Climate Sensitivity in Mature Versus Pre-Reproductive Saguaro Determined from Spine Isotopic Signals

by

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ABSTRACT

The saguaro cactus is vital to the unique biodiversity of the Sonoran Desert. Many animals rely on it for survival and shelter. Due to its thick waxy exterior, it is able to retain a massive amount of water in its succulent stems, making it an ideal habitat for many birds that are native to the region. It also holds a large cultural significance for many native tribes of this region. Thus, it is important to understand how these magnificent plants are affected by changing environmental conditions in order to protect them in the future. Measurements of carbon isotope ($\delta^{13}C$) signals in saguaros spine tissues have been shown to be a robust approach for evaluating plant responses to climate variation over multiple seasons and years. This study investigated growth and $\delta^{13}C$ signals in young saguaros (< 2.5 m tall) at a location north of Phoenix to compare with large saguaros (> 4 m tall) that actively flower in the spring. Adolescent saguaros were examined to determine how they responded to variation in moisture between the years 2012 through 2016. As with a previous study by Hultine et al. (2018), this research demonstrated a strong relationship between height growth and precipitation. Stem growth was also highly correlated with the number of areoles produced across both young and old plants. Winter and late summer moisture from the previous year had a significant effect on stem growth, and a legacy effect could be seen in the low growth rates in 2014 which may be a result of low precipitation during the previous fall and winter. This study also established that the adolescent saguaro may be less sensitive to seasonal changes than the mature saguaro. When comparing the large plants with the adolescent plants, spine $\delta^{13}C$ values were similar between mature and adolescent plants, indicating that perhaps flower does not impose a significant added cost to saguaro water budgets.

However, identifying the cost of flowering requires more investigation than that from only spine carbon isotope measurements and coarse measurements of plant productivity.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my committee Co-Chair, Kevin Hultine, for always willingly teaching me plant physiology in a way that helped to get me excited about the beautiful desert. He has been the most patient with me at every step of the way even before I actually completed even one ecology course. While walking me through complex processes, he explained every piece of material in a way that was not only easy to understand but inspiring to learn. This research is built on the foundation of the tireless contributions he and the researchers at the Desert Botanical Gardens (DBG) have made to the study of saguaros and I truly would not have been able to take on this endeavor without him. Also, I'd like to thank the DBG for allowing me to use the lab and resources, and for their cooperation with ASU and the Biology department.

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CHAPTER 1

INTRODUCTION

The saguaro cactus (*Carnegiea gigantea*) is native to the Sonoran Desert and is considered a foundation species that is vital to many of the other organisms that live in the region (Angelini, Altieri, Silliman & Bertness, 2011). As one of the largest desert regions in the world, the Sonoran Desert spans 120,000 square miles and includes parts of Arizona, California, and northern Mexico (Salmón, 2012). Since the saguaro only grows in the Sonoran Desert, and since it takes a very long time for a saguaro to propagate, replenishing lost saguaro populations does not happen easily.

The unique aspects of the saguaro make it a vital part of the survival of many insects and animals living in this area. Since they have massive amounts of storage tissue, and also have a thick, waxy exterior, saguaros are able to retain substantial amounts of water in their large succulent stems (Turner & Funicelli, 2000). This makes saguaro stems perfect homes for many birds during the hot summer days. Woodpeckers are the first to make small holes along the main trunk since they are able to break through the saguaro's tough skin. These burrows (called boots) eventually become homes for other birds once the woodpeckers vacate their self-established domains. This is why saguaros are sometimes called bird motels (Wildlife Interactions, 2015). Vulnerable animals, such as the lesser long-nosed bat, use the saguaro as a food source by consuming the fruit or the nectar from the flowers that grow near the top of the plant (Flemming et al., 2001). Moreover, saguaro requires pollinating animals like nectar feeding bats to complete pollination for their propagation. Even though specialized species like the lesser long-nosed bat are less reliable pollinators for cacti, saguaros are still one of the few food sources for lesser long-nosed bats during their migration (Flemming et al., 2001). Migration patterns of bats are anticipated to coordinate with the timing of flowering. As shifts in flowering phenology change due to a warming climate, there could be critical impacts on seed dispersal, fruit set, population structure and geographic distribution of cacti species (Flemming et al., 2001). This symbiotic relationship between the saguaro and animals is a delicate balance, and sustaining healthy saguaro populations is important for the continuation of this unique ecosystem.

The saguaro also has important cultural connections as well. The saguaro cactus have a very significant role to play in the Tohono O'odham community and are a crucial element to the tribe's culture and religion. Tohono O'odham and other Native tribe spiritual leaders and elders stress their original premise for all living things. However, within the Tohono O'odham tribe the connection with the saguaro is very strong (Pater & Siquieros, 2000). For instance, in Tohono O'odham lore there is a story of the first emergence of saguaros that says there was a man who was given the responsibility of disposing of all the saguaro seed but a mischievous coyote cleverly deceived the individual into accidentally carrying them all over the southern slopes of the desert mountains where they grow to this day (Pater & Siquieros, 2000). The Tohono O 'odham use the fruit for rain ceremonies and for food and wine. The ribs of the saguaros are used for various types of structural purposes like building homes and fences, and have even been used as a type of calendar stick by carving notches into the saguaro. This community truly believes in living with their surroundings, instead of making their surroundings live for them (Pater & Siquieros, 2000). It's amazing how interconnected a culture can be to a plant, and this story only made me more fascinated about the versatility of the saguaro and better understand how precious it is. When I go back and consider how much it is dependent on by humans and wildlife, it is for these reasons that it is important to better understand the needs of the saguaro, as this understanding will allow us to play a more active role in its conservation and perhaps even develop new symbiotic ways of living with this organism ourselves.

