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Daily to Decadal Patterns of Precipitation, Humidity, and Photosynthetic Physiology Recorded in the Spines of the Columnar Cactus, Carnegiea Gigantea

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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 $\delta^{13}C$ and $\delta^{18}O$ records from three spines grown in series over a 70 day period. We also constructed a 22 year record of $\delta^{13}C$ 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 $\delta^{13}C$ values of spines; both related to vapor pressure deficit (VPD). Our data suggest that stomatal conductance is unlikely to be the determinant of $\delta^{13}C$ 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 $\delta^{13}C$ variation in spines. Intra-annual and interannual variability of $\delta^{18}O$ 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.


1. Introduction

[2] Climatic and physiological variations are integrated and recorded in the stable isotope ratios of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) 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
using the methods used to construct a chronology of saguaro spines
climate data. In the work of
link climate to plant or ecosystem productivity and to inform
standing of isotopic variability in spines, can be used to
provided by a spine series, when coupled with an under-
drivers of isotopic variability in spines. The temporal context
identify and evaluate probable physiological and climatic
ferent temporal scales of saguaro spine growth and to
understanding of the processes and environmental para-
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 \( \delta^{13}C \), \( \delta^{18}O \) and F14C. 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 \( \delta^{13}C \) and \( \delta^{18}O \) variability found in spines over scales of days, weeks and years; (3) \( \delta^{13}C \) variation in spines is most likely not driven by stomatal conductance; and (4) F14C and \( \delta^{13}C \) 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

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. Saguaro 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 timescales. 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, F14C, \( \delta^{13}C \), and \( \delta^{18}O \)

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 \( \delta^{13}C \) and \( \delta^{18}O \) 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.

The F14C and \( \delta^{13}C \) 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, \( \delta^{13}C \) variability might provide a useful record of past climate changes. While the F14C is based on the dilution and removal of anthropogenic \( ^{14}C \) in the atmosphere produced by atmospheric nuclear testing [Reimer et al., 2004], the \( \delta^{13}C \) chronometer is based on interspine \( \delta^{13}C \) 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 CO2 for growth and this has consequences for the \( \delta^{13}C \) of cactus tissue and spines. Thorough reviews of CAM physiology and isotope systematics can be found in the works of Dodd

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.
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-1,5-bisphosphate carboxylase (Rubisco).

Aside from long-term changes in atmospheric $\delta^{13}$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 $\delta^{13}$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 photosynthetic production and carbon allocation to spine growth. In this case, carbon isotope discrimination ($\Delta$) 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]:

$$\Delta = a + (b_4 - a)p_t/p_a$$  

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 $p_t/p_a$ 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 $p_t/p_a$ 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 $p_t/p_a$, would account for measurable variation in $\delta^{13}$C of the photosynthetic pool [Roberts et al., 1997]. Indeed, drought and high nighttime atmospheric VPD have been previously shown to reduce stomatal conductance, $p_t/p_a$ and $\Delta$ (increase in $\delta^{13}$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].

The second possible mechanism of $\delta^{13}$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, MacDougall 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 photosynthetic pool will be partially derived from Rubisco fixation of atmospheric CO2 (leading to tissues with $\delta^{13}$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 $\delta^{13}$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 $\delta^{13}$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 photosynthetic pools isolating it from long-term, integrated carbohydrate reservoirs. As such, we predict that short-term changes in $\Delta^{13}$C are most likely recorded permanently in spine tissue $\delta^{13}$C.

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 $\delta^{13}$C discrimination. As conductance for CO2 diffusion from intercellular airspaces to the cytosol decreases, $\Delta$ 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 $\Delta$). In saguaro’s, the result of this would be to decrease $\delta^{13}$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.

Variability in the $\delta^{18}$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 $\delta^{18}$O of water in the stem that add complexity to our interpretation of what determines $\delta^{18}$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

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
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). 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.

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 epiflourescent and normal transmitted-light microscopes to examine banding patterns.

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

2.2.1. Subdaily Records

We investigated whether changes in $\delta^{13}$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 $\delta^{13}$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 $\delta^{13}$C and $\delta^{18}$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 $\delta^{13}$C and 0.2‰ for $\delta^{18}$O.

