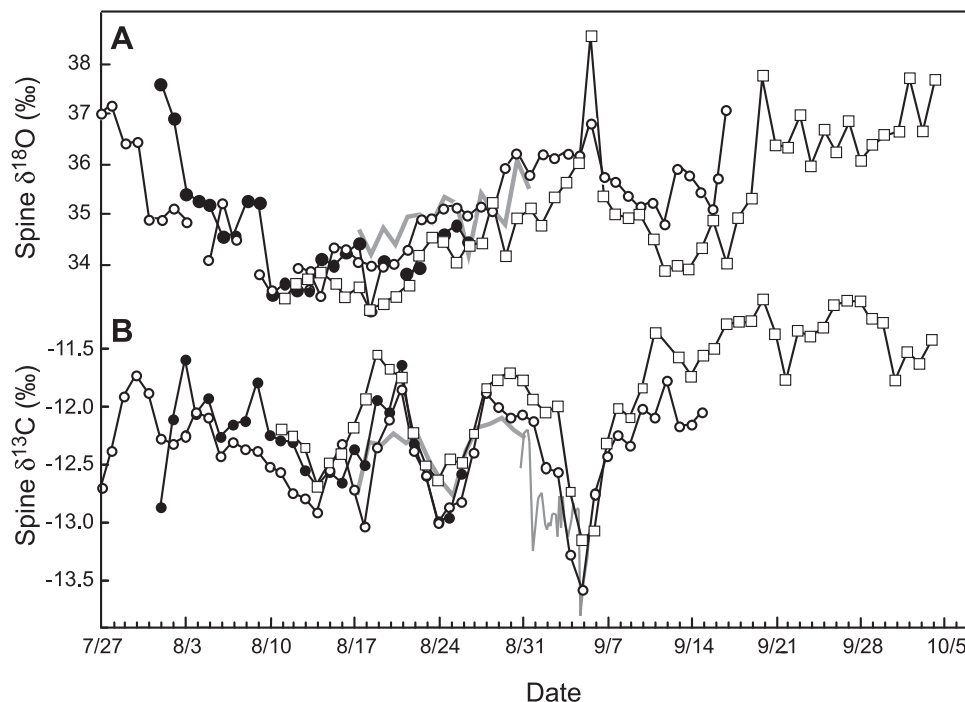


Figure 5. Daily resolution (a) $\delta^{18}\text{O}$ and (b) $\delta^{13}\text{C}$ record from three, overlapping saguaro spines grown between August and October 2006. Open circles, solid circles, and squares are the spines taken from the closest areole, the second closest areole, and the third closest areole to the apex, respectively. Thick and thin gray lines are the adjusted average of the other four spines (Figure 4) and the high-resolution $\delta^{13}\text{C}$ record (Figure 3b) respectively.

with concurrent changes in VPD that alter vertical and radial gradients in the $\delta^{18}\text{O}$ of stem water.

3.2.3. Annual Records

[22] The spine series collected from SNPE A showed high intra-annual variation in carbon isotope ratios (from -9.8‰ to -13.4‰), but low interannual variation (Figure 7). The F^{14}C measurements revealed that the cactus was at least 22 years old, consistent with the number of peaks in the $\delta^{13}\text{C}$ record. These peaks are consistently found during high VPD months of each year (i.e., May and June). The time period of the $\delta^{13}\text{C}$ cycle is short in the early part of the record but gradually lengthens as the cactus grew taller. While presented as continuous records, the records are censored between the months of November and March when spines are not growing [English et al., 2007]. Stem growth and areole generation rates derived from the dated spine time series (Figure 8) are consistent with other studies of cactus growth [Pierson and Turner, 1998; Drezner, 2003].

4. Discussion

4.1. Transverse Bands Within Single Spines

[23] Data from repeat photography, anatomical analysis and marked spines all suggest that daily initiation and cessation of growth corresponds to transverse banding on cactus spines, and that each transverse band (one dark/light couplet) represents 1 day. The relationship between banding and diurnal growth potentially holds true for other species of cactuses with transverse banding in their spines. We found a 1:1 relationship between the number of growing days and the number of transverse bands produced (results not

shown) on a spine of *Ferocactus wislizeni* (Engelm.) Britt. and Rose using similar methods to those described above. Transverse bands are present on the spines of many other species of cactuses near the Desert Laboratory, including *F. wislizeni*, *Stenocereus thurberi* (Engelm.) Buxbaum, and *O. engelmannii* (ex. Engelm.) Salm-Dyck.

