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Certain *Agave* Species Exhibit the Capability to be Moderately Productive Under
Conditions of High Salt and Drought Stress

Steven James Bergsten

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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December 2013

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ABSTRACT

Certain *Agave* Species Exhibit the Capability to be Moderately Productive Under Conditions of High Salt and Drought Stress

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Master of Science

Water availability and arable lands are increasingly limiting resources in many parts of the U.S., particularly in semi-arid and arid regions. As a means of addressing food and fuel demands associated with burgeoning population growth, highly productive and water-use efficient crops need to be identified. One potential crop, *Agave*, merits consideration and evaluation due to its putative capability to provide sustenance and energy despite growing in water-limited regions and on marginal soils. However, little is known regarding the productivity these succulent plants will have under growing conditions of the Southwest, where high concentrated saline soils are abundant, and water is often limited. The objectives of these studies were to determine the effects of high levels of salinity and different volumetric water content levels (VWC) on plant growth, biomass accumulation, and nutrient uptake.

I used a hydroponic study to compare the effects of four salinity treatments (0.5, 3, 6, and 9 dS m⁻¹) on productivity of four *Agave* species (*Agave parryi*, *Agave utahensis* ssp. *kaibabensis*, *Agave utahensis* ssp. *utahensis*, and *Agave weberi*). In a second study, an automated irrigation system was established to examine four pre-determined VWC threshold set-points and simulated a gradient of well-watered to drought conditions, to evaluate how *A. weberi* would respond to varying levels of water availability. Salinity concentrations did not significantly affect root and plant dry weight accumulation in *A. weberi*, but all other agave plants experienced less biomass accumulation under high saline conditions (>6 dS m⁻¹). Seedlings of *A. utahensis* were two times more likely to die in the two highest saline treatments (6 and 9 dS m⁻¹) than the two lower treatments (0.5 dS m⁻¹ and 3 dS m⁻¹). Calcium, Mg, S, Mn levels decreased in both *A. parryi* and *A. weberi* at higher salinity levels. *Agave weberi* was able to tolerate salinity, but it also experienced lower biomass production ≤3 dS m⁻¹. In the water-stress study, *Agave weberi* plants experienced a decrease of 2.11 g as compared to plants in the highest treatment. Plants in the intermediate VWC treatments had similar dry mass values as those in the highest treatment, which suggests that this species could have moderately high yields under limited water conditions, and consequently should be evaluated as a potential bioenergy crop for semi-arid regions, such as the U.S. Southwest.

Agave shows considerable potential to be grown in arid and semi-arid regions that are moderately high in salinity and have limited water availability. Indeed, the cultivation of *Agave* as a crop appears to be a viable option for many areas of the Southwest. While some of the *Agave* species evaluated were quite productive under moderate salt and water stress, it is uncertain if growth will be significantly reduced if under these stress conditions for periods longer than 3 months.

Keywords:

Agave, *Agave parryi*, *Agave utahensis*, *Agave weberi*, automated irrigation, drought stress, hydroponic, nutrient uptake, salt stress, volumetric water content, water-use efficiency

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Ryan Stewart. He has been very dedicated in helping me understand how to use the scientific method, and has spent countless hours aiding in projects and revising my thesis. I would also like to thank Cali McMurtrey, Yue Shen, and Westen Archibald for helping me take measurements on several of my studies.

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Chapter 1

Evaluation of the Effects of Salinity on Productivity and Nutrient Levels of *Agave* Species
Important to Agriculture and Environmental Restoration in the Southwestern U.S.

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Manuscript prepared for submission to the *Journal of Arid Environments*

ABSTRACT

Agave exhibits potential to be used in saline soils as a feedstock source for cattle, bioenergy production, and for reestablishment in burned areas. However, little is known regarding the productivity levels of *Agave* when grown in saline soils in the semi-arid U.S. Southwest. Hydroponic experiments were carried out to evaluate the effects of salinity on biomass accumulation and nutrient uptake of *Agave parryi*, *A. utahensis* ssp. *kaibabensis*, *A. utahensis* ssp. *utahensis*, and *A. weberi*. Salinity treatments (0.5, 3, 6, and 9 dS m⁻¹) were imposed in each experiment. Both subspecies of *A. utahensis* were sensitive to salt treatments and experienced high mortality and lower plant dry weight in the higher salinity treatments. *Agave parryi* was more tolerant and only experienced a decrease in plant dry weight in the 9 dS m⁻¹ treatment. *Agave weberi* was the most tolerant of the species to high salinity and did not have a significant reduction in growth even in the 9 dS m⁻¹ treatment. Calcium, Mg, S, and Mn levels decreased in both *A. parryi* and *A. weberi* at higher salinity levels. *Agave parryi* also had a decrease of K and P in the higher salt treatments. The decrease in nutrients was not severe enough to cause any nutrient deficiencies across species. *Agave* plants tolerate salinity at higher levels than previously thought, which suggests that they can grow in more areas of the Southwest than previously expected.

Keywords: Century plant; *Agave parryi*; *Agave utahensis*; *Agave weberi*; hydroponics; salt stress; bioenergy

INTRODUCTION

In much of the U.S., groundwater levels have been declining dramatically (Konikow, 2013), which could potentially lead to local and regional water shortages, especially in areas that require large water inputs for crop production (Dominguez-Faus et al., 2009). Water shortages in the US southwest are of particular concern, considering many semi-arid areas receive less than 500 mm of rainfall per year (FAO, 1989). Such limitations increase the need for identifying plant species that can be productive despite constraints on water availability. One potential group of plants that could be utilized for agricultural and environmental restoration purposes in the U.S. Southwest is the *Agave* genus. *Agave* constitutes several species that utilize the Crassulacean acid metabolism (CAM) photosynthetic pathway (Nobel, 2010). The CAM pathway enables *Agave* species to utilize low amounts of water relative to plants that photosynthesize through the C₃ and C₄ pathways. Agaves exchange carbon dioxide at night, resulting in a relatively lower amount of water that transpires out of their leaves and consequently relatively higher values of water-use efficiency (WUE) (Nobel, 1984; Nobel, 1994).

Most agaves lack sufficient cold hardiness to survive winters in the US southwest, but some species appear to be sufficiently cold hardy. *Agave parryi*, *Agave utahensis*, and *Agave weberi* can tolerate temperatures as low as -19.6, -17.5, and -9.8°C, respectively (Nobel, 1984; Nobel and Smith, 1983; Parida and Das, 2005), suggesting they have potential to be grown in more northern regions at elevation levels as high as 850 m. *Agave parryi* is native to the southwest US, and is found in mountainous areas of central and northern Arizona, southwestern New Mexico, and northern Chihuahua (Minnis and Plog, 1976). *Agave utahensis* populates mountain slopes of southern Utah, southern Nevada, northwestern Arizona, and southeastern

California (Baldwin et al., 2012; Welsh et al., 1993). The native distribution of *A. weberi* ranges from southern Texas to San Luis Potosi and Tamaulipas, Mexico (eMonocot Team, 2013).

Traditionally, agaves were used for food, alcoholic and non-alcoholic beverages, syrup, clothing, and cordage (Castetter et al., 1938). *Agave* roasting pits used by various Native American tribes are scattered throughout the Southwest (Castetter et al., 1938; Greer, 1965). However, *A. parryi*, *A. utahensis*, and *A. weberi* have great potential to be cultivated nowadays for ornamental use (Irish and Irish, 2000), medicine (Cruse, 1973), livestock forage (Fuentes-Rodriguez, 1997), soil erosion control (McDaniel, 1985), desert grassland ecosystem reestablishment (Lindsay et al., 2011), and production of agave nectar (or syrup) (Narváez-Zapata and Sánchez-Teyer, 2010). Indeed, *Agave* shows promise to be cultivated for syrup production in the Southwest, given that interest has grown in using it as an alternative sweetener (Garcia-Aguirre et al., 2009). *Agave parryi* and *A. utahensis* particularly have potential to be used for desert ecosystem reestablishment due to the fact that they are native to the Southwest. In addition, work is ongoing in evaluating the potential of *Agave* as a bioenergy crop (Conlu et al., 2011; Davis et al., 2011; Escamilla-Trevino, 2012; Holtum et al., 2011; Nunez et al., 2011), which is underscored by the high productivity of *Agave mapisaga* and *Agave salmiana*. Both were reported by Nobel (1991) to have yielded 38 and 42 Mg ha⁻¹ yr⁻¹, respectively, which exceeds that of other bioenergy feedstock crops, such as corn (15-19 Mg ha⁻¹ yr⁻¹) (Dohleman and Long, 2009) and switchgrass (10-12 Mg ha⁻¹ yr⁻¹) (Heaton et al., 2008). The high biomass and putative cold hardiness of mature *Agave weberi* plants suggest that this species could be produced as a bioenergy crop (Irish and Irish, 2000).