In order to preserve limited resources, depending on how much precipitation has fallen during the winter and previous fall, saguaros may not produce many fruits or flowers during the next spring or summer (Hultine et al., 2018). This could impact the surrounding animals that depend on this plant for food, as well as have an impact on the population structure of the cactus itself. If saguaros aren't producing flowers, then fruit and nectar sources will be unavailable for nectivores, frugivores and other animals that rely on saguaros as a food source. Since precipitation in the Sonoran Desert ranges from below 100 mm in the northwest to 500 mm in the far south, it is important to better understand how cacti in these regions respond to variation in precipitation and evaporative demand, and how it could affect their environment (Hultine et al., 2018). Likewise, rising temperatures coupled with increased drought intensity and duration that are predicted by global circulation models may have a profound impact on the reproductive output of saguaro cactus in the coming decades.

There is potentially a high cost of flowering in terms of water allocation from the stem for desert succulent plants. It takes a saguaro about 35 years to begin flowering ("How Saguaros Grow"). Water allocation, in order to support flowering in arid conditions, can hinder the survival and future growth. After a single period of reproduction, Nobel (1977) was able to deduce that the water cost of flowering is enough to explain vegetative plant death. He did this by modeling water costs connected to the redirection of water from leaves to inflorescences, or flowering (Reekie & Bazzaz, 2011). Because of the potentially high flowering cost, researchers are looking into whether or not protected species of plants, such as the saguaro, are in danger of lower reproduction rates or producing less fruit and nectar in the future due to changes in climate. According to current data, it can be deduced that under conditions of drought the saguaro may be forced to limit its flowering output (Renzi, Peachey & Gerst, 2019).

Saguaros, like almost all cactus plants, use the Crassulacean Acid Metabolism, (CAM) photosynthetic pathway. This is a carbon-concentrating photosynthetic pathway that allows plants to acquire CO_2 at night, while water loss is minimized. CAM plants then undergo the carbon fixation process of photosynthesis during the day. This temporal separation of the two processes has developed to limit the time that stomata are open at night, when water loss would be less likely. $\delta^{13}C$ in CAM plants such as saguaro decreases with drought stress, largely because stomatal conductance is reduced, limiting the diffusion of CO_2 for photosynthesis (Hultine, et al., 2018). Plants that use CAM photosynthesis have a very different carbon isotope composition in their tissues than plants that use the more common C3 photosynthetic pathway (Hultine et al., 2018). This is because the primary photosynthetic enzyme in CAM plants is PEP Carboxylase, which has a higher affinity for the heavier naturally occurring stable isotope $\delta^{13}C$. This is different from the primary photosynthetic enzyme of C3 plants, Rubisco, which has a higher affinity for the lighter more common isotope $\delta^{12}C$ in atmospheric CO_2.

These enzymes are important because they convert CO_2 into sugars during photosynthesis. Despite these different affinities, the carbon isotope ratios of both CAM plants and C3 plants can reflect plant stress and exposure to droughts and heat waves (Winter & Holtum, 2002). Therefore, the isotopic signals in saguaro spines - determined by the ratio of $\delta^{12}C$ / $\delta^{13}C$ relative to a known standard and expressed in the notation $\delta^{13}C$ - are similar to measuring isotopic signals in the growth rings of trees in that they can provide insight on climate responses through time (Hultine, et al., 2018).

For example, carbon isotope ratios were measured in the spines of saguaro from six locations varying in mean annual temperature and precipitation in the northern Sonoran Desert (Appendix A; Hultine et al., 2018). The spines were collected from near the apex of the stems, representing the most recent growth, to compare with the climate conditions from each of the six locations (Hultine, et al., 2018). This study allowed the examination of the climate conditions that support fitness responses and growth of the giant saguaro. It also helped develop a way for evaluating possible impacts of future temperature surges particularly at night, as well as the impact of aridity on this foundation species and other biota in the region. The study was able to deduce that complex climate conditions from the previous summer and winter were coupled to current year's photosynthetic physiology recorded through isotope $\delta^{13}C$ and stem growth.

Much like $\delta^{13}C$, which is used to help monitor changes in the photosynthetic processes, $\delta^{18}O$ can be used to monitor changes in the water balance of the plant. Taking samples from the apex, or the top of the saguaro where new spines grow, and then sampling spines down the axis of the stem can provide a picture of how $\delta^{13}C$ and $\delta^{18}O$

vary through time (English et al., 2007), and potentially identify years in which long-lived cactus plants such as saguaro begin to produce flowers (Hultine et al., 2018). Before flowering begins, the spines located at the apex record the seasonal wet/dry cycle and can clearly show a constant annual fluctuation of $\delta^{18}O$ in pre-reproductive plants (Hultine et al., 2018). Because of the ability to examine a clear cycle when looking at the spines that form prior to a plant reaching reproductive maturity, changes should be detectable in this cycle once the saguaro begins producing flowers due to the high levels of water investment into flower production.

My research expands upon previously published work, which studied the isotopic signals of mature saguaro plants to determine how saguaros respond to seasonal climate variation (Hultine et al., 2018), by now analyzing isotopic signals on spines from pre-reproductive saguaros. By comparing water costs of flowering between adult and adolescent saguaros, we can add to the science community's understanding of this plant's resource allocation throughout its long lifespan. Due to COVID-19, there were a few setbacks in the research process of this thesis over the past year and a half. The pandemic has put limitations on my research such as the inability to participate in field work, cut off my access to the lab, and made it difficult to reach out for assistance from my professors and members of my lab group. Nevertheless, after pairing down this research to an exploration of $\delta^{13}C$, climate, growth data and areole production I was able to produce empirical data that adds to the academic research on water consumption in saguaros. The question that this research answers is whether flowering has a significant water cost to the plants overall water budget using growth data, areole production data, climate, and isotopic data.