[24] Saguaro spines grew rapidly during early daylight hours (Phases II and III), yet it is still unclear if they grow continuously or if PAR and other factors influence their growth rate [Nobel, 1988]. This is in agreement with studies from other CAM plants that found tissue growth was greatest during Phase III [Gouws et al., 2005]. A thunderstorm on the morning (~ 10 A.M.) of the first day during the repeat photography measurements of a single spine's growth (Figure 2) coincided with lower spine growth rates compared to the second, cloudless day. This suggests that low PAR, and subsequent reductions in metabolic processes, such as decarboxylation and assimilation, may instantaneously reduce spine growth. Spine elongation at night, however, may result from increased turgor pressure throughout the cactus and hydration of living cells near the spine base rather than from addition of new tissue. Likewise, spine retraction (5% of the daily linear growth) is probably related to desiccation of the soft spine tissue during the driest part of the day [Nobel, 2006]. Further work is needed to establish if there is a relationship between band width and PAR.

[25] Spines on saguaro grown while the plant is less than ~ 3 m tall contain ~ 30 to 60 transverse bands, reflecting the number of days over which a spine grew. For a short period (\sim weeks), a spine at the apex will grow at the same time as a

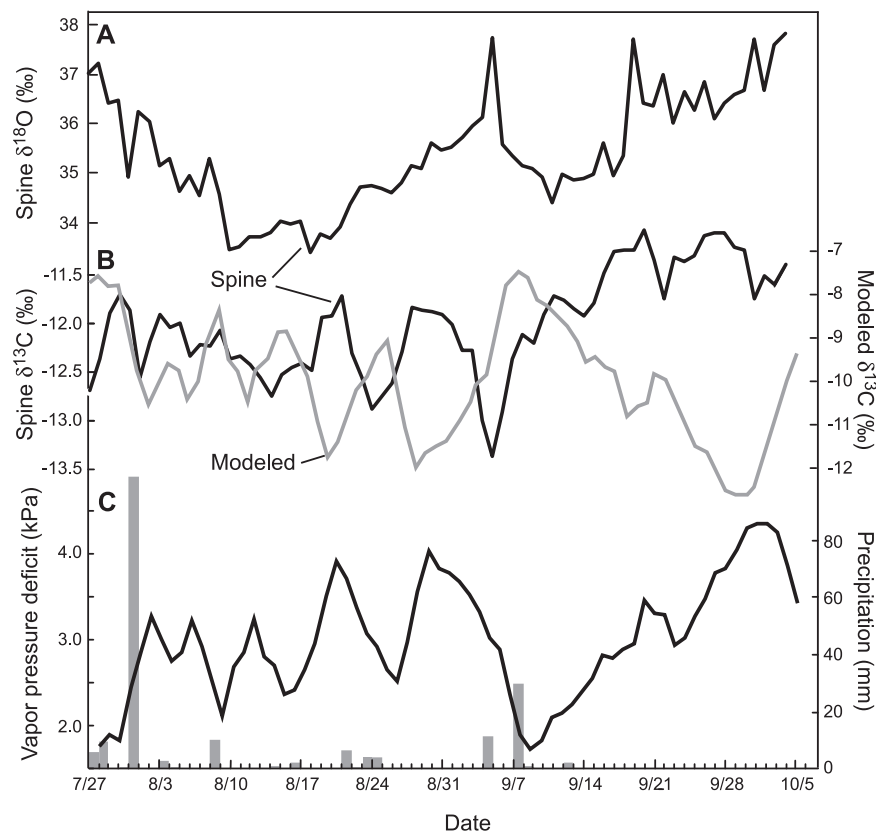


Figure 6. Averaged daily (a) $\delta^{18}\text{O}$ and (b) $\delta^{13}\text{C}$ record and modeled $\delta^{13}\text{C}$ compared to environmental parameters between August and October 2006. Bold gray line is the modeled $\delta^{13}\text{C}$ record. (c) Bold black line is the estimated 3 day running average of nighttime vapor pressure deficit for Tumamoc Hill (see text), and gray bars indicate daily total precipitation at Tumamoc Hill.

spine grown previously along the same rib. The temporal overlap of spine growth allows diurnal isotopic records to be combined precisely to create diurnally resolved records that span the entire spine growing season.

4.2. Subdaily, Daily, and Annually Resolved Stable Isotope Records

4.2.1. Subdaily Records

[26] Although we chose a relatively high-resolution sampling scheme for the 5 day $\delta^{13}\text{C}$ record (Figure 3), we could not identify any regular pattern of $\delta^{13}\text{C}$ variation over diurnal cycles that was attributable to early morning or late afternoon CO_2 assimilation by Rubisco. There was, however, a decline in $\delta^{13}\text{C}$ over these several days. The absence of a daily $\delta^{13}\text{C}$ pattern may be due to the use of stored carbohydrates, generated during the previous day's Phase II, III or IV, for the regeneration of PEP during Phase I. Drawing from a larger storage pool of carbohydrates would expectedly swamp any short-term variability of $\delta^{13}\text{C}$ photosynthate produced from CO_2 assimilated the same morning or afternoon. Sutton *et al.* [1981] noted that C sources for tissues (e.g., the roots or the epidermis) of cactus may be decoupled from one another over weekly and longer timescales, such that the tissues from which spines emerge (e.g., the epidermis) are most likely drawing from a carbohydrate pool that exhibits more rapid changes in $\delta^{13}\text{C}$ than tissues further from the epidermis and chlorenchyma. These