However, salinity could severely impede cultivation of *A. parryi*, *A. utahensis*, and *A. weberi* in many parts of the Southwest. In the U.S. alone, 8.5 million ha of land are considered

saline or sodic (Massoud, 1976), with 5 million ha located in the western U.S. (Bohn et al., 1985). Excess salinity can decrease water availability to plants because the osmotic pressure of the soil solution increases as the salt concentration increases, resulting in stunted plant growth (Abrol et al., 1988). In addition, high salt concentrations can reduce cell expansion in root tips and young leaves, leading to stomatal closure and reduced water uptake (Munns and Tester, 2008). Excessive salt absorption can cause plants to suffer ionic stress due to ion accumulation in shoots (Munns and Tester, 2008), leading other nutrient ions such as Ca^{2+} , K^+ , and Mg^{2+} to become deficient because they have to compete with high NaCl uptake (Khan et al., 1999).

The impact of high salinity on yield of many C_3 and C_4 crops is already well documented. Corn (*Zea mays*) yield decreased by 23% at an electrical conductivity (EC) level of 3.4 dS m^{-1} (Katerji et al., 1996). Likewise, soybean (*Glycine max*) had a 56% decrease in yield at 6.7 dS m^{-1} (Katerji et al., 1998). The effects that salinity has on CAM plants have also been evaluated in some species. Cladodes of *Opuntia ficus-indica* decreased 40% in growth at 4.2 dS m^{-1} NaCl (Nerd et al., 1991). Pineapple (*Ananas comosus*) can grow in soil with an EC range from 3.0-6.0 dS m^{-1} before experiencing a significant decline in growth (Ayers and Westcot, 1985). *Aloe vera* started to experience a decrease in leaf number and root dry weight at EC levels higher than 6 dS m^{-1} (Moghbeli et al., 2012).

The tolerance of *Agave* to salinity tends to vary depending on species. Nobel and Berry (1985) found that EC levels above 3 dS m^{-1} greatly decreased elongation of roots and shoots of *Agave deserti* seedlings. Schuch and Kelly (2008) found that *Agave parryi* shoot and root dry weights decreased significantly at an EC of 5 dS m^{-1} . In addition ten-month-old *A. sisalana* plants exposed to 10 dS m^{-1} NaCl and 10 dS m^{-1} CaCl_2 had a reduction in dry weight of 40%

after 5 months (El-Gamassy et al., 1974). In another study, the dry weight of *A. sisalana* significantly decreased in EC levels of 6.25 dS m⁻¹, and decreased by 46% in EC levels of 25 dS m⁻¹ (El-Bagoury et al., 1993). However, in contrast to these findings, Miyamoto (2008) found that salinity did not have any significant impacts on *Agave americana* growth at levels up to 9 dS m⁻¹. Variation in the degree of salinity tolerance obviously exists in the genus.

If salinity severely impacts growth of most *Agave* species, cultivating or reestablishing these species for commercial or environmental purposes may not be feasible in the Southwest. This may particularly be the case in degraded, marginal lands where salinity exceeds normal thresholds. However, the tolerance of *Agave* to salinity appears to vary depending on species, suggesting that some species may be more tolerant to salinity than generally assumed.

In order to gain a better understanding of how agaves respond to high salinity, more species need to be evaluated, particularly those of agricultural and environmental interest. The main objective of this study was to determine the impact treatments ranging from low to high salinity have on the productivity of *A. weberi*, *A. parryi*, and two subspecies of *A. utahensis*, *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis*. Another objective of the study was to determine the impacts that salinity have on nutrient uptake of essential plant elements, considering that is another variable that can be used to measure productivity. We hypothesized that 1) *A. weberi*, which is a closely related species of *A. americana*, a plant that has been found to tolerate salinity levels as high as 9 dS m⁻¹ (Miyamoto, 2008), will also be tolerant to salinity and withstand levels as high as 9 dS m⁻¹ without experiencing a significant decrease in growth. 2) *A. parryi* will have a significant reduction in growth after 3 dS m⁻¹ based on findings found by Schuch and Kelly (2008), which suggest *A. parryi* is not very tolerant to salinity. 3) The two subspecies of *A. utahensis*, will also be sensitive to salinity past levels of 3 dS m⁻¹ due to the fact

that they primarily grow on hillsides where salinity is often low (Hara, 1992) and thus have not had to evolve to tolerate high saline concentrations. 4) As salinity levels increase past 6 dS m⁻¹, nutrient uptake in *A. parryi* and *A. weberi* will decrease, but not to deficient levels.

MATERIALS AND METHODS

Experimental design, plant material and location

Four separate hydroponic experiments were established in order to analyze the following *Agave* taxa: *A. parryi*, *A. utahensis* ssp. *kaibabensis*, *A. utahensis* ssp. *utahensis*, and *A. weberi*. The two subspecies of *A. utahensis* were evaluated in this study to gain a more comprehensive understanding of how the species responds overall to salinity. *Agave parryi* and *A. weberi* plants used in the study were 6-month-old clones propagated through tissue culture (Rancho Tissue Technologies, Rancho Santa Fe, CA). *Agave utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* plants were grown from seed obtained from Phoenix Desert Nurseries (Phoenix, AZ). The study was conducted under greenhouse conditions at Brigham Young University in Provo, UT. The first two experiments, which included *A. utahensis* ssp. *kaibabensis* and *A. weberi* began on 15 Nov 2012 and concluded after 75 days. The third and fourth experiments, which included *A. parryi* and *A. utahensis* ssp. *utahensis* began on 20 Feb 2013 and ended after 60 days. Each experiment was arranged in a completely randomized block design, with a container containing four plants of the same species serving as the experimental unit. Four salinity treatments (0.5, 3, 6, and 9 dS m⁻¹) were established, with each treatment being replicated 4 times, resulting in a total of 16 containers used in the design. Buckets were randomly placed in four rows approximately 30 cm apart on a greenhouse bench. Plants were grown under supplemental light conditions (12 h daily) with an average temperature of 25 ± 5°C during the

light period and an average temperature of $15 \pm 2^\circ\text{C}$ during the dark period. Relative humidity averaged 47% during the study.

Agave parryi. Sixty-four plants were thoroughly washed to remove soil particles from roots. Plants were transferred to 16 polyethylene containers (depth = 24.1 cm; width = 23.5 cm, volume = 7.6 L) covered in aluminum foil, containing 7.5 L dilute, modified Steinberg nutrient solution (Nichols et al., 2012; Steinberg, 1953) and placed randomly on a greenhouse bench. The modified Steinberg (1953) solution contained 1337 μM calcium nitrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$], 287 μM magnesium nitrate [$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], 246 μM ammonium nitrate [NH_4NO_3], 130 μM potassium phosphate [K_2HPO_4], 40 μM ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$], 4.5 μM manganese chloride [$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$], 12 μM boric acid [H_3BO_3], 1.2 μM zinc sulfate [$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$], 0.3 μM copper sulfate [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$], 0.2 μM sodium molybdate [$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$], 128 μM potassium nitrate [KNO_3], 131 μM potassium chloride [KCl], 132 μM potassium sulfate [K_2SO_4], 185 μM magnesium sulfate [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$], 46 μM N-(2-Hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid trisodium salt [HEDTA], and 94 μM iron chloride [$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$]. Each plant was inserted into neoprene net cup lids and placed inside 5.1-cm foam-net pots (Atlantis Hydroponics, College Park, GA). Each foam-net pot was placed inside an opaque plastic lid situated on top of each container. Air stones were placed at the base of each container to aerate the roots and solution. Plants were grown in the pre-treatment solution for 14 days prior to transfer into treatments.