CHAPTER 2

MATERIALS AND METHODS

In order to address this question, my process was to conduct research in conjunction with my mentor plant physiologist/ecophysiologist, Kevin Hultine, PhD, at the Desert Botanical Garden (DBG). Our methods strategy was four pronged, 1) literature review, 2) lab orientation, 3) sample preparation and 4) data analysis. My literature review work began in May of 2019. I gathered, studied, and synthesized available information on the topic to develop my understanding of the previous work. Kevin Hultine provided me with articles to help begin my research, and then I supplemented that information through literature that I collected from the ASU library, and other internet searches of peer reviewed studies to comprise a robust list of sources that are cited within. My mentor and I conferred together in order to build up my basis of this field of study. The purpose of this stage of the process was to better understand the current state of research in this area and to identify how our new research could add value to the community of study.

In my lab orientation in 2019 at the end of my undergrad, Professor Hultine introduced me to the lab where I would eventually be working to process spines. I learned where the collected spines were stored in the lab at the DBG, how I'd eventually weigh, package and record the samples when the time came. In 2020, Kevin brought out all of the adolescent samples that I would need to process from years 2012-2016. These samples consisted of approximately 190 spines each of 2.5 cm to 7.5 cm in length, representing five different cacti studied over those years. The site that holds these cacti is located in the Cave Creek Regional Park, elevation of 601m, and the coordinates are

33°48’43.63” N 111°59’21.19” W. I started with the first year samples, and separated the samples by cacti label number. Working on one sample at a time, I would split the spine in the middle with pliers in order to gain access to only the center portion of the spine. Then, using a razor blade and holding the spine firmly, I spliced the spine in half vertically, and then repeated that again, in order to cut off a portion of the spine that I would weigh to be between 0.5 and 1.0 mg. In order to weigh the spine sample, I used a tin capsule and placed it on a microbalance. Once the sample met the required weight for analysis I recorded the weight, then I folded the tin box onto itself using two forceps in order to make the capsule small enough to analyze with a mass spectrometer. After that, I put samples in the microplate wells that corresponded to the numbers on the weigh sheet. I repeated that process for all samples. When completed, I covered the microplates and placed them in a box to be analyzed at the University of Arizona, Environmental Isotope Laboratory.

Understanding the methods used in Hultine’s study on *reproductive* saguaros is important because I used those methods to study *pre-reproductive* saguaros. From Hultine’s last study referenced above, his methods and materials are outlined here because I used these similar methods, since this research is a continuation of the previous work.

As outlined in “Relationships Among Climate, Stem Growth, and Biomass $\delta^{13}C$ in the Giant Saguaro Cactus (Carnegiea Gigantea)”, Hultine et al. 2018 conducted research at six sites in the Sonoran Desert. Five sites were located in southern Arizona and one site was located near the Pinacate Biosphere Reserve in Puerto Penasco, Sonora in Mexico. These sites were chosen because they presented a suitable range in average precipitation. The researchers studied six to nine single-stemmed saguaro plants from each site. The saguaros had a height range from 2.25 to 4.00 meters. This height was selected because this is the size that saguaro stems achieve their highest growth rates, and because this height is above the minimum necessary for reproductive maturity to be achieved, plus the stem apex could be assessed with a five-meter-tall orchard ladder. Annual growth was measured during the 2014 growing season at each site, and over four consecutive growing seasons (2013 – 2016) at two of the Arizona locations. The samples that are used in this current study are from just one site in Cave Creek instead of six different sites like the previous study. For future research, when field work resumes, more samples will be collected from all the sites.

In the Hultine et al. (2018) research, colored fabric paint was used on the stem and the spines to identify each year's growth. By coloring the tips of the spines that were located at the apex of every saguaro, stem growth was able to be measured and recorded by the DBG. Painting spines annually yields important information because annual stem growth and a number of areoles produced are accurately measured year over year. Furthermore, the age of the individual spines is recorded on the plant. The distance between the apex and the areole was measured with field tape, in addition the number of areoles produced in a season was recorded. The relationship between growth, climate, and spines $\delta^{13}C$ values were evaluated from the collected spines. The researchers collected 399 spine samples and analyzed them for $\delta^{13}C$ signals. Spine samples were split with a razor blade and weighed on a microbalance and loaded into a tin capsule. Samples were sent to the University of Arizona Environmental Isotope Laboratory to be analyzed. Stem volume was measured repeatedly at two of the Arizona sites for three years. Ribs around each stem were counted and stem diameter and mean rib height were measured. These measurements were used to calculate stem volume. I also applied this method to my sample group as well.

Hultine et al (2018) measured spine $\delta^{13}C$ and stem growth from a broad range of climate conditions from the northern Sonoran Desert. These parameters included precipitation (P), vapor pressure deficit (D) and a moisture index (M_i) derived from P / D (Hultine et al., 2018), during periods of growth and spine production, and to evaluate the potential long-lasting effects from climate conditions. Regression analysis was performed to relate their data collection to precipitation, as well as to relate stem growth to the number of areoles produced. Temporal relationships between stem growth and $\delta^{13}C$ with precipitation and M_i at two locations were also evaluated over four consecutive years. I used the same methods for analyzing the adolescent cacti data. I then used a t-test to compare my data set to better understand any differences or similarities to how they are affected by environmental pressures and be able to better understand the cost of flowering on water allocation in saguaro.

CHAPTER 3

RESULTS

Table 1: Results from the linear regression of site stem growth and areole production for mature plants, collected between the years 2012 and 2016 against total precipitation, VPD and precipitation divided by VPD.