2.2.2. Daily Records

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 $\delta^{13}$C and $\delta^{18}$O and rainfall, VPD and modeled $P_{e}/P_{n}$ (Data Sets S1–S4 and Table S1). Single marked spines from four other randomly selected cacti...
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-Specra 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 \( \delta^{13}C \) and \( \delta^{18}O \) analysis.

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 (PAR < 500 \( \mu \)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 cacti.

### 2.2.3. Annual Records

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\(^\circ\)N, 110.73\(^\circ\)W, 870 m elevation). We sampled and recorded the height of one spine from \( \delta^{13}C \) 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 \(^{14}C\) 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 \(^{14}C\) 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 (2\( \times \) age range) [English et al., 2007]. For the \( \delta^{13}C \) 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.

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

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

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**Figure 3.** High-resolution \( \delta^{13}C \) 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 \( \delta^{13}C \) 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.
Within that same interval (bands within an interval of time was the number of days) of Model II nested ANOVA the main effect on the number of tissue in cross section 0.3

Spines produce a single transverse band for each day of 16% (Day 1) and 33% (Day 2) to the total daily growth of at noon. Continued growth occurred overnight and added evening spine length had recovered to the length achieved 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%

thunderstorm on Day 1 reduced total daily PAR by 22% compared to Day 2, which was cloudless (Figure 2c).

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 δ¹³C (Figure 3). However, we did observe a trend of decreasing δ¹³C 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 δ¹³C 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 CO₂ 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 δ¹³C and δ¹⁸O 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 δ¹³C variation between spines from different plants between 17 and 31 August (Figure 4). The variability in δ¹⁸O 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 δ¹³C and δ¹⁸O 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), δ¹³C 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 δ¹³C model solely dependent on nighttime stomatal conductance altered by VPD (Figure 6). Notable increases in both δ¹³C and VPD occurred on 21 and 30 August, while significant decreases in both occurred near 16 and 25 August and 7 September.

[21] Spine δ¹⁸O decreased rapidly after periods of heavy rain (∼ > 10 mm, on 31 July and 7 September), and increased during periods with little or no rainfall (Figure 6). There is a notable positive δ¹⁸O spike in early September that coincided with a decrease in δ¹³C, 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 ¹⁸O. The spike on 7 September is possibly an analytical error, or the result of high δ¹⁸O 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 ¹⁸O mixed with stem waters and decreased δ¹⁸O of spines grown at the apex. To some degree, the effect of precipitation and drought on the δ¹⁸O of apical stem water, and thus on spine δ¹⁸O, is confounded
3.2.3. Annual Records

The spine series collected from SNPE A showed high intra-annual variation in carbon isotope ratios (from $-9.8\%_0$ to $-13.4\%_0$), 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 $d^{13}C$ record. These peaks are consistently found during high VPD months of each year (i.e., May and June). The time period of the $d^{13}C$ 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

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.

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.

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 (~weeks), a spine at the apex will grow at the same time as a
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 $\delta^{13}C$ record (Figure 3), we could not identify any regular pattern of $\delta^{13}C$ variation over diurnal cycles that was attributable to early morning or late afternoon CO$_2$ assimilation by Rubisco. There was, however, a decline in $\delta^{13}C$ over these several days. The absence of a daily $\delta^{13}C$ 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 $\delta^{13}C$ photosynthesize 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 $\delta^{13}C$ 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 $\delta^{13}C$ are apparent, but significant shifts are observed over the entire 6 days of record. $\delta^{13}C$ 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 $\delta^{13}C$ and VPD records over 70 days to evaluate two hypotheses for what drives daily $\delta^{13}C$ variability in spines: (1) short-term $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 6. Averaged daily (a) $\delta^{18}O$ and (b) $\delta^{13}C$ record and modeled $\delta^{13}C$ compared to environmental parameters between August and October 2006. Bold gray line is the modeled $\delta^{13}C$ 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.
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 (b4 in equation (1)). We were unable to find any published relationships between VPD and pi/pa for any cactus, but Roberts et al. [1997] found that for two species of the CAM genus Clusia pi/pa is significantly related to VPD (pi/pa = −0.1858 x VPD + 0.7835). Although Clusia 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 Clusia-derived linear relationship of VPD and pi/pa to test the impact of nighttime stomatal opening on d13C in saguaro.