The initial nutrient concentrations used during the treatment period were the same as the pre-treatment, except NaCl concentration was adjusted to reach the desired electrical conductivity (EC) level for each treatment. Electrical conductivity was measured using a HM Digital COM-100 EC meter (Atlantis Hydroponics, College Park, GA). Salinity was supplied at

four levels (0.6, 3, 6, and 9 dS m⁻¹) of NaCl in nutrient solutions buffered at a pH of 6. Solution pH was initially adjusted and then maintained daily with sodium hydroxide (NaOH) and hydrochloric acid (HCl). Two weeks after the treatment started, the modified Steinberg (1953) solution was slightly increased to 1671 μM calcium nitrate [Ca(NO₃)₂*4H₂O], 359 μM magnesium nitrate [Mg(NO₃)₂*6H₂O], 308 μM ammonium nitrate [NH₄NO₃], 147 μM potassium phosphate [K₂HPO₄], 45 μM ammonium sulfate [(NH₄)₂SO₄], 5 μM manganese chloride [MnCl₂*4H₂O], 14 μM boric acid [H₃BO₃], 1.3 μM zinc sulfate [ZnSO₄*7H₂O], 0.3 μM copper sulfate [CuSO₄*5H₂O], 0.2 μM sodium molybdate [Na₂MoO₄*2H₂O], 192 μM potassium nitrate [KNO₃], 197 μM potassium chloride [KCL], 199 μM potassium sulfate [K₂SO₄], 248 μM magnesium sulfate [MgSO₄*7H₂O], 59 μM HEDTA, and 141 μM iron chloride [FeCl₃*6H₂O]. Nutrient solutions in each container were replenished every 2 weeks. When replenishing the solutions, the whole container was replaced with a new solution in deionized water to ensure that salinity level would remain relatively constant over the course of the experiments. While harvesting, shoots and roots were separated for further analysis.

Agave utahensis ssp. kaibabensis. Seeds were germinated by placing them on cheesecloth placed on 4-mm stainless steel screens in 9.5-cm deep rectangular plastic trays. The screens were immersed with 2 L of diluted modified Steinberg solution. The germination solution contained 977 μM calcium nitrate [Ca(NO₃)₂*4H₂O], 210 μM magnesium nitrate [Mg(NO₃)₂*6H₂O], 180 μM ammonium nitrate [NH₄NO₃], 113 μM potassium phosphate [K₂HPO₄], 35 μM ammonium sulfate [(NH₄)₂SO₄], 1.8 μM manganese chloride [MnCl₂*4H₂O], 5 μM boric acid [H₃BO₃], 0.5 μM zinc sulfate [ZnSO₄*7H₂O], 0.1 μM copper sulfate [CuSO₄*5H₂O], 0.08 μM sodium molybdate [Na₂MoO₄*2H₂O], 112 μM potassium nitrate [KNO₃], 115 μM potassium chloride [KCL], 117 μM potassium sulfate [K₂SO₄], 145 μM magnesium sulfate [MgSO₄*7H₂O], and 11

μM iron-ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid (Fe-EDDHA). Germination and subsequent elongation of seedlings were carried out over a 21-day period at $\sim 25^{\circ}\text{C}$. The pre-treatment procedure applied to *A. parryi* was also used for *A. utahensis ssp. kaibabensis*, except, during the treatment period, nutrient solutions were replenished every 4 weeks instead of every 2 weeks.

Agave utahensis ssp. utahensis. Seedlings of *A. utahensis ssp. utahensis* were propagated similarly to those of *A. utahensis ssp. kaibabensis*. They were also pre-treated and treated with the same modified Steinberg solutions as the *A. parryi* plants, except that 128 seedlings comparable in size were transferred to opaque plastic lids placed on top of containers (8 plants per container). After a 14-day pre-treatment period, 64 seedlings uniform in size were then selected out of the 128 seedlings and transferred to new containers (4 plants per container) with opaque plastic lids. The treatment protocol for *A. parryi* was used for *A. utahensis ssp. utahensis* except seedlings were initially replenished every 2 weeks for the first 30 days. They were then replenished every 4 weeks due to slow nutrient uptake. Seedlings were harvested after 60 days in treatment.

Agave weberi. Plants of *A. weberi* were pre-treated and treated with the same modified Steinberg solution as the *A. parryi* plants. However, during the treatment period, nutrient solutions were replenished 4 weeks after treatment started. Following the initial replenishment, nutrients were replenished every 2-weeks.

Mortality count, dry weight and elemental analysis

At the end of each experiment, a mortality count was taken to determine the percent mortality of plants in each treatment. Shoots and roots of plants in all experiments were surface washed and then oven dried at 65°C for a minimum of 72 hours to a uniform dryness and then

weighed. Shoots of *A. parryi* and *A. weberi* were subsequently ground using a mortar and pestle for elemental analysis. Shoots of *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* were not ground because there was not enough dry material to use for elemental analysis. In order to measure the concentrations of Ca, Mg, K, P, S, Zn, Cu, and Na in the shoots, ground samples were digested in nitric-perchloric acid and analyzed by inductively coupled plasma atomic emission spectroscopy (IRIS Intrepid II XSP, Thermo Electron Corporation, Franklin, MD). Total N was analyzed using a nitrogen analyzer (LECO CHN628 series, LECO Corporation, St. Joseph, MI).

Statistical analysis

The average dry shoot, dry root, and nutrient concentrations were calculated from the four plants in each bucket. Statistical analyses were performed using Statistical Analysis System (SAS, version 9.3, SAS Institute, Cary, NC). A check for normality for each analysis was accomplished with quantile-quantile plots for residuals, and by running the Shapiro-Wilk, Cramer-von Mises, and Anderson-Darling tests. Data for shoot dry weight, root dry weight, total dry weight, and nutrient uptake were analyzed using analysis of variance (ANOVA) with mean separation using the Tukey-Kramer test at the 0.05 level of significance ($P \leq 0.05$).

RESULTS

Mortality

Mortality of seedlings of *A. utahensis* ssp. *kaibabensis* in the 6 and 9 dS m⁻¹ salt treatments were more than double of those in the control and 3 dS m⁻¹ salt treatments (Table 1). As EC levels increased, there was a proportional increase in mortality of *A. utahensis* ssp. *utahensis* seedlings. Mortality in the 9 dS m⁻¹ treatment was approximately 25, 50, and 75%

higher than in the 6, 3, and 0.6 dS m⁻¹ treatments. All *A. parryi* and *A. weberi* plants survived, regardless of treatment.

Shoot, root, and total dry weight

Shoot, root, and total dry weights of *A. parryi* were only significantly different between plants in the control and 9 dS m⁻¹ treatments. Plants in the control treatment had the highest dry weight values (Table 2).

Seedlings of *A. utahensis* ssp. *kaibabensis* in the 6 dS m⁻¹ and 9 dS m⁻¹ treatments had 1.5 to 2 times less shoot dry weight than those in the control treatment (Table 2). Significant treatment differences in root dry weight were only manifested in the 9 dS m⁻¹ salinity treatment. Roots in this treatment had nearly 2.5 times less dry weight than of those in the control treatment (Table 2). Total dry weight of plants in the control treatment was significantly higher than those in the 6 and 9 dS mdS m⁻¹ treatments. Also, total dry weight of plants in the 3 dS m⁻¹ treatment was significantly higher than of those in the 9 dS m⁻¹ treatment (Table 2).

Agave utahensis ssp. *utahensis* seedlings in the control treatment had more than two times as much shoot dry weight than those in the 9 dS m⁻¹ treatment (Table 2). Shoot dry weight of control seedlings also were 1.8 times greater than seedlings in the 6 dS m⁻¹ treatment (Table 2). Seedlings in the control treatment had 2.6 and 4.5 times more root dry weight, respectively, than seedlings in the 6 and 9 dS m⁻¹ treatments (Table 2). Treatment differences in total dry weight followed that of shoot and root dry weights where dry weights decreased as salinity levels increased.

There were no significant differences in shoot, root, or total dry weights of *A. weberi* among the four treatments (Table 2). However, similar to *Agave parryi* plants, the shoot dry weight and total dry weight tended to decrease as salt concentration increased.