Mature				
Cave Creek Parameters	Stem growth		Areole Production	
	F	P	F	P
Precipitation				
July – October	3.87E-05	0.9861	0.008512	0.9349
October - September	13.51	0.0667	12.7	0.07054
October - September *2 yrs	0.6743	0.4979	0.5492	0.5358
September - August	1.056	0.4121	1.011	0.4206
July -October previous year	1.584	0.3352	2.112	0.2833
September - August * 2 yrs	0.2301	0.6788	0.3022	0.6377
Monsoon * 2 yrs	0.7204	0.4854	1.068	0.02223
July (previous year) - August	3.607	0.198	3.65	0.1962
October - March	0.105	0.7766	0.04177	0.857
VPD				
July – October	0.03961	0.8606	0.1056	0.7761
October - September	0.06387	0.8241	0.144	0.7408
October - September *2 yrs	0.7241	0.4844	1.078	0.4082
September - August	0.4442	0.5737	0.6832	0.4954
July -October previous year	2.946	0.2282	4.084	0.1807
September - August * 2 yrs	0.6791	0.4965	1.017	0.4193
Monsoon * 2 yrs	0.7029	0.49	1.0511	0.4131
July (previous year) - August	1.091	0.4058	1.591	0.3344
October - March	0.05539	0.8358	0.1382	0.7458
precipitation/VPD				
July – October	0.003591	0.9577	0.03196	0.8746
October - September	1.909	0.3011	2.519	0.2534
October - September *2 yrs	0.05757	0.8327	0.1373	0.7465
September - August	2.092	0.285	2.584	0.2492
July -October previous year	1.802	0.3115	2.362	0.2641
September - August * 2 yrs	1.029	0.4172	1.107	0.4031
Monsoon * 2 yrs	0.4381	0.5761	0.6857	0.4947
July (previous year) - August	4.337	0.1727	5.734	0.1389
October - March	0.03159	0.8753	0.003113	0.9606

Tables 1 and 2 were used to view any relationships between the climate data and the various growth data of large and small saguaros. It is interesting that growth did not correlate with any of the climate parameters in the adolescent cacti, while growth in the large saguaros was only correlated with precipitation that fell from October of the previous year to September of the current year (Table 1). Likewise, the number of areoles produced per year (an important indicator of reproductive potential) was only correlated in large saguaros with monsoon moisture that fell over two consecutive growing seasons (Table 2), while areole production was not correlated with any of the climate parameters measured in the small saguaros (Table 1). However, this data, even though it's only within a relatively short four-year time span, sets a foundation for future research that can expand on these data. We can use these numbers and compare them against what we might observe in another four years, to give us a fuller picture of how growth and reproduction are affected by climate variability. The ability to capture these data now, adding to the previous data from multiple sites over many years, will allow us to gain deeper insights into how these plants adapt to climate change.

Table 2: Results from linear regression of site stem growth and areole production for adolescent plants, collected between the years 2012 and 2016 against total precipitation, VPD and precipitation divided by VPD

Adolescent				
Cave Creek Parameters	Stem growth		Areole Production	
	F	P	F	P
precipitation				
July – October	0.6911	0.4668	0.2782	0.6344
October - September	0.1084	0.7636	0.1612	0.7149
October - September *2 yrs	2.063	0.2464	2.84	0.1905
September - August	0.2875	0.629	0.3041	0.6197
July -October previous year	0.1797	0.7002	0.08136	0.794
September - August * 2 yrs	2.744	0.1962	2.222	0.2329
Monsoon * 2 yrs	0.1653	0.7116	0.0349	0.8637
July (previous year) – August	0.05863	0.8243	0.07457	0.8025
October - March	2.185	0.2359	1.861	0.2658
VPD				
July – October	2.164	0.2376	1.121	0.3675
October - September	2.788	0.1936	1.507	0.3072
October - September *2 yrs	1.49	0.3094	0.7443	0.4517
September - August	3.286	0.1676	1.674	0.2863
July -October previous year	0.222	0.6697	0.1114	0.7605
September - August * 2 yrs	0.4492	0.5507	0.1691	0.7085
Monsoon * 2 yrs	0.8568	0.4229	0.3925	0.5754
July (previous year) – August	1.102	0.3709	0.5612	0.5082
October - March	0.2062	0.6806	0.02832	0.8771
precipitation/VPD				
July – October	0.9734	0.3966	0.4243	0.5612
October - September	0.1161	0.7558	0.02431	0.886
October - September *2 yrs	0.1211	0.7508	0.3303	0.6058
September - August	0.02517	0.884	0.06507	0.8151
July -October previous year	0.1419	0.7315	0.0639	0.8168
September - August * 2 yrs	0.1224	0.7496	0.05479	0.83
Monsoon * 2 yrs	0.2181	0.6723	0.04678	0.8426
July (previous year) – August	0.004657	0.9499	0.000744	0.98
October - March	2.646	0.2023	2.044	0.2481

Stem growth was well correlated between the adult and adolescent saguaro over these five years, with the year 2014 being a standout difference (Figure 1). The only year out of the five where the adolescent plants out grew their mature counterparts was in the year 2014. The stem growth average for adult plants varied from a low of 11.12 cm to a high of 20.62 cm, while the stem growth of adolescent plants varied from an average low of 8.00 cm to a high of 19.70 cm. The mean of all mature areole production varied from a low of 3.00 to a high of 7.75, whereas the adolescent plants had an areole production that varied from a low of 2.00 to a high of 6.00. We learned from previous research from Nobel (1986), English et al. (2010a) that areoles increase as the stem height increases because areoles are evenly spaced on the stem as it grows. This new data reflects the information from the Hultine et al. (2018) study, which also demonstrated a growth and fitness relationship. These data demonstrate that stem growth was highly correlated with the number of areoles produced across both young and old plants. The margin of difference in the averages between mature and adolescent areole production was greater than the margin for height growth since the adolescent plants out grew the mature group in 2014. In Hultine et al. (2018) it was theorized that winter and late summer moisture from the previous year has a great effect on stem growth. In support of that claim, adolescent stem growth in 2014 could possibly be a result of the previous year’s precipitation of 405 mm, with a VPD of 1.86 and a moisture index of 218 (Table 3). However, for both groups, both stem growth and areole production dropped in 2015 and 2016 reflecting dryer conditions.