two conclusions are supported by the high-resolution isotope record (Figure 3), where over 1–2 days, no immediate changes in $\delta^{13}\text{C}$ are apparent, but significant shifts are observed over the entire 6 days of record. $\delta^{13}\text{C}$ in diurnal bands may not be reliable for tracking hour-to-hour changes in environmental or physiological parameters, but may be capable of doing so at daily intervals or greater. It is also possible that the internal diffusion limitations leading to a reduction in discrimination during Phase II and an increase in discrimination during Phase I muted isotopic variability [Griffiths *et al.*, 2007]; however, it seems unlikely these results would balance each other so well as to leave no observable signal.

4.2.2. Daily Records

[27] We compared the average $\delta^{13}\text{C}$ and VPD records over 70 days to evaluate two hypotheses for what drives daily $\delta^{13}\text{C}$ variability in spines: (1) short-term $\delta^{13}\text{C}$ variability in spines is determined by nighttime VPD-driven changes in stomatal conductance associated with nighttime photosynthetic gas exchange (i.e., p_i/p_a); and (2) short-term $\delta^{13}\text{C}$ variability in spines is determined by VPD-driven changes in daytime stomatal opening that alter the balance of PEPC and Rubisco assimilated atmospheric CO_2 . To test the first hypothesis, we modeled $\delta^{13}\text{C}$ as if it were determined only by changes in p_i/p_a associated with nighttime gas exchange and stomatal conductance moderated by VPD and temperature. In the model, we used local temperature