Nutrient concentrations

The only significant difference in C concentrations of *A. parryi* was between the 3 and 9 dS m⁻¹ treatment with more being in the 3 dS m⁻¹ treatment. Nitrogen concentrations were only significantly different between the 3 and 6 dS m⁻¹ treatments, with more N in the 6 dS m⁻¹ treatment. There were no significant differences between the control, 6, and 9 dS m⁻¹ treatments. Dried shoot samples showed significant differences in Ca, K, Mg, Mn, Na, P and S (Table 3). Calcium, K, Mg, Mn, and S concentrations all decreased significantly with an increase in salt treatment. Each of these elements had significant decreases in the 6 and 9 dS m⁻¹ treatments relative to the control. Phosphorus concentration was significantly lower in the 9 dS m⁻¹ treatment relative to the 3 and 6 dS m⁻¹ treatments. There was also no significant difference in P concentration between the control and 9 dS m⁻¹ treatments (Table 3).

There were no significant differences between any of the treatments for *A. weberi* in terms of C concentration. Nitrogen concentration in plants in the control treatment was significantly lower than of those in the other treatments (Table 3). There were no significant differences in K, P, Zn and Fe among plants in the four treatments. However, Ca, Mg, S and Mn all significantly decreased in concentration as salinity increased, particularly in the 6 and 9 dS m⁻¹ treatments. Copper concentrations were significantly lower in plants in the control and 9 dS m⁻¹ treatments compared to the 3 dS m⁻¹ treatment. Sodium concentration significantly increased as salinity treatment increased (Table 3).

DISCUSSION

Growth of both subspecies of *A. utahensis* was severely reduced at high salt concentrations. The poor response of these plants to salinity may have been due to their exposure

to high levels of salt stress as seedlings. Their ability to adapt to salt stress was low compared to the more established and putatively stress-tolerant *A. parryi* and *A. weberi* plants. Nobel et al. (1985) reported that 12-day old seedlings of *A. deserti* also performed poorly in high salinity, with a 50% decrease in root growth occurring at 5.6 dS m^{-1} , and a similar decrease in shoot growth occurring at 9.3 dS m^{-1} (Nobel and Berry, 1985).

To date, research has not been carried out determining how mature plants of *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* respond to high salinity. However, using soil-based data collected by Nobel and Berry (1985), Hara (1992) estimated that EC values of field-grown *A. utahensis* range between $2.5\text{-}3.2 \text{ dS m}^{-1}$. This suggests that the natural habitat of *A. utahensis* is generally not very saline, which possibly explains why the species is fairly intolerant of high salt concentrations. Another reason that *A. utahensis* ssp. *kaibabensis* and *A. utahensis* ssp. *utahensis* seedlings performed poorly may have been due to their inability to osmotically adjust to high salt levels. Mature *Agave* plants typically tolerate high salt concentrations through osmotic adjustment, where moisture content decreases and Na^+ and Cl^- ions increase in the shoots (Schuch and Kelly, 2008). *Agave* plants will also exude salt from their leaves to try and adjust to salt stress. However, since the plants of both taxa in our study were only seedlings, their capacity to store salt was possibly limited. Although not directly measured, such adjustment mechanisms may have been at play with the more mature and larger *A. parryi* and *A. weberi* plants.

Data from the study suggests that *A. parryi* performs well in EC levels up to 6 dS m^{-1} , which correlates with another study that analyzed the salinity tolerance of 1-year-old *A. parryi* plants grown under greenhouse conditions (Miyamoto, 2008). Miyamoto (2008) found that plant growth was not restricted within the range of $6\text{-}8 \text{ dS m}^{-1}$, but was severely reduced at 9.4 dS m^{-1} .

Contrary to our findings, Schuch and Kelly (2008) found in an outdoor study that shoot and root dry weights of potted *A. parryi* plants decreased by 33% when irrigated with water with an EC of 5 dS m⁻¹ relative to those exposed to 0.6 dS m⁻¹. This suggests that even within species, some conspecific *Agave* plants may be more tolerant to salinity than others.

Out of the four species evaluated, *A. weberi* was the most tolerant to high salinity levels, with no significant difference in growth among treatments. Other species of agaves have also performed well in high salinity. Miyamoto (2008) found that *Agave americana* plants were considerably tolerant of high salt levels, and did not have any significant decreases in growth when irrigated with 9.4 dS m⁻¹ water. In addition, soil samples taken from the root zones of field-grown *A. americana* had EC levels ranging between 7.0-8.0 dS m⁻¹ (Hara, 1992; Nobel and Berry, 1985). Interestingly, the EC levels of soils underneath *Agave salmiana*, *Agave lechuguilla*, and *Agave fourcroydes* were in the range of 13-16 dS m⁻¹, 17-20 dS m⁻¹, and 44-47 dS m⁻¹, respectively, which indicates that some *Agave* species are amazingly tolerant to high levels of salinity.

To our knowledge, studies evaluating optimal nutrient concentrations of *A. parryi* and *A. weberi* have not been carried out. Moreover, identifying nutrient deficiencies in *Agave* species can be difficult because visual symptoms are not always obvious (Ruiz-Luna et al., 2011). Indeed, nutritional deficiencies may not be manifest for up to 12 months. As a result, it was difficult to visually determine if plants in our study were suffering from nutrient deficiencies. However, by taking into consideration reported nutrient concentrations of ecologically similar *Agave* species (*A. americana*, *A. deserti*, *A. fourcroydes*, *A. lechuguilla*, *A. salmiana*, and *A. utahensis*) (Nobel and Berry, 1985), estimates can be made of nutrient threshold levels of both *A. parryi* and *A. weberi*. For *A. parryi* and *A. weberi*, N uptake significantly differed between

treatments, but did not appear to be associated with salinity level. *Agave weberi* had significantly less N in the control group than the 6 and 9 dS m⁻¹ treatments, while *A. parryi* had significantly less N in the 3 dS m⁻¹ treatment than the 6 dS m⁻¹ treatment. The decrease in N in the lower treatments is inconsistent with that found in other studies, where N tended to decrease with an increase in salinity (Al-Rawahy et al., 1992; Feigin et al., 1991; Pessarakli, 1991). However, salinity level does not necessarily affect overall N uptake (Maksimovic and Ilin, 2012). Salt-stressed plants may continue to accumulate N, even if there is a reduction in yield or dry matter.

The decrease in N, however, did not appear to impair plant growth and productivity in the lower treatments, with both species having more dry weight in the control and 3 dS m⁻¹ treatments than the 6 and 9 dS m⁻¹ treatments. Compared to the average N content of six *Agave* species (1.19%) evaluated by Nobel and Berry (1985), the lowest N levels in *A. parryi* (1.97%) and *A. weberi* (3.28%) in our study were relatively high. This suggests that the plants in these treatments were likely not impaired by N deficiency. The fact that N uptake does not decrease with an increase in salinity is noteworthy because N can be the most limiting element for *Agave* growth (Nobel and Berry, 1985), as it is primarily responsible for the growth of leaves, crowns, and roots (Lock, 1969).

In terms of the other essential nutrients that significantly decreased, Ca may have been the only element that became deficient at high NaCl concentrations. Calcium levels in the 9 dS m⁻¹ treatment of *A. parryi* (1.35%) and *A. weberi* (1.44%) were noticeably lower than of other *Agave* species where the average concentration was 4.16% (Nobel and Berry, 1985). Even in the control treatments, the Ca levels were still fairly low for *A. parryi* (2.75%) and *A. weberi* (2.87%) as compared to that reported by Nobel and Berry (1985). However, Lock (1962) reported that mature leaves of field-grown *A. sisalana* in Tanzania, had similar levels of Ca

(1.4%), indicating that not all *Agave* need high Ca concentrations in their leaves to be productive. Furthermore, seedlings of *A. deserti* did not significantly vary in growth with Ca levels ranging from 0.0008 to 0.02% (Nobel and Berry, 1985). *Agave* plants have been found to grow in soil Ca levels ranging from 0.015% to 0.01% (Nobel and Berry, 1985).