Table 3: Climate means for Cave Creek between the years 2012 and 2016. This includes precipitation, VPD, and moisture index (precipitation/VPD).

Parameters	Precipitation (mm)	VPD	Precipitation/VPD
2012			
July – October	110	2.14	51
October – September	185	1.8	103
October - September *2 yrs	393	1.97	200
September – August	164	1.9	86
July -October previous year	45	3.21	14
September - August * 2 yrs	373	2.03	184
Monsoon * 2 yrs	161	2.64	61
July (previous year) - August	196	2.16	91
October – March	75	1.23	61
2013			
July – October	130	2.13	61
October – September	280	1.82	154
October - September *2 yrs	465	1.81	257
September – August	241	1.82	133
July -October previous year	110	2.14	51
September - August * 2 yrs	405	1.86	218
Monsoon * 2 yrs	248	2.06	120
July (previous year) - August	323	1.87	173
October – March	150	1.29	116
2014			
July – October	131	2.02	65
October – September	234	1.81	130
October - September *2 yrs	515	1.81	284
September – August	257	1.84	140
July -October previous year	130	2.13	61
September - August * 2 yrs	499	1.83	273
Monsoon * 2 yrs	268	1.99	135
July (previous year) - August	320	1.88	170
October – March	102	1.33	77
2015			
July – October	36	3.33	11
October – September	260	2.11	123
October - September *2 yrs	494	1.96	252
September – August	303	2.03	149
July -October previous year	131	2.02	65

September - August * 2 yrs	560	1.94	290
Monsoon * 2 yrs	226	2.44	93
July (previous year) - August	390	2.05	190
October – March	144	1.24	116
2016			
July – October	62	3.34	19
October – September	229	2.15	106
October - September *2 yrs	489	2.13	230
September – August	227	2.14	106
July -October previous year	36	3.33	11
September - August * 2 yrs	530	2.09	254
Monsoon * 2 yrs	153	3.01	51
July (previous year) - August	262	2.36	111
October – March	155	1.21	128

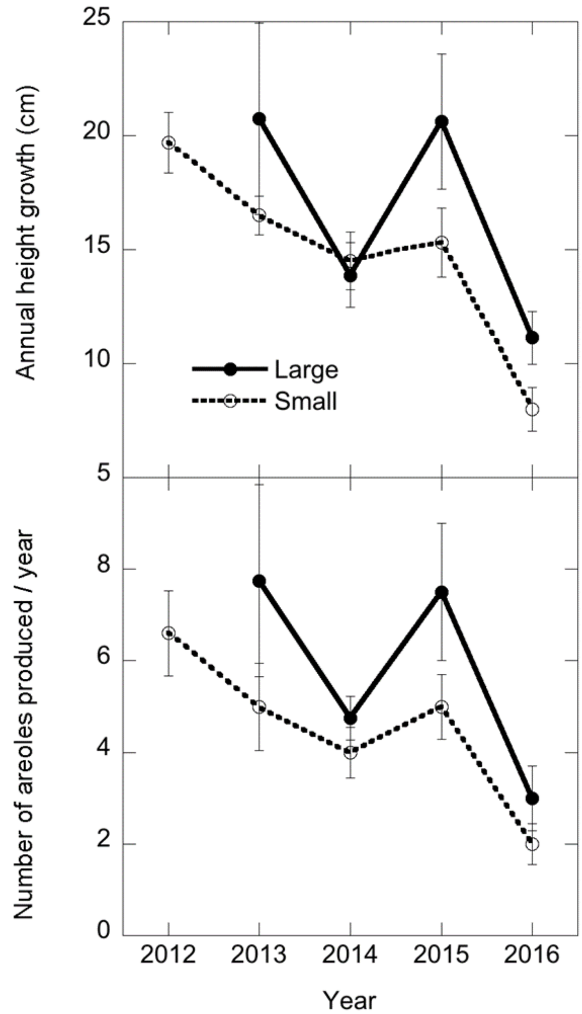

Figure 1: The annual growth and areole production compared between the years 2012 and 2016

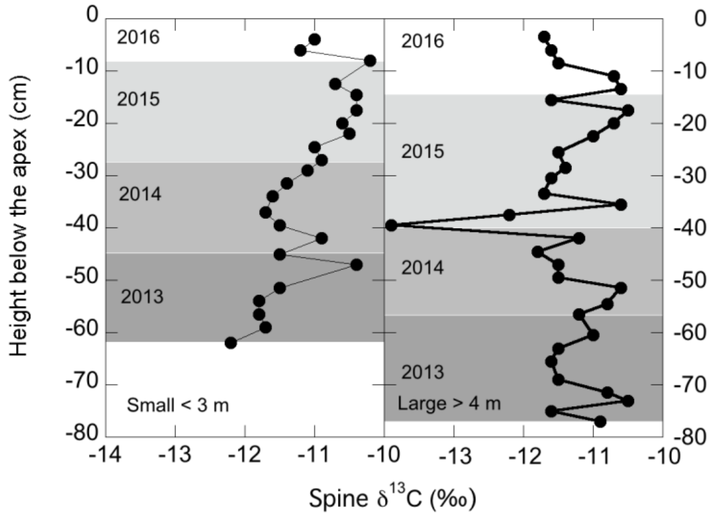

Figure 2: Time series of $\delta^{13}C$ abundance in spines produced over four consecutive growing seasons from one adolescent and one mature saguaro at the Cave Creek location. The range of values for the adolescent plant is from approximately -10.1‰ to -12.3‰, and the range for the mature plant is between approximately -10.4‰ and -13.9‰.