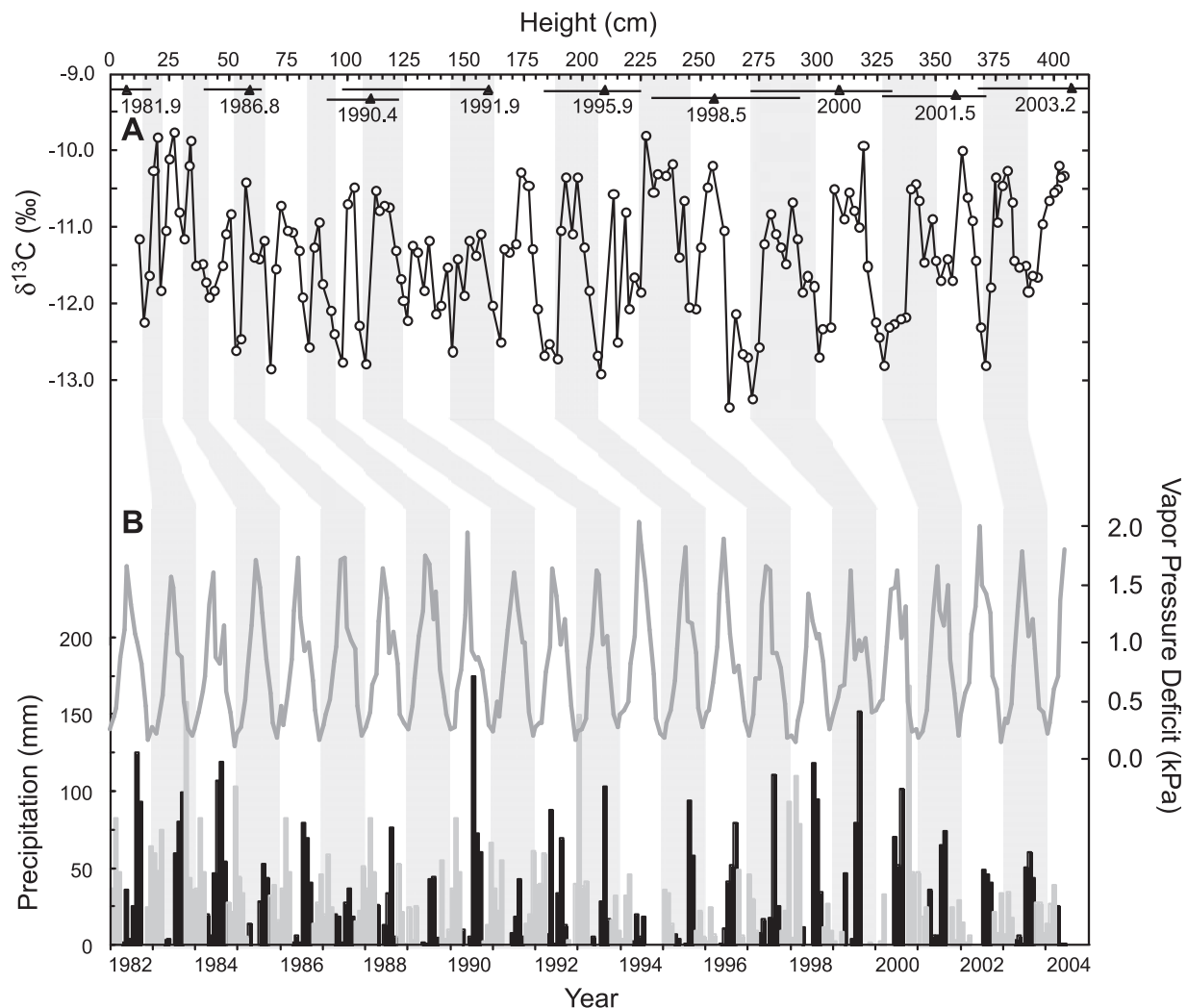


Figure 7. (a) The $\delta^{13}\text{C}$ record in spine tips spanning ~ 4 m of a naturally occurring saguaro (SNPE A) in Saguaro National Park, Arizona. Solid triangles with lines are corrected $F^{14}\text{C}$ ages and error. Open circles are $\delta^{13}\text{C}$ values from spine tips. Thick gray line is monthly nighttime vapor pressure deficit calculated from PRISM data. (b) Monthly precipitation values from nearby climate stations are shown for October–March (gray bars) and April–September (black bars, see text for data sources). Light gray bands denote our interpretation of correlated years.

and relative humidity measured over the 70 day period to calculate VPD and temperature-related changes in the net discrimination associated with carboxylation by PEPC (b_4 in equation (1)). We were unable to find any published relationships between VPD and p_i/p_a for any cactus, but Roberts *et al.* [1997] found that for two species of the CAM genus *Chusia* p_i/p_a is significantly related to VPD ($p_i/p_a = -0.1858 \times \text{VPD} + 0.7835$). Although *Chusia* trees are not ideal cactus analogs and the relationship is probably different for saguaro, the direction of the slope and intercept are likely to be similar. Therefore, we chose to apply the *Chusia*-derived linear relationship of VPD and p_i/p_a to test the impact of nighttime stomatal opening on $\delta^{13}\text{C}$ in saguaro.

[28] The modeled spine $\delta^{13}\text{C}$ is positively offset by $\sim 2.3\%$ and negatively correlated (simple linear regression, $r^2 = 0.08$, $p < 0.02$) with the observed average 70 day record of $\delta^{13}\text{C}$ (Figure 6). The offset is likely due to post- CO_2 assimilation processes not accounted for in the model, for example, the

$\sim 3\%$ shift in $\delta^{13}\text{C}$ as carbon derived from autotrophic stem tissue is synthesized into lignified tissue like spines [see Boutton, 1996; Badeck *et al.*, 2005] or the influence of mesophyll conductance on Phase I discrimination [Griffiths *et al.*, 2007]. Other possible sources of model error are the use of stored carbon with lower $\delta^{13}\text{C}$ values and a steady state contribution to the available C pool from Rubisco derived atmospheric CO_2 . Radiocarbon on spines from other saguaro [English *et al.*, 2007] and other $\delta^{13}\text{C}$ studies [Sutton *et al.*, 1981] suggest that stored carbohydrates do not contribute significantly to spine growth. At shorter timescales of days and weeks, the negative correlation of $\delta^{13}\text{C}$ might be caused by a small delay between CO_2 assimilation and carbon synthesis into spine tissue as described above (section 4.2.1). However, if this were the case we might expect to see decreasing spine $\delta^{13}\text{C}$ a week after the VPD minima on 8 September (Figure 6). Instead, spine $\delta^{13}\text{C}$ increases after 14 September. This suggests that spine $\delta^{13}\text{C}$

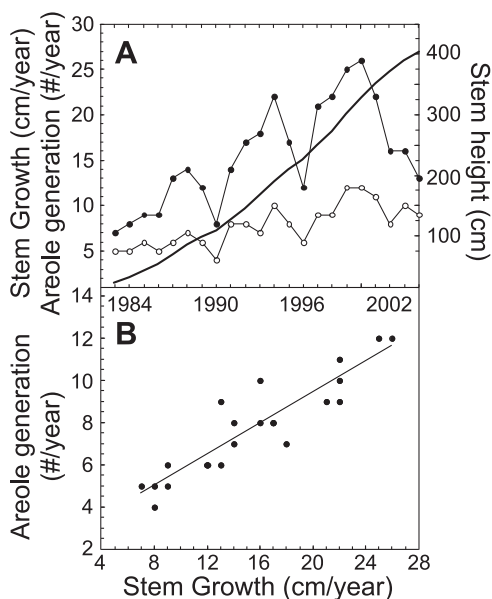


Figure 8. Stem growth and areole production rates of a naturally occurring saguaro in Saguaro National Park East (SNPE A). (a) Estimated annual growth rate of SNPE A cactus (solid circles) and the number of areoles produced each year (open circles). Also shown is total height of the cactus (bold black line) and the year (x axis). (b) Relationship of areole production to estimated stem growth. Solid line is a simple linear regression ($t_{21} = 9.96$, $p < 0.0001$).

is not recording delayed changes in the $\delta^{13}\text{C}$ of carbohydrate pools controlled by nighttime p_i/p_a .