The modeled spine d13C is positively offset by ~2.3‰ and negatively correlated (simple linear regression, r² = 0.08, p < 0.02) with the observed average 70 day record of d13C (Figure 6). The offset is likely due to post-CO2 assimilation processes not accounted for in the model, for example, the ~3‰ shift in d13C 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 d13C values and a steady state contribution to the available C pool from Rubisco derived atmospheric CO2. Radiocarbon on spines from other saguaro [English et al., 2007] and other d13C 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 d13C might be caused by a small delay between CO2 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 d13C a week after the VPD minima on 8 September (Figure 6). Instead, spine d13C increases after 14 September. This suggests that spine d13C
is not recording delayed changes in the $\delta^{13}$C of carbohydrate pools controlled by nighttime $p_i/p_o$.

[29] In contrast, the mean 70 day record of $\delta^{13}$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 $p_i/p_o$ as the principal determinant of spine $\delta^{13}$C, we propose that $\delta^{13}$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 $\delta^{18}$O values during periods of low VPD, and such periods would be pervasive during the monsoon season. Additionally, 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 $\Delta^{13}$C during Phase I as a function of assimilation rate [Griffiths et al., 2007]. Furthermore, although these hypotheses may explain the pattern of $\delta^{13}$C variation during monsoon periods, the relationship that underlies it probably changes throughout the growing season such that other factors might drive $\delta^{13}$C variation at other times of the year.

[30] Daily to weekly variation in $\delta^{18}$O was less over the 70 day study period than that in $\delta^{13}$C (Figure 4). What explains the discrepancy in the variability of $\delta^{18}$O and $\delta^{13}$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 $\sim 1.5$ 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 cactus. 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 $\delta^{18}$O of spine tissue. As such, daily variation in VPD or other climate parameters would not be expected to alter $\delta^{13}$C in spine tissue at the same pace as $\delta^{13}$C. Our data suggest that, over days and weeks, variation in spine $\delta^{18}$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 $\delta^{18}$O. This is consistent with changes in spine $\delta^{18}$O that occur after the uptake of rainwater (with $\delta^{18}$O values of $\sim -5$ to $-10\%$ in Tucson) on 31 July and 7 September.

[31] Over longer timescales, seasonal decreases in spine $\delta^{18}$O should occur as plant uptake of monsoon or winter rainfall decreases the average $\delta^{18}$O value of stem water. At the same time, we would expect spine $\delta^{13}$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 $\delta^{13}$C values, so if assimilation were higher in the monsoon and early spring, discrimination would be increased and spine $\delta^{13}$C values would be lower. In southern Arizona, seasonal precipitation and VPD are confounded when considered as factors that affect spine $\delta^{18}$O and $\delta^{13}$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 $\sim 30$ to 60 days of spine growth of an individual spine. However, $\delta^{13}$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 $\delta^{13}$C spine series reflects the onset of spine growth under high water availability in March and April ($\sim 13\%$), followed by a hot, premonsoon drought in May and June ($\sim 10\%$), and then the arrival of the monsoon in July and August ($\sim 13\%$) after which spine growth slows as the cactuses enter dormancy for the winter.
In contrast to this consistent seasonal pattern, the interannual variability of $\delta^{13}C$ does not appear to be coupled to that of annual rainfall (Figure 7). So while $\delta^{13}C$ and F14C may be useful for establishing the spine-series chronology in this plant, this plant is not a good choice for evaluating interannual VP variability. Analogous to observations in dendrochronological studies [McCrae and Loder, 2004], the position of this cactus in a shallow water may make it less sensitive to annual or decadal variations in VP or precipitation. Cacti located on hillslopes and away from water drainages may show greater sensitivity to annual and decadal variation in VP 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 VP, rainfall or other climate data from the $\delta^{13}C$ variation in spine series. The other application of F14C and $\delta^{13}C$ in spines is to derive accurate annual growth rates of cactus stems (Figure 8). In addition to confirming that areole this characteristic. Spines exhibit more negative responses. The transverse bands present in saguaro spines possibly other cacti and stem succulents with sequen-

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