It appears that the adolescent saguaro is less sensitive to seasonal changes than the more mature plant. However, if the outlier at -13.9‰ at the beginning of 2015 were removed from the time series of the mature saguaro, both plants would have values that range between -12 and -10‰: values that are typical for constitutive CAM plants (Winter & Holtum, 2002). Unlike the mature plant, the adolescent plant showed a gradual progression towards higher $\delta^{13}C$ values, particularly in 2015 (Figure 2). These patterns suggest that the plant was operating with an improved water balance and conducting higher rates of photosynthesis compared to other years, even though the monsoon season was drier and hotter relative to other years spine isotopic signals were measured (Table 3). These patterns underscore the complexity of evaluating climate sensitivity in succulent stemmed plants that rely on long-term water storage for survival.

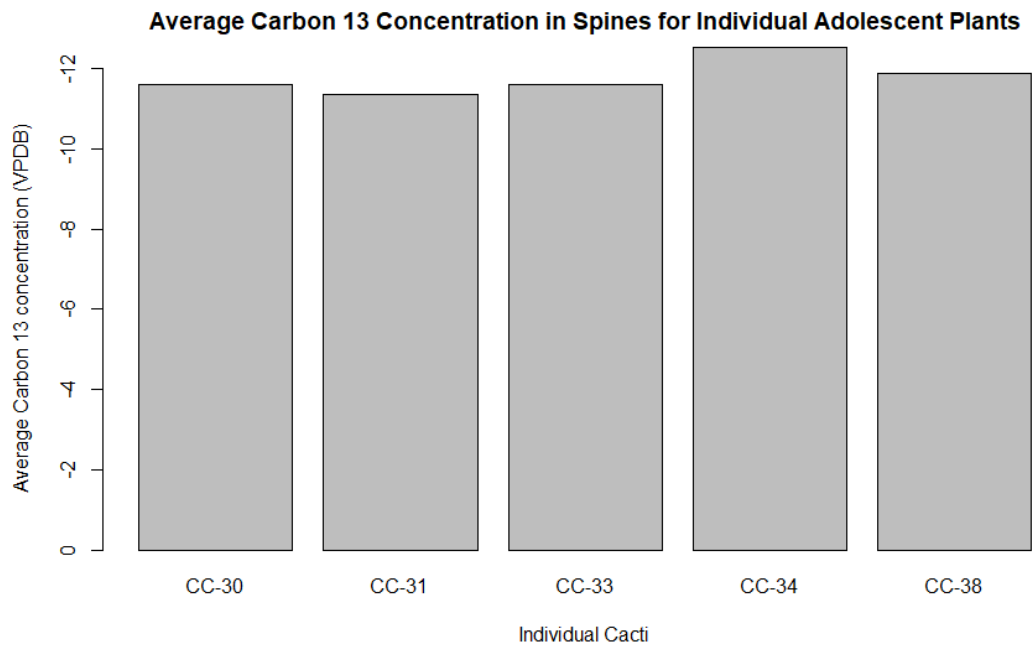

Figure 3: A comparison of $\delta^{13}C$ abundance in adolescent saguaro spines produced in 2014 at Cave Creek.

The originally intention was to show $\delta^{13}C$ data in Figures 3 and 4 over multiple years. However, the pandemic altered those plans. Due to constraints from COVID-19, access to the lab was quite difficult. Not only was no one allowed in the lab for approximately eight months, but once researchers were allowed access to the lab at the DBG, only one person was allowed in the lab at a time. What this restricted was the ability to send out and receive data and samples in a timely manner, and it prevented quality checks and assurances. In a recent survey of doctoral researchers, it was found that the pandemic lockdown impaired the ability to collect data, discuss ideas, and disseminate findings (Byrom, 2020). It is notable to include limitations of the lockdown as a constraint on this study's results, because it will be critical for future researchers to learn from this in order to mitigate these issues should another lockdown occur. Since access to the samples in this current study were limited, not all the samples could be analyzed correctly in time. Nevertheless, we were able to process $\delta^{13}C$ data for all of the target plants for the year 2014. This was a particularly interesting year, as seen in the stem growth and areole production data above in Figure 1, which made including the $\delta^{13}C$ data for this study beneficial.

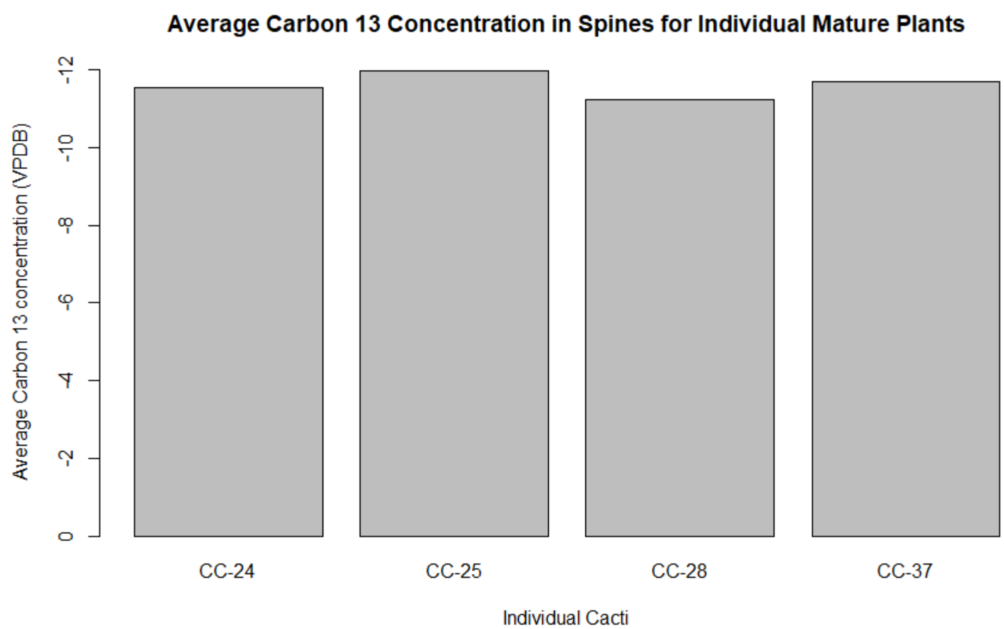

Figure 4: A comparison of the abundance of $\delta^{13}C$ in the spines produced in mature saguaro in 2014 at Cave Creek.