Although decreases of K and P were statistically significant in *A. parryi*, their low concentrations (1.97% K, 0.40% P) were still above the average values found by Nobel and Berry (1985). Furthermore, the lowest S concentrations in *A. parryi* (0.19%) and *A. weberi* (0.25%) were still above that found in well-watered and fertilized *Agave angustifolia* (Ruiz-Luna et al., 2011). Despite the significant decrease in Mg and Mn in both *A. parryi* and *A. weberi*, the lowest Mg levels in *A. parryi* (0.52%) and *A. weberi* (0.55%) were relatively similar to the average of the six *Agave* species mentioned above (0.55%) (Nobel and Berry, 1985). Also, Mn levels in the highest NaCl treatments of *A. parryi* (65 ppm) and *A. weberi* (39 ppm) were considerably larger compared to the average (18 ppm) of the six species evaluated by Nobel and Berry (1985). Studies on micronutrient deficiency symptoms in *A. sisalana* showed that deficiencies occurred below about 10 ppm Mn, 2 ppm Cu, and 5 ppm Zn (Lock, 1962; Pinkerton, 1971). The plants in our study thus appeared to not be experiencing micronutrient deficiencies. Despite a decrease in nutrient uptake at higher salt concentrations, actual physiological impairment to the plants appeared to be minimal.

The relations between salinity and mineral nutrition are extremely complex (Grattan and Grieve, 1999), and predicting the specific nutrients that would be deficient are difficult. Based on studies of other plants, it is common to have a decrease in most essential elements with an increase in salinity (Parida and Das, 2005), but whether they lead to decreases in plant growth and productivity depends upon the nutrient in question, salinity level and composition of salts,

plant species, and environmental factors (Grattan and Grieve, 1999). *Agave parryi* and *A. weberi* responded comparatively well to high concentrations of salinity, but in order to identify specific nutrient deficiency threshold levels for each species, long-term experiments need to be carried out evaluating each essential nutrient separately.

CONCLUSION

Based on the results of our study, it appears that several *Agave* species show variation in response to high levels of salinity. However, age and stage of development may play a factor in the degree of tolerance expressed. *Agave utahensis* seedlings were very sensitive to high levels of salinity, with growth and survival greatly decreasing in higher salinity treatments. As such, the potential for *A. utahensis* to be established in burned areas high in salinity does not seem promising. However, it is important to note that mature plants of *A. utahensis* have not been evaluated in saline environments, and may be more tolerant to salt stress than seedlings. In contrast, *A. parryi* and *A. weberi* plants were relatively tolerant to high levels of salinity. Consequently, *Agave parryi* and *A. weberi* have great potential to be utilized in saline environments of the Southwest for restoration efforts in burned areas, as well as for commercial uses, such as for syrup production and bioenergy.

ACKNOWLEDGEMENTS

We are thankful to three undergraduate students, Cali McMurtrey, Yue Shen, and Westen Archibald, for helping us carry out this experiment.

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TABLES

Table 1. Percent Mortality

Percent mortality of *Agave utahensis* ssp. *kaibabensis* and *Agave utahensis* ssp. *utahensis* exposed to four levels of salinity.

Percent mortality was calculated by averaging the number of mortalities in each hydroponic container, and then averaging those averages across each treatment.

Taxa and treatment	Percent mortality
<i>Agave utahensis</i> ssp. <i>kaibabensis</i>	
0.6 dS m ⁻¹	19
3 dS m ⁻¹	19
6 dS m ⁻¹	44
9 dS m ⁻¹	44
<i>Agave utahensis</i> ssp. <i>utahensis</i>	
0.6 dS m ⁻¹	0
3 dS m ⁻¹	27
6 dS m ⁻¹	50
9 dS m ⁻¹	75

Table 2. Dry Weights

Four separate experiments evaluating the effects 0.6-9 dS m⁻¹ salinity levels have on shoot, root and total dry weight of four *Agave* species. *Agave parryi* and *Agave utahensis* were in treatment for 60 days, while *Agave weberi* and *Agave utahensis kaibabensis* were in treatment for 75 days.

<i>Agave parryi</i>	Shoot dry weight (g)			Root dry weight (g)			Total dry weight (g)		
0.6 dS m ⁻¹	1.63	A ^Z	±0.085	0.541	A	±0.035	2.17	A	±0.039
3.0 dS m ⁻¹	1.47	AB	±0.085	0.509	AB	±0.035	1.97	AB	±0.039
6.0 dS m ⁻¹	1.30	AB	±0.085	0.486	AB	±0.035	1.79	AB	±0.039
9.0 dS m ⁻¹	1.16	B	±0.085	0.402	B	±0.035	1.57	B	±0.039
<i>Agave utahensis ssp. kaibabensis</i>									
0.6 dS m ⁻¹	0.0387	A	±0.003	0.0071	A	±0.001	0.0459	A	±0.004
3 dS m ⁻¹	0.0328	AB	±0.003	0.0057	AB	±0.001	0.0386	AB	±0.004
6 dS m ⁻¹	0.0201	B	±0.003	0.0045	AB	±0.001	0.0246	BC	±0.004
9 dS m ⁻¹	0.0170	B	±0.003	0.0029	B	±0.001	0.0199	C	±0.004
<i>Agave utahensis ssp. utahensis</i>									
0.6 dS m ⁻¹	0.0235	A	±0.002	0.0063	A	±0.001	0.0298	A	±0.003
3 dS m ⁻¹	0.0179	AB	±0.002	0.0046	AB	±0.001	0.0225	AB	±0.003
6 dS m ⁻¹	0.0131	B	±0.002	0.0024	B	±0.001	0.0155	B	±0.003
9 dS m ⁻¹	0.0113	B	±0.002	0.0014	B	±0.001	0.0128	B	±0.003
<i>Agave weberi</i>									

0.6 dS m ⁻¹	2.93	A	±0.137	0.787	A	±0.211	3.71	A	±0.257
3 dS m ⁻¹	2.93	A	±0.137	0.921	A	±0.211	3.85	A	±0.257
6 dS m ⁻¹	2.77	A	±0.137	0.902	A	±0.211	3.67	A	±0.257
9 dS m ⁻¹	2.44	A	±0.137	0.743	A	±0.211	3.55	A	±0.257

^Z Different letters indicate means that are significantly different from each other (Tukey test, $P \leq 0.05$, ANOVA)

Table 3. Nutrient Concentrations

Nutrient concentrations of two separate experiments evaluating the effects of salinity on nutrient uptake of *Agave weberi* and *Agave parryi*.

Nutrient Unit	C %	N %	K %	P %	Ca %	Mg %	S %
<i>Agave parryi</i>							
0.6 dS m ⁻¹	42.4 AB ^Z ±0.26	2.09 AB ±0.04	2.11 A ±0.02	0.41 AB ±0.003	2.75 A ±0.05	0.70 A ±0.01	0.24 A ±0.005
3 dS m ⁻¹	43.1 A ±0.26	1.97 B ±0.04	2.07 AB ±0.02	0.42 A ±0.003	2.22 B ±0.05	0.63 B ±0.01	0.22 AB ±0.005
6 dS m ⁻¹	42.2 AB ±0.26	2.15 A ±0.04	2.01 BC ±0.02	0.42 A ±0.003	1.71 C ±0.05	0.55 C ±0.01	0.21 BC ±0.005
9 dS m ⁻¹	41.4 B ±0.26	1.99 AB ±0.04	1.97 C ±0.02	0.40 B ±0.003	1.44 D ±0.05	0.52 C ±0.01	0.19 C ±0.005
<i>Agave weberi</i>							
0.6 dS m ⁻¹	44.5 A ±0.17	3.28 B ±0.21	2.08 A ±0.02	0.44 A ±0.02	2.87 A ±0.11	0.83 A ±0.03	0.35 AB ±0.01
3 dS m ⁻¹	43.9 A ±0.17	4.10 AB ±0.21	2.11 A ±0.02	0.52 A ±0.02	2.76 A ±0.11	0.78 A ±0.03	0.38 A ±0.01
6 dS m ⁻¹	43.8 A ±0.17	4.21 A ±0.21	2.06 A ±0.02	0.47 A ±0.02	1.81 B ±0.11	0.61 B ±0.03	0.31 BC ±0.01
9 dS m ⁻¹	43.8 A ±0.17	4.11 A ±0.21	2.04 A ±0.02	0.45 A ±0.02	1.35 B ±0.11	0.55 B ±0.03	0.25 C ±0.01