[29] In contrast, the mean 70 day record of $\delta^{13}\text{C}$ in spines is positively correlated with nighttime VPD. We have no direct evidence that ties VPD to a changing balance of PEPC and Rubisco assimilated CO_2 . However, having ruled out nighttime p_i/p_a as the principal determinant of spine $\delta^{13}\text{C}$, we propose that $\delta^{13}\text{C}$ variability in spines ensues from daytime acquisition of CO_2 by Rubisco during CAM Phases II and IV when VPD is low. Low VPD allows stomata to remain open longer in the morning and thus directly fix a greater proportion of atmospheric CO_2 with Rubisco than when VPD is high and stomata are closed. This results in lower spine $\delta^{13}\text{C}$ values during periods of low VPD, and such periods would be pervasive during the monsoon season. Alternatively, variable discrimination due to diffusion limitation of CO_2 at night altered by mesophyll conductance coupled to assimilation rates or temperature may also explain the changes found in the carbon isotope ratios of spines. These relationships remain to be confirmed by gas exchange observations, but are consistent with the work of many other studies that have shown CO_2 assimilation during the day in CAM plants during times of low water stress, including saguaro [MacDougal and Working, 1933; Conde and Kramer, 1975; Osmond et al., 1979a, 1979b; Nobel, 1988; Lajtha et al., 1997] and variable $\Delta^{13}\text{C}$ during Phase I as a function of assimilation rate [Griffiths et al., 2007]. Furthermore, although these hypotheses may explain the pattern of $\delta^{13}\text{C}$ variation during monsoon periods, the relationship that underlies it probably changes throughout the growing season

such that other factors might drive $\delta^{13}\text{C}$ variation at other times of the year.

[30] Daily to weekly variation in $\delta^{18}\text{O}$ was less over the 70 day study period than that in $\delta^{13}\text{C}$ (Figure 4). What explains the discrepancy in the variability of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in spine tissue over short timescales? The turnover rate of the oxygen and carbon reservoirs in saguaro is uncertain, although Nobel [1977] estimated that a mature barrel cactus, *Ferocactus acanthodes* (Lemaire) Britton and Rose, will transpire ~ 1.5 times its mass in water each year and utilize most of a year's carbohydrate production for growth and reproduction that same year. We hypothesize that the travel time of ^{16}O enriched rainfall from the soil to the apex where spine tissue is synthesized is greater than the travel time of carbohydrates produced in chlorenchyma near the apex of the saguaro. Water traveling from the base of the saguaro to the apex also undergoes mixing with water previously stored in the plant and this mutes the strength and smooths the arrival of any rainfall pulse recorded in the $\delta^{18}\text{O}$ of spine tissue. As such, daily variation in VPD or other climate parameters would not be expected to alter $\delta^{18}\text{O}$ in spine tissue at the same pace as $\delta^{13}\text{C}$. Our data suggest that, over days and weeks, variation in spine $\delta^{18}\text{O}$ is determined by uptake and transpiration of water in the stem and that short-term changes in VPD have only a small effect on spine $\delta^{18}\text{O}$. This is consistent with changes in spine $\delta^{18}\text{O}$ that occur after the uptake of rainwater (with $\delta^{18}\text{O}$ values of ~ -5 to -10‰ in Tucson) on 31 July and 7 September.

[31] Over longer timescales, seasonal decreases in spine $\delta^{18}\text{O}$ should occur as plant uptake of monsoon or winter rainfall decreases the average $\delta^{18}\text{O}$ value of stem water. At the same time, we would expect spine $\delta^{13}\text{C}$ values to decline due to lowered VPD associated with the monsoon and winter and increase due to higher VPD associated with the premonsoon and postmonsoon drought. Alternatively, changes in mesophyll conductance may also play a role in altering spine $\delta^{13}\text{C}$ values, so if assimilation were higher in the monsoon and early spring, discrimination would be increased and spine $\delta^{13}\text{C}$ values would be lower. In southern Arizona, seasonal precipitation and VPD are confounded when considered as factors that affect spine $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. Seasonal and annual isotopic variations in spine series implicate these mechanisms and both warrant further examination in controlled experiments.

4.2.3. Annual Records

[32] We evaluated annual isotopic variability in saguaro spines by analyzing the narrow tip of single spines (the top 2–3 mm) across consecutively produced areoles along 4 m of a single cactus rib. The isotope spine series from the cactus SNPE A (Figure 7) represents decades of growth, but each tip represents only 2 or 3 days of recorded isotopic variability over ~ 30 to 60 days of spine growth of an individual spine. However, $\delta^{13}\text{C}$ varies at a periodicity consistent with the independent chronology established by F^{14}C dating of the same spine series. The seasonal cycle in this $\delta^{13}\text{C}$ spine series reflects the onset of spine growth under high water availability in March and April (-13‰), followed by a hot, premonsoon drought in May and June (-10‰), and then the arrival of the monsoon in July and August (-13‰) after which spine growth slows as the cactuses enter dormancy for the winter.