Unlike Figure 2, which only reports isotope data for one plant over multiple years, Figure 3 reports isotope data for all five small plants in 2014, and Figure 4 reports isotope data for four large plants in 2014. Neither the large plants or the adolescent plants produced large ranges in mean spine $\delta^{13}C$ values, perhaps indicating that stem water storage buffered all of the plants from annual and intra-annual fluctuations in climate conditions. However, there are a few individuals that stand out slightly more than others, such as saguaro CC-34 for the adolescent with a $\delta^{13}C$ abundance of -12.54‰ and saguaro CC- 25 for the mature with a $\delta^{13}C$ abundance of -11.98‰ (Figure 5).

In 2014, height growth was similar between adolescent and mature plants (Figure 1) and spine $\delta^{13}C$ abundance was also similar between adolescent and mature saguaros (Figure 5). Although the comparison is limited to a single year, the fact that adolescent and mature saguaros had similar growth and $\delta^{13}C$ abundance indicates that the cost of flowering in mature saguaros is not easily detectable from spine carbon isotope measurements and coarse measurements of plant productivity.

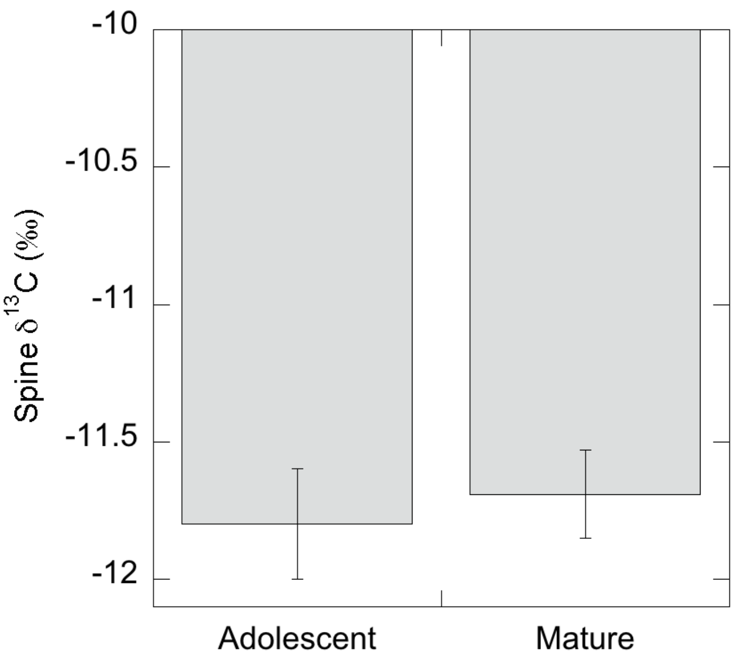

Figure 5: Mean $\delta^{13}C$ values in spines produced in 2014 in adolescent and mature saguaro plants occurring at Cave Creek. Error bars represent the standard error of the means.

CHAPTER 4

DISCUSSION

Saguaros will only flower if they are growing, meaning that they are getting all the water and nutrients that they need to flourish. If they are not reproducing that would indicate that they are using their energy for something the plant has deemed to be more important, like survival. The wait to test the reproduction of a saguaro is a long one, as it is 35 years before a fully-grown plant begins to flower ("How Saguaros Grow"). This extended life cycle makes it difficult to quickly gather information on this long-lived plant species. However, time isn’t the only constraint that has led to a lack of research on this topic. Location has a part to play as well since the saguaro does not grow in any other part of the world other than the Sonoran Desert. Nevertheless, there are several published studies regarding saguaro reproduction completed over the past few decades that focus on flowering phenology, and complex responses to climate in adult and adolescent saguaros (Steenbergh and Lowe 1977, Drezner 2008, Crimmins et al. 2010, Renzi et al. 2019). These previous studies provide ongoing data from saguaro research plots, which help to create a foundation for more accurate learning on how these plants survive and reproduce. However, the challenge of sample locations and the infrequency of study in the conditions needed does not make critical analysis easy. This highlights the importance of contemporary studies like those from Hultine et al. (2018) that examined the factors that contribute to the fitness and population structure of the saguaro cactus amongst multiple locations. In the 2018 study, the results demonstrated that growth and fitness are influenced by climate conditions and recent legacies. By analyzing isotopic comparisons among various locations in the Sonoran Desert, their study provided foundational information on the overarching effect of climate on giant cacti. That study became the basis for this continued research, and utilized the saguaro plots and previously gathered samples as a foundation for this analysis. Therefore, the main goal of this current paper was to produce empirical data that adds to the academic research of water consumption in saguaros that continues the work of previous studies.

The Hultine et al. (2018) study focused on other factors that affect mature saguaro growth besides precipitation, such as evaporative demand (i.e. vapor pressure deficit) of the affected stem and areole production. To expand on the previous study with this current study, precipitation, VPD and moisture index (precipitation divided by VPD) were examined alongside growth and areole production data from adolescent plants. Some results from our current study indicate that there is no significance between the climate data and the various growth data used. This is shown through the results from the regression that was conducted where the P values of much of the data was above the significance that was set as 0.05. This research put alongside previous work might lead to conclude that there is a higher water cost for mature plants than for the adolescent. Meaning that because mature plants flower, climate shifts or seasonal changes have a larger effect on their overall growth or well-being, meanwhile adolescent plants are not as affected because they are not allocating their resources to reproduction. This makes the growth in large saguaros more variable with larger reductions during dry years that the adolescent plants. The American Southwest has a more rapid rate of climate change than anywhere else within our continent excluding the possibility of the arctic (Overpeck and Udall, 2010). Over time, as temperatures continue to rise throughout the years each summer, adolescent saguaros have a higher chance of survival since they are not shifting their resources from growth to reproduction. As we learned from the Hultine et al (2018) study, growth and climate relationships are more complex than previously thought. The plants aren’t just affected by precipitation in the current year, rather they have a legacy effect from multiple seasons. Since precipitation and temperature are arguably the most important factors in a plant’s reproduction (Crimmins et al., 2010), this research creates a reference point for how adolescent plants, which are the future of the plants' newer population, will survive.