Nutrient Unit	Mn ppm	Cu ppm	Zn ppm	Fe ppm	Na ppm
<i>Agave parryi</i>					
0.6 dS m ⁻¹	109 A ±3.7	4.1 A ±0.24	18 A ±0.52	80 A ±16.8	1326 D ±505.6
3 dS m ⁻¹	101 A ±3.7	3.7 A ±0.24	16 B ±0.52	75 A ±16.8	8952 C ±505.6
6 dS m ⁻¹	79 B ±3.7	4.0 A ±0.24	16 B ±0.52	80 A ±16.8	18001 B ±505.6
9 dS m ⁻¹	65 B ±3.7	3.8 A ±0.24	15 B ±0.52	81 A ±16.8	23373 A ±505.6
<i>Agave weberi</i>					
0.6 dS m ⁻¹	85 A ±4.7	3.3 B ±0.24	29 A ±16.3	76 A ±3.7	1086 D ±483.9
3 dS m ⁻¹	89 A ±4.7	4.8 A ±0.24	30 A ±16.3	81 A ±3.7	9917 C ±483.9
6 dS m ⁻¹	54 B ±4.7	3.8 AB ±0.24	27 A ±16.3	78 A ±3.7	19710 B ±483.9
9 dS m ⁻¹	39 B ±4.7	3.6 B ±0.24	27 A ±16.3	80 A ±3.7	22910 A ±483.9

^Z Different letters indicate means that are significantly different from each other (Tukey test, $P \leq 0.05$, ANOVA)

Chapter 2

Measurement of the Influence of Low Water Availability on the Productivity of a Potential Bioenergy Crop, *Agave weberi*, Using a Novel Automated Irrigation System

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Manuscript prepared for submission to *Global Change Biology Bioenergy*

ABSTRACT

In recent years, research has focused on determining the potential of *Agave* to be utilized for bioenergy production due to its ability to grow in arid and marginal lands. However, little is known regarding its productivity under limited water conditions. Most *Agave* species can tolerate low soil-moisture levels, but it is unclear at what point productivity will be significantly constrained. Using an automated irrigation system under greenhouse conditions, we evaluated the effects of low to high volumetric water content (VWC) levels on biomass accumulation and nutrient uptake of a putative bioenergy crop, *Agave weberi*. Plants were exposed to four constant VWC levels (0.05, 0.12, 0.19, and 0.26 m³ m⁻³). Shoot dry weight of plants in the 0.26 m³ m⁻³ treatment was significantly higher than those in the 0.05 m³ m⁻³ treatment, but not than those in the intermediate treatments. Both chlorophyll concentration and nutrient uptake decreased as VWC level decreased. Although plants were fairly productive under moderately dry soil conditions, it would be expected that over time, plants receiving high levels of irrigation would have greater growth than plants in dry soil moisture levels given that *Agave* will switch from CAM to C₃ photosynthesis under conditions of high water availability.

Keywords: *Agave*, century plant, automated irrigation, drought stress, nutrient uptake, *Agave weberi*

INTRODUCTION

Ethanol from corn (*Zea mays*) currently dominates the renewable fuels market in the U.S. (Coyle, 2007), but attempting to grow more corn to satisfy energy needs could be difficult because cultivation of this crop requires large amounts of water and synthetic fertilizer for optimal growth (Dominguez-Faus *et al.*, 2009). Producing such crops will increasingly strain

already limited water sources, particularly in semi-arid and arid parts of the world (White & Nackoney, 2003). Indeed, water availability continues to decline in many parts of the U.S. As a case in point, consumption of groundwater averaged $9.2 \text{ km}^3 \text{ yr}^{-1}$ in the U.S. between the years 1900-2008, but more than $25 \text{ km}^3 \text{ yr}^{-1}$ were depleted between the years 2000-2008 (Konikow, 2013). Sparse rainfall also limits potential productivity (FAO, 1989). Most conventional food or energy crops simply cannot be grown sustainably in these regions. Highly productive energy crops requiring limited water need to be identified.

Agave, which is a genus of succulent plants native to arid and semi-arid parts of Central and North America, shows promise as a stress-tolerant, bioenergy crop for semi-arid and arid regions, such as the southwestern U.S. *Agave* is comprised exclusively of species that utilize the Crassulacean acid metabolism (CAM) photosynthetic pathway (Matiz *et al.*, 2013, Nobel, 2009). This pathway enables *Agave* species to have high water-use efficiency (WUE) because they open their stomates at night resulting in less water being transpired relative to other plants that use the C_3 and C_4 photosynthetic pathways (Davis *et al.*, 2011). Comparatively, the WUE of C_3 , C_4 , and CAM plants average 3-12, 6-24, and 32-98 $\text{g CO}_2 \text{ L}^{-1} \text{ H}_2\text{O}$, respectively (Nobel, 2009). Clearly, CAM plants assimilate CO_2 at a much lower transpirational cost, which indicates that agaves could be grown as energy crops in arid and semi-arid regions of the U.S., an area encompassing over 10 million km^2 (Huntsinger & Starrs, 2006). According to some estimates (Nobel *et al.*, 1992, Nunez *et al.*, 2011, Somerville *et al.*, 2010), using agaves for bioenergy production could result in higher yields than other energy crops, such as corn ($15\text{-}19 \text{ Mg ha}^{-1}$) (Dohleman & Long, 2009), miscanthus ($29\text{-}38 \text{ Mg ha}^{-1}$), and switchgrass ($10\text{-}12 \text{ Mg ha}^{-1}$) (Heaton *et al.*, 2008). Moderate levels of management and resource inputs led to yields of 38 and $42 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for

Agave mapisaga and *Agave salmiana*, respectively (Nobel *et al.*, 1992). Indeed, considerable potential exists for *Agave* to help satisfy growing energy needs in drier regions of the U.S.

Most *Agave* species, however, lack sufficient cold hardiness to survive winters in the dry, high elevation regions, such as the U.S. Southwest. However, *Agave weberi*, a highly productive species whose native distribution extends from southern Texas to San Luis Potosi and Tamaulipas, Mexico (eMonocot Team, 2013, Irish & Irish, 2000), withstands temperatures down to -10°C (Nobel & Smith, 1983). Consequently, the species could likely be grown much farther north and at higher elevations outside of its current cultivated range in Mexico. *Agave weberi* is widely cultivated in Mexico for production of alcoholic beverages, such as mescal, due to its high starch and sugar content (Gentry, 1982). It is also cultivated for fiber production (Nobel, 1994), and is often used as living fences around houses (Gentry, 1982). No information appears to be available, though, regarding *A. weberi* productivity potential. However, a closely related species, *A. sisalana*, was reported to have a biomass productivity of 5 Mg ha⁻¹ yr⁻¹ in Tanzania while growing in less-than-optimal growing conditions (Lock, 1962). We assume that with modest increases in irrigation and fertilizer input, the yield of *A. weberi* would likely exceed this threshold. However, further examination of the productivity of *A. weberi*, particularly with varying levels of water availability, is warranted in order to accurately assess its true agronomic potential.

Limited water availability, however, could impede *Agave* productivity in the U.S. Southwest, considering that most of the region receives no more than 500 mm of annual rainfall (FAO, 1989). On average, precipitation across the native range of *A. weberi* ranges between 100-1000 mm (Instituto de Geografía, 1990). Hara (1992) reported that *A. americana* grown in soil with a volumetric water content (VWC) level of 5.7% had reduced growth compared to

conspecific plants grown in soils with VWC levels of 14.6%. However, some *Agave* species perform comparatively greater than others in terms of growth under similar watering profiles. *Agave salmiana* yielded 10 Mg ha⁻¹ yr⁻¹ under 320 mm of annual rainfall (Nobel & Meyer, 1985) compared to *A. lechuguilla*, which produced only 4 Mg ha⁻¹ yr⁻¹ when subjected to 427 mm of rainfall (Nobel & Quero, 1986). Wide inter- and intraspecific variation in growth in response to varying water availability indicates that some *Agave* species may be highly productive despite constraints on soil moisture.

A reduction in water availability may also impede productivity by reducing nutrient uptake. Generally, most plants under drought stress experience a decrease in nutrient uptake due to decreased transpiration rates and active transport of nutrients (Alam, 1994; Viets, 1972). These decreases can even be severe enough to cause nutrient deficiencies (Beringer & Trolldenier, 1978; Hu & Schmidhalter, 2005; Turner, 1985). Drought stress can also cause a reduction in chlorophyll content, which often impedes productivity of various plants (Alberte et al., 1977; Ohashi et al., 2006; Zhang et al., 2002). To our knowledge, the effects of drought stress on nutrient uptake and chlorophyll content have not been evaluated in *Agave*. However, both variables could be important in identifying the productivity of *Agave* under different water levels.