[33] In contrast to this consistent seasonal pattern, the interannual variability of $\delta^{13}\text{C}$ does not appear to be coupled to that of annual rainfall (Figure 7). So while $\delta^{13}\text{C}$ and F^{14}C may be useful for establishing the spine-series chronology in this plant, this plant is not a good choice for evaluating interannual VPD variability. Analogous to observations in dendrochronological studies [McCarroll and Loader, 2004], the position of this cactus in a shallow wash may make it less sensitive to annual or decadal variations in VPD or precipitation. Cactuses located on hillslopes and away from water drainages may show greater sensitivity to annual and decadal variation in VPD or rainfall because they are unable to reach their full water-holding capacity in March and April preceding the premonsoon drought. It is possible that with the inclusion of more climatological information we might tease out VPD, rainfall or other climate data from the $\delta^{13}\text{C}$ variation in spine series. The other application of F^{14}C and $\delta^{13}\text{C}$ in spines is to derive accurate annual growth rates of cactus stems (Figure 8). In addition to confirming that areole generation in saguaro is positively correlated ($t_{21} = 9.96$, $p < 0.0001$) to apical growth [Nobel, 1986; Buskirk and Otis, 1994], our data indicate that SNPE A had a growth rate 1.8 times faster than that predicted by the local growth model for saguaro in this population [Drezner, 2003; Steenbergh and Lowe, 1977]. This degree of growth variation is not uncommon across the range of this species [Drezner, 2005], but we were surprised to find it within a single population. Several lines of evidence suggest that the growth rate we derived is correct: (1) the independent agreement between the F^{14}C and $\delta^{13}\text{C}$ record; (2) the cactus lies within a small plot where all saguaro plants were surveyed and permanently tagged by Steenbergh and Lowe [1977], however, SNPE A has neither a tag nor is noted on the 1977 survey map (Plot 41A, provided by C. Funicelli) suggesting that it was either not present or not visible in 1977; and (3) the shape of the growth curve (Figure 8) described by the F^{14}C and $\delta^{13}\text{C}$ record over the life of this cactus is similar to that of other saguaro [Drezner, 2003]. We hypothesize that the position of this cactus in a shallow wash, and therefore its ability to recharge its stem water to full capacity before the onset of drought each year, has contributed to its rapid growth. While this example demonstrates the utility of F^{14}C and $\delta^{13}\text{C}$ dating of spine time series, it is also a caution against using population-derived growth models [Drezner, 2003] to infer the age of an individual cactus.

5. Conclusions

[34] Stable isotope time series from saguaro spines, and possibly other cactuses and stem succulents with sequentially added and durable tissues, can be used to resolve daily to annual patterns of climatic variability and physiological responses. The transverse bands present in saguaro spines are accurate chronometers of daily growth, and other species of cactuses with transverse bands in their spines likely share this characteristic. Spines exhibit more negative $\delta^{13}\text{C}$ coincident with lower VPD and higher water status during favorable periods during the growing season. This characteristic is found in other CAM plants [Griffiths et al., 2007] and may result from CO_2 fixed by Rubisco in Phases II and IV of the CAM cycle or greater Phase I assimilation rates. We have not ruled out that changes in nighttime p_i/p_a also influence $\delta^{13}\text{C}$, but

this effect is not apparent in the records presented here. Intraspine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variability along the 5 day high-resolution spine transect and the 70 day composite spine time series, suggest that daily changes of physiological (the balance of PEPC/Rubisco CO_2 fixation, p_i/p_a , and plant water status) and environmental parameters (VPD, precipitation) are recorded in spine isotopes at weekly timescales, but the precise relationships to $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ have yet to be determined for saguaro. Once the drivers of diurnal, weekly and annual spine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variability are reasonably well understood, and the temporal dynamics of cortical carbohydrate reservoirs are resolved, interspine isotopic time series can be used to create exceptionally detailed records of past biotic (e.g., cactus growth) and abiotic properties.