The original proposal for this project was to gather samples from six locations throughout the Sonoran Desert: Cave Creek, Cascabel, Kofa Mountains, Papago Park, Pinacate, and Tumamoc Hill; this would have mirrored the locations studied in the Hultine et al 2018 study. This project originally would have examined a sample set of five adolescent saguaro cacti from each location, while observing five variables: precipitation, VPD, moisture index, $\delta^{13}C$ abundances, and $\delta^{18}O$ abundances for each plant. However, in February 2020, COVID-19 became a global pandemic and put the world into lockdown which changed the modality of this project. This unprecedented constraint forced the difficult decision to reduce the scope of the project in order to produce enough of an analysis in time. Therefore, the sample locations were reduced from six to one (Cave Creek), and the variables were reduced from five to four (reducing the number of $\delta^{13}C$ years examined and eliminating the examination of $\delta^{18}O$ altogether).

Many academic institutions and researchers in STEM fields experienced similar constraints where their original plans for 2020 were thwarted and they had to start anew, affecting not only the modality of the research but also the survival of the projects due to changes in funding (Radecki, and C. Schonfeld, 2021). In addition to changes in scope, this current study also had to extend the time frame into another semester because there wasn't enough time to get back into the lab with the limited lab time and resources available to fit it all in before the original end date. This extension required addition funding proposals for scholarships as well. The reduced scope, extended time frame, and additional costs due to the global pandemic are notable as these lessons will not only help future researchers in STEM, but it has also laid an easy roadmap of where an extension of this study can continue: add $\delta^{18}O$ abundance data, examine the other sample locations.

When continuing this study in the future, other isotopes should be added in order to get a clearer understanding of how the saguaro allocates water resources. There is currently not much research examining the relationship between long lived desert plant photosynthetic processes, changes in water balance, and reproduction. This additional isotope data can help us understand the tradeoffs between these two areas (Williams et al., 2014). Since, the isotopes composition of $\delta^{13}C$ and $\delta^{18}O$ in the spines of saguaros are relatively stable, measuring those factors over time can help tell the story of how saguaros respond to climate change. Specifically, a change in $\delta^{18}O$ and $\delta^{13}C$ abundance may be detected during the onset of flowering which is a time during the saguaros life where water is allocated to flow to flowers (Williams et al., 2014). For instance, saguaros that receive more precipitation will start reproducing earlier since they are smaller in size than other plants. Compared to saguaros in a more arid area that might need to hold off on reproducing till they are more mature because of limited resources. This isotope records seasonal wet/dry cycles that can be used to observe the difference in usage between large and small plants when compared. Small plants have strong annual cycles because they don't use up their water resources for flowering and fruit, while large plants water balance is disrupted because the mature plants are losing water through their flowers and fruit (Williams et al.). Much like $\delta^{13}C$, $\delta^{18}O$ is a non-destructive tool that can be used to monitor this water balance fluctuation (Williams et al., 2014). Examining $\delta^{13}C$ is useful, but it is only part of the story. By adding in another isotope that is necessary for the plants' water balance, we can learn more about how and when a plant allocates resources to reproduction in times of stress (i.e., climate change).

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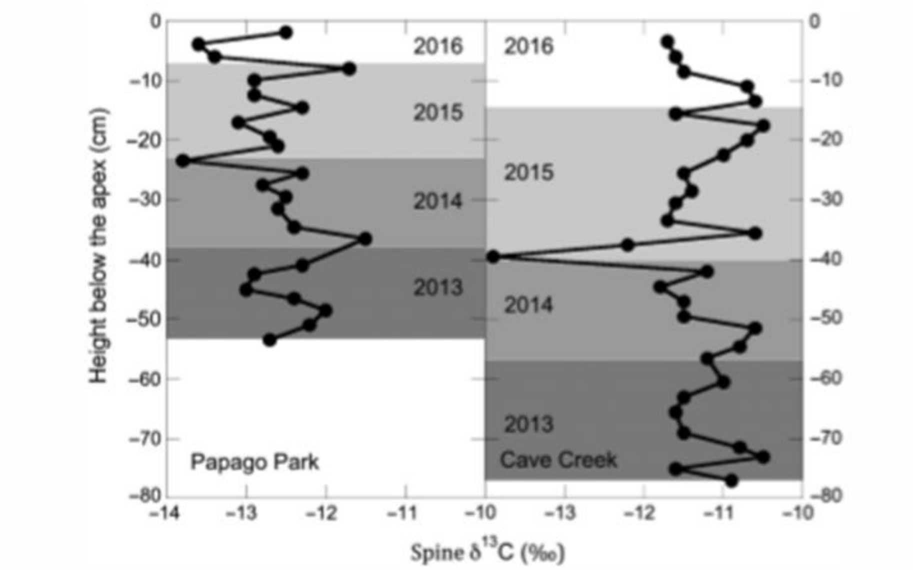
APPENDIX A

TIME SERIES OF MATURE SAGUARO FROM HULTINE ET AL. 2018



Appendix A: δ^{13}C series of spines collected over four growing seasons from one saguaro located at Papago Park and the other at Cave Creek. The δ^{13}C is slightly higher at Papago Park which is the drier and hotter location (Hultine et al., 2018).