The main objective of this study was to determine the productivity threshold of *A. weberi* across a range of VWC levels. We conducted an exploratory experiment in a greenhouse setting to evaluate the impacts of varying VWC levels on *A. weberi* productivity. Previous studies evaluating the effects of water stress have been difficult due to the challenges in imposing biologically relevant results (Kim *et al.*, 2012, Nemali & van Iersel, 2006). Common methods for imposing drought stress include withholding irrigation until wilting occurs (Harb *et al.*, 2010,

Kawaguchi *et al.*, 2004) or by subjecting plants to osmoticum (Kreps *et al.*, 2002) or other means of desiccation (Seki *et al.*, 2002). In container-based systems, withholding water hastens the development of drought stress faster than under natural conditions, making it difficult to infer results to the field (Nemali & van Iersel, 2006). Moreover, recent research indicates that molecular-level responses to drought are highly dependent of the method of drought-stress imposition (Bray, 2004). Also, precise control of water availability under controlled conditions allows for a more integrated understanding of water stress impacts given the constancy of the method and minimization of variability (Granier *et al.*, 2006, Harb *et al.*, 2010).

As a means of deriving relevant measures of *A. weberi* response to water stress, we developed an automated irrigation system based on the design of Nemali and van Iersel (2006), which was used to maintain constant VWC levels in each container. Optimal precipitation and soil moisture values for high productivity of *A. weberi* are not documented, but based on physiological responses of other *Agave* species to water stress (Hara, 1992, Nobel & Meyer, 1985, Nobel & Quero, 1986), we hypothesized that *A. weberi* exposed to fairly dry soil-moisture levels ($0.05 \text{ m}^3 \text{ m}^{-3}$) would have equivalent growth levels when grown under moist soil conditions ($0.26 \text{ m}^3 \text{ m}^{-3}$). We also hypothesized that nutrient uptake and chlorophyll content in *A. weberi* would decrease in dry soil moisture levels ($0.05 \text{ m}^3 \text{ m}^{-3}$), but not enough to cause any significant decrease in plant growth.

MATERIALS AND METHODS

Experimental design, growing conditions, and plant care

This study was conducted under greenhouse conditions at Brigham Young University, Provo, UT, USA. The experiment began on 29 Jan 2013, and concluded after 80 days. The

experiment was arranged as a randomized complete block design, with individual potted *A. weberi* plants serving as the experimental units. Plants were 6-month-old clones propagated through tissue culture (Rancho Tissue Technologies, Rancho Santa Fe, CA). Four irrigation treatments representing different VWC levels (0.05, 0.12, 0.19, and 0.26 m³ m⁻³) were established, with each treatment replicated 8 times (N = 32). Each container (replicate) was filled with a 4:1:1 mix of sand, calcined clay, and pea-sized gravel. Potted plants were randomly placed in 8 rows approximately 30 cm apart on a greenhouse bench. Plants were grown under supplemental light conditions (12 h daily) with an average temperature of 25 ± 5°C during the light period and an average temperature of 15 ± 2°C during the dark period. Relative humidity during the study period averaged 47%. Each pot was fertilized with 500 mL of a 20-20-20 NPK fertilizer before the experiment began.

Irrigation system

An automated irrigation system, which was based on that developed by Nemali and van Iersel (2006), was set up to regulate the amount of water applied to each *A. weberi* plant. The VWC levels were determined by creating a calibrated soil-moisture curve specific to the soil medium mentioned above. The moisture curve was determined by regressing raw sensor output (mV) against gravimetric measurements in dry-to-completely saturated medium. These data were fed into a program in a CR1000 datalogger (Campbell Scientific, Logan, UT) to maintain relatively consistent soil-moisture content values in each pot. Moisture sensors (10HS ECH₂O, Decagon Devices, Pullman, WA), which were attached to the logger through a multiplexer (AM16/32, Campbell Scientific), measured water content in each container. Drip emitters, connected to solenoid valves (Rainbird DV/DVF series, Rain Bird Corporation, Tucson, AZ), would turn on or off through relay devices (SDM-CD16AC, Campbell Scientific) when soil

volumetric water content values were below or above pre-determined threshold (treatment) levels.

Dry weight, elemental analysis, plant height, and chlorophyll count

At the end of the experiment, shoots and roots of plants were oven dried at 65°C for a minimum of 72 hours to uniform dryness and then weighed. Shoots were also ground using a mortar and pestle for elemental analysis. Concentrations of Ca, Mg, K, P, S, Zn, Cu, and Na in shoots were determined by digesting ground samples in nitric-perchloric acid and analyzed by inductively coupled plasma atomic emission spectroscopy (IRIS Intrepid II XSP, Thermo Electron Corporation, Franklin, MD). Total N was analyzed at the end of the experiment using an N analyzer (LECO CHN628 series, LECO Corporation, St. Joseph, MI).

At the end of the experiment, the height of each plant was taken by measuring the length from the base of the plant to the top of the agave rosette. Chlorophyll concentration was measured before harvesting of plants using a hand held CCM-200 Plus meter (Apogee Instruments, Inc., Logan, UT) in the three most recently unfolded leaves of each plant. Three measurements were taken on each leaf, and were subsequently averaged.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS version 9.3, SAS Institute, Cary, NC). All end-of-harvest measurements (i.e., shoot dry mass, root dry mass, chlorophyll count, and nutrient concentrations) were analyzed using analysis of variance (ANOVA) with mean separation using the Tukey-Kramer test at the $P \leq 0.05$ level of significance. A check for normality for each analysis was accomplished with quantile-quantile plots for residuals, and by running the Shapiro-Wilk, Cramer-von Mises, and Anderson-Darling

tests. A log transformation was done on nutrient concentrations of Fe, Na, and Zn in order to achieve normality.

RESULTS

Shoot, root, total dry weight, and plant height

Shoot, total dry weight, and plant height of plants were lower in the 0.05 m³ m⁻³ treatment compared to those in the 0.26 m³ m⁻³ treatment. However, no statistical differences were found between plants in the other treatments or relative to the 0.05 and 0.26 m³ m⁻³ treatments (Table 1). There were also no differences in root dry weight among treatments (Table 1).

Chlorophyll count

There were no significant differences in chlorophyll count between plants in the different irrigation treatments in the two most recently unfolded leaves of each plant (leaf 1 and leaf 2) (Table 2). However, in leaf 3, which was the oldest of leaves sampled, chlorophyll count was significantly lower in plants in the 0.05 m³ m⁻³ water treatment in comparison to those in the 0.26 m³ m⁻³ treatment. Chlorophyll counts of plants in the 0.19 and 0.12 m³ m⁻³ treatments were not significantly different from the 0.05 or 0.26 m³ m⁻³ treatments.

Nutrient concentration

While not different than those in the 0.19 and 0.26 m³ m⁻³ treatments, plants in the 0.05 m³ m⁻³ treatment had significantly less percent N than those in the 0.12 m³ m⁻³ treatment. Carbon, Mg, S, Mn, Cu, Zn, Fe, and Na concentrations were not significantly different in plants in any of the treatments. Potassium was significantly lower in plants in the 0.05 m³ m⁻³ water treatment relative to the 0.26 and 0.12 m³ m⁻³ treatments. Phosphorus was significantly lower in plants in the 0.12 and 0.05 m³ m⁻³ treatments than of those in the 0.26 and 0.19 m³ m⁻³

treatments. Calcium was significantly lower in plants in the 0.05 m³ m⁻³ treatment compared to those in the 0.26 and 0.19 m³ m⁻³ treatments. Calcium in plants in the 0.12 m³ m⁻³ was significantly lower than of those in the 0.26 m³ m⁻³ treatment (Table 3).