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References

- Abramoff, M. D., P. J. Magelhaes, and S. J. Ram (2004), Image processing with ImageJ, *Biophotonics Int.*, *11*, 36–42.
- Badeck, F. W., G. Tcherkez, S. Nogues, C. Piel, and J. Ghashghaie (2005), Post-photosynthetic fractionation of stable carbon isotopes between plant organs—A widespread phenomenon, *Rapid Commun. Mass Spectrom.*, *19*, 1381–1391, doi:10.1002/rcm.1912.
- Bernacchi, C. J., A. R. Portis, H. Nakano, S. von Caemmerer, and S. P. Long (2002), Temperature response of mesophyll conductance: Implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis in vivo, *Plant Physiol.*, *130*, 1992–1998, doi:10.1104/pp.008250.
- Black, C. C., and C. B. Osmond (2003), Crassulacean acid metabolism: 'Working the night shift,' *Photosynth. Res.*, *76*, 329–341, doi:10.1023/A:1024978220193.
- Boutton, T. W. (1996), Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change, in *Mass Spectrometry of Soils*, edited by T. W. Boutton and S. I. Yamasaki, pp. 47–82, Marcel Dekker, New York.
- Buskirk, R. E., and G. W. Otis (1994), Annual waxy bands on a Costa Rican cactus, *Biotropica*, *26*, 229–232, doi:10.2307/2388815.
- Conde, L. F., and P. J. Kramer (1975), Effect of vapor pressure deficit on diffusion resistance in *Opuntia-compressa*, *Can. J. Bot.*, *53*, 2923–2926, doi:10.1139/b75-321.
- Dodd, A. N., A. M. Borland, R. P. Haslam, H. Griffiths, and K. Maxwell (2002), Crassulacean acid metabolism: Plastic, fantastic, *J. Exp. Bot.*, *53*, 569–580, doi:10.1093/jexbot/53.369.569.
- Drezner, T. D. (2003), Saguaro (*Carnegiea gigantea*, Cactaceae) age-height relationships and growth: The development of a general growth curve, *Am. J. Bot.*, *90*, 911–914, doi:10.3732/ajb.90.6.911.
- Drezner, T. D. (2005), Saguaro (*Carnegiea gigantea*, Cactaceae) growth rate over its American range and the link to summer precipitation, *Southwest. Nat.*, *50*, 65–68, doi:10.1894/0038-4909(2005)050<0065:SCGCGR>2.0.CO;2.
- Drezner, T. D., and R. C. Balling (2002), Climatic controls of saguaro (*Carnegiea gigantea*) regeneration: A potential link with El Niño, *Phys. Geogr.*, *23*, 465–475, doi:10.2747/0272-3646.23.6.465.
- English, N. B., D. Dettman, D. R. Sandquist, and D. G. Williams (2007), Past climate changes and ecophysiological responses recorded in the isotope ratios of saguaro cactus spines, *Oecologia*, *154*, 247–258, doi:10.1007/s00442-007-0832-x.