DISCUSSION

Studies evaluating drought stress on other potential bioenergy crops such as corn (Efeoglu *et al.*, 2009), miscanthus (Clifton-Brown *et al.*, 2002), and switchgrass (Stroup *et al.*, 2003), had to achieve drought stress by manually irrigating and taking soil moisture measurements regularly. Such approaches can be labor intensive and complicate efforts to replicate natural drought conditions for extended periods of time due to the variability of VWC levels in heterogeneous media. The automated irrigation system designed in this study proved to be effective in evaluating the impacts of pre-determined, specific VWC levels on *A. weberi* growth, given that moisture levels remained relatively constant throughout the duration of the study ($\pm 2\%$). The ease by which otherwise highly productive, ostensibly water-stress-tolerant *Agave* germplasm can be quickly screened through this system shows considerable promise (Bauweraerts *et al.*, 2013, Jones, 2007, Kim *et al.*, 2012, Nemali & van Iersel, 2008). Such a system should be considered for future experiments evaluating the effects of water stress on plant growth.

Interestingly, the productivity of *A. weberi* was not significantly reduced by moderately low VWC levels. Only plants at very low VWC levels had significantly less growth, indicating that *A. weberi* can exhibit moderate amounts of growth despite growing in VWC levels as low as 12%. These results go against that found in other studies where a decrease in water availability typically leads to statistically significant declines in growth (Clifton-Brown & Lewandowski,

2000, Earl & Davis, 2003, Nobel *et al.*, 1989). *Opuntia amclea* and *O. ficus-indica* have been reported to have notably high productivity ($46 \text{ Mg ha}^{-1} \text{ yr}^{-1}$), but only when irrigated and fertilized daily (Nobel *et al.*, 1992). Accordingly, the ability of *A. weberi* plants in lower VWC treatments to have statistically similar growth as plants in the highest treatment underscores the need for further evaluating this potential energy crop for dryland regions.

Although growth was similar between all treatments with the exception of the lowest, it is likely that over time plants receiving high amounts of water would experience an increase in growth due to a switch in the photosynthetic pathway. The basis for our assumption lies in the fact that agaves are opportunistic plants, which will start to open their stomates earlier in the day and will even switch from CAM to C_3 photosynthesis when water is abundant (Lüttge, 2010, Matiz *et al.*, 2013, Nobel & Hartsock, 1979). After ten weeks of daily watering, mature plants of *A. deserti* switched from CAM photosynthesis to C_3 photosynthesis, where it predominantly had daytime CO_2 uptake. *Agave weberi* plants grown in a hydroponic system experienced similar results, where stomatal opening started to occur during the day, particularly in the afternoon, after 8 weeks of growing under well-watered conditions (Stewart and Bergsten, unpublished data).

While some *Agave* species such as *A. mapisaga* and *A. salmiana* have been recorded to have extremely high productivity, it was probably due to the fact that these plants were irrigated near field capacity, which facilitated high stomatal conductance during the day resulting in an increase in carbon uptake (Lüttge, 2010, Matiz *et al.*, 2013). Indeed, without additional resource inputs, yields of these species were reported to reach only $25 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ (Nobel, 1991). Clearly, water can be a limiting factor in *Agave* growth.

Other variables besides shoot dry weight also indicated that plants in lower water treatments were not as productive. Chlorophyll count data showed that there was a decrease in chlorophyll in the oldest leaves as VWC treatment decreased. Furthermore, the relatively lower concentrations of N, K, P, and Ca in the 0.05 and 0.12 m³ m⁻³ treatments indicated that nutrient uptake may have been inhibited due to limited water availability. This is likely due to a decrease in transpiration rates and impaired active transport and membrane permeability, which reduces nutrient uptake by the roots and nutrient transport from the roots to the shoots (Alam, 1994, Viets, 1972).

CONCLUSION

The potential for *A. weberi* to be cultivated in the U.S. Southwest or other semi-arid regions for bioenergy production may be limited. Most of this region receives low amounts of precipitation (FAO, 1989), and many groundwater reserves are already being overpumped (Postel *et al.*, 1996), reducing the potential of using water resources in this area for *Agave* cultivation. Sustaining *Agave* crop yields comparable to corn or miscanthus grown in high-rainfall regions will likely only be manageable through large irrigation inputs, and might not be economically or environmentally feasible in the U.S. Southwest. However, the natural variation and plasticity found within the *Agave* genus (Nobel, 1994) suggests that moderately high-yielding accessions could be identified that tolerate low soil-moisture conditions inherent to semi-arid regions. Less-than-ideal yields may be obtained, but if supplemented with other alternative sources of energy, such as wind and solar energy, it could become a reliable energy crop for semi-arid regions. Determining the viability of *Agave* as an energy crop should continue to be pursued. Undoubtedly, long-term experiments evaluating the responses of *A. weberi* and other *Agave* species to drought and other environmental stresses are certainly warranted.

ACKNOWLEDGEMENTS

We are thankful to three undergraduate students, Cali McMurtrey, Yue Shen, and Westen Archibald, for helping us carry out this experiment.

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TABLES

Table 1. Dry Weights

The effect of varying volumetric water content levels on growth variables of *Agave weberi* over an 80-day period.

Treatment	Shoot dry weight			Root dry weight			Total dry weight			Plant height		
(m ³ m ⁻³)	(g)			(g)			(g)			(cm)		
0.26	6.32	A	±0.42	4.10	A	±0.42	10.2	A	±0.71	10.1	A	±0.38
0.19	4.85	AB	±0.42	3.47	A	±0.42	8.07	AB	±0.71	8.9	AB	±0.38
0.12	5.23	AB	±0.42	3.69	A	±0.42	8.93	AB	±0.71	9.31	AB	±0.38
0.05	4.21	B	±0.42	3.16	A	±0.42	7.37	B	±0.71	8.5	B	±0.38

^ZDifferent letters indicate means that are significantly different from each other (Tukey test, $P \leq 0.05$, ANOVA).

Table 2. Chlorophyll Content

Average chlorophyll content levels of the three most recently unfolded leaves of each *Agave weberi* plant separated by treatment. Leaf 1 was the youngest leaf.

Treatment (m ³ m ⁻³)	Leaf 1	Leaf 2	Leaf 3
0.26	88 A ±4.2	104 A ±5.1	112 A ±6.1
0.19	91 A ±4.2	95 A ±5.1	96 AB ±6.1
0.12	95 A ±4.2	111 A ±5.1	98 AB ±6.1
0.05	90 A ±4.2	103 A ±5.1	87 B ±6.1

²Different letters indicate means that are significantly different from each other (Tukey test, $P \leq 0.05$, ANOVA).

Table 3. Nutrient Concentrations

Nutrient concentrations of *Agave weberi* in each volumetric water content treatment.

Treatment	C			N			K			P			Ca			Mg			S		
(m ³ m ⁻³)	%			%			%			%			%			%			%		
0.26	43.5	A	±0.2	0.43	AB	±0.04	1.94	A	±0.02	0.20	A	±0.01	2.37	A	±0.08	0.79	A	±0.02	0.10	A	±0.01
0.19	43.8	A	±0.2	0.38	AB	±0.04	1.93	AB	±0.02	0.18	A	±0.01	2.19	AB	±0.08	0.79	A	±0.02	0.10	A	±0.01
0.12	43.6	A	±0.2	0.47	A	±0.04	1.95	A	±0.02	0.15	B	±0.01	2.03	BC	±0.08	0.76	A	±0.02	0.10	A	±0.01
0.05	44.1	A	±0.2	0.33	B	±0.04	1.88	B	±0.02	0.13	B	±0.01	1.84	C	±0.08	0.72	A	±0.02	0.10	A	±0.01

Treatment	Mn			Cu			Zn			Fe			Na		
(m ³ m ⁻³)	ppm			ppm			ppm			ppm			ppm		
0.26	595.6	A	±42.9	1.94	A	±0.17	5.83	A	±0.86	51.8	A	±8.8	475.6	A	±42.5
0.19	590.8	A	±42.9	1.73	A	±0.17	5.20	A	±0.86	37.4	A	±8.8	474.9	A	±42.5
0.12	639.1	A	±42.9	2.06	A	±0.17	6.36	A	±0.86	37.2	A	±8.8	535.6	A	±42.5
0.05	541.1	A	±42.9	1.96	A	±0.17	5.36	A	±0.86	54.9	A	±8.8	580.4	A	±42.5

^ZDifferent letters indicate means that are significantly different from each other (Tukey test, $P \leq 0.05$, ANOVA)