- Farquhar, G. D., K. T. Hubick, A. G. Condon, and R. A. Richards (1989), Carbon isotope fractionation and plant water-use efficiency, in *Stable Isotopes in Ecological Research*, edited by P. W. Rundel et al., pp. 21–40, Springer, New York.
- Francey, R. J., C. E. Allison, D. M. Etheridge, C. M. Trudinger, I. G. Enting, M. Leuenberger, R. L. Langenfelds, E. Michel, and L. P. Steele (1999), A 1000-year high precision record of $\delta^{13}\text{C}$ in atmospheric CO_2 , *Tellus, Ser. B*, *51*, 170–193.
- Ghil, M., et al. (2000), Advanced spectral methods for climatic time series, *Rev. Geophys.*, *40*(1), 1003, doi:10.1029/2000RG000092.
- Gibson, A. C., and P. S. Nobel (1986), *The Cactus Primer*, Harvard Univ. Press, Cambridge, Mass.
- Godinez-Alvarez, H., T. Valverde, and P. Ortega-Baes (2003), Demographic trends in the Cactaceae, *Bot. Rev.*, *69*, 173–203, doi:10.1663/0006-8101(2003)069[0173:DTITC]2.0.CO;2.
- Gouws, L. M., C. B. Osmond, U. Schurr, and A. Walter (2005), Distinctive diel growth cycles in leaves and cladodes of CAM plants: Differences from C_3 plants and putative interactions with substrate availability, turgor and cytoplasmic pH, *Funct. Plant Biol.*, *32*, 421–428, doi:10.1071/FP05074.
- Griffiths, H., U. Lüttge, K.-H. Stimmel, C. E. Crook, N. M. Griffiths, and J. A. C. Smith (1986), Comparative ecophysiology of CAM and C_3 bromeliads. III. Environmental influences on CO_2 assimilation and transpiration, *Plant Cell Environ.*, *9*, 385–393, doi:10.1111/j.1365-3040.1986.tb01752.x.
- Griffiths, H., A. B. Cousins, M. R. Badger, and S. von Caemmerer (2007), Discrimination in the dark: Resolving the interplay between metabolic and physical constraints to phosphoenolpyruvate carboxylase activity during the crassulacean acid metabolism cycle, *Plant Physiol.*, *143*, 1055–1067, doi:10.1104/pp.106.088302.
- Lajtha, K., K. Kolberg, and J. Getz (1997), Ecophysiology of the saguaro cactus (*Carnegiea gigantea*) in the Saguaro National Monument: Relationship to symptoms of decline, *J. Arid Environ.*, *36*, 579–590, doi:10.1006/jare.1996.0240.
- Lange, O. L., and E. Medina (1979), Stomata of the CAM plant *Tillandsia recurvata* respond directly to humidity, *Oecologia*, *40*, 357–363, doi:10.1007/BF00345331.
- MacDougal, D. T., and E. B. Working (1933), The pneumatic system of plants, especially trees, *Publ. 441*, Carnegie Inst. of Wash., Washington, D. C.
- Mauseth, J. D. (2006), Structure–function relationships in highly modified shoots of Cactaceae, *Ann. Bot.*, *98*, 901–926, doi:10.1093/aob/mcl133.
- McCarroll, D., and N. J. Loader (2004), Stable isotopes in tree rings, *Quat. Sci. Rev.*, *23*, 771–801, doi:10.1016/j.quascirev.2003.06.017.
- Nobel, P. S. (1977), Water relations and photosynthesis of a Barrel Cactus, *Ferocactus acanthodes*, in the Colorado Desert, *Oecologia*, *27*, 117–133, doi:10.1007/BF00345817.
- Nobel, P. S. (1986), Relation between monthly growth of *Ferocactus acanthodes* and an environmental productivity index, *Am. J. Bot.*, *73*, 541–547, doi:10.2307/2444260.
- Nobel, P. S. (1988), *Environmental Biology of Agaves and Cactus*, Cambridge Univ. Press, New York.
- Nobel, P. S. (2006), Parenchyma–Chlorenchyma water movement during drought for the hemiepiphytic cactus *Hylocereus undatus*, *Ann. Bot.*, *97*, 469–474, doi:10.1093/aob/mcj054.
- Osmond, C. B., M. M. Ludlow, R. Davis, I. R. Cowan, S. B. Powles, and K. Winter (1979a), Stomatal responses to humidity in *Opuntia inermis* in relation to control of CO_2 and H_2O exchange patterns, *Oecologia*, *41*, 65–76, doi:10.1007/BF00344837.
- Osmond, C. B., D. L. Nott, and P. M. Firth (1979b), Carbon assimilation patterns and growth of the introduced CAM plant *Opuntia inermis* in Eastern Australia, *Oecologia*, *40*, 331–350, doi:10.1007/BF00345329.
- Pierson, E. A., and R. M. Turner (1998), An 85-year study of saguaro (*Carnegiea gigantea*) demography, *Ecology*, *79*, 2676–2693.
- Rasband, W. S. (2009), *ImageJ*, U.S. Natl. Inst. of Health, Bethesda, Md. (Available at <http://rsb.info.nih.gov/ij/>)
- Reimer, P. J., T. A. Brown, and R. W. Reimer (2004), Discussion: Reporting and calibration of post-bomb ^{14}C data, *Radiocarbon*, *46*, 1299–1304.
- Roberts, A., A. M. Borland, and H. Griffiths (1997), Discrimination processes and shifts in carboxylation during the phases of Crassulacean acid metabolism, *Plant Physiol.*, *113*, 1283–1292.
- Robinson, H. (1974), Scanning electron microscope studies of the spines and glochids of the opuntioideae (*cactaceae*), *Am. J. Bot.*, *61*, 278–283, doi:10.2307/2441606.
- Steenbergh, W. F., and C. H. Lowe (1977), Ecology of the saguaro. II. Reproduction, germination, establishment, growth, and survival of the young plant, *Sci. Monogr. Ser. 8*, Natl. Park Serv., Washington, D. C.
- Steenbergh, W. F., and C. H. Lowe (1983), Ecology of the saguaro. III. Growth and demography, *Sci. Monogr. Ser. 17*, Natl. Park Serv., Washington, D. C.
- Sutton, B. G., I. P. Ting, and R. Sutton (1981), Carbohydrate metabolism of cactus in a desert environment, *Plant Physiol.*, *68*, 784–787, doi:10.1104/pp.68.3.784.
- Turner, R. M., J. E. Bowers, and T. L. Burgess (1995), *Sonoran Desert Plants: An Ecological Atlas*, Univ. of Arizona Press, Tucson.
- Wang, E., C. J. Smith, W. J. Bond, and K. Verburg (2004), Estimations of vapour pressure deficit and crop water demand in APSIM and their implications for prediction of crop yield, water use, and deep drainage, *Aust. J. Agric. Res.*, *55*, 1227–1240, doi:10.1071/AR03216.
- Winter, K., and J. A. Holtum (2002), How closely do the $\delta^{13}\text{C}$ values of crassulacean acid metabolism plants reflect the proportion of CO_2 fixed during day and night?, *Plant Physiol.*, *129*, 1843–1851, doi:10.1104/pp.002915.
- Wolf, B. O., and A. E. McKechnie (2003), Nutrient dynamics in a desert bird community; The functional importance of columnar cacti, *Integr. Comp. Biol.*, *43*, 864.

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