Exploring the Potential of *Agave* as a Biofuel Crop on Arid Land

Rhea Conlu, Diana Ha, and Jeffery Shen
School of Life Sciences, University of Nevada, Las Vegas

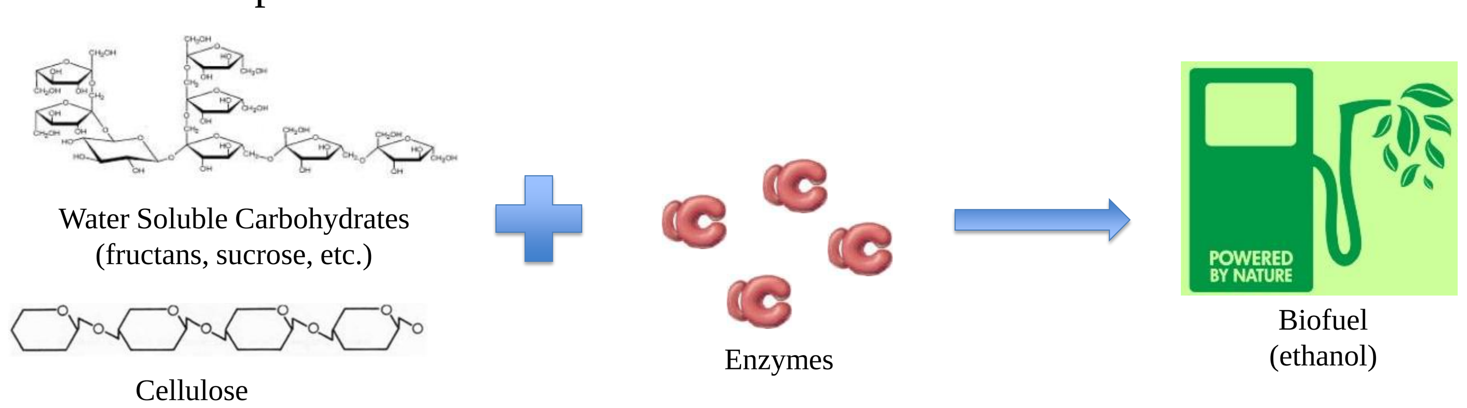
Introduction

As residents of a desert environment, the main concern stemming from climate change is the future availability of water. Decreasing water levels forces us to consider not only conservation, but also the smarter use of the resulting waste water. The genus *Agavaceae* comprises over 200 species of plants, many of which are capable of growing in very harsh, arid environments, including the Nevada desert. The plant has adapted in various ways to a lack of hydration, including the storage of water throughout its tissues and a Crassulacean acid metabolism (CAM), where its sugar production and carbon fixation - around 87% - is done mainly at night [2]. This allows the stomata to remain shut during the day, and reduces the amount of water lost through transpiration. Because of this drought tolerance, it can be cultivated on the 18% of the earth's landmass which is semi-arid land, much of which is no longer being used for agricultural production [3]. Global warming coupled the imminent depletion of world fossil fuels has shifted the focus of alternative energy toward the production of biofuels.

Throughout its history, the agave has been grown and harvested as a source of alcohol and as a sugar substitute because of its high concentration of fructans, which are oligomers of fructose units bound to a sucrose. The pinãs, or heads, of these plants contain a high amount of fructan polymers such as inulin, which can be hydrolyzed to free fructose [1]. Because the sugars can thereafter be fermented, *Agave* is considered an economically important plant. It is estimated that the *Agave* can yield 3,000-10,500 liters/ha of ethanol, which is more than other potential sources of biofuels such as corn, sugarcane, and poplar [3]. These predictions, combined with a higher projected productivity than corn and the plant's ability to flourish with less water and nitrogen requirements, makes a compelling argument for further research on the Agave.

What makes *Agave* an ideal biofuel crop?

- High total sugar density and content
- High genetic diversity
- Does not compete with agricultural land
- Is not a food crop
- Requires little water
- CAM metabolism minimally affected by climate change/ increasing CO2
- High cellulosic biomass



How do other biofuel crops compare?

Product /Process	Corn	Agave(head)	Sugar Cane
Feedstock needed for 1L EtOH	3 kg	6 kg	11.6 kg
Feedstock Production (Tonnesha^{-1} year $^{-1}$)	12	110	74
Years to harvest	1	6	1
Biomass produced (Tonnesha^{-1} year $^{-1}$)	25	200 (whole plant)	74
EtOH production (L tonne^{-1} year $^{-1}$)	399	125	86
Water requirements	High	Very low	Very high
Field Labor	High	Low	High
Environmental impact (fertilizers, insecticides)	Very high	Very low	High
Agricultural value of land	Very high	Very low	Very high
Demand as food	Very high	None	High
Sugar content (Brix)	5%-11%	27%-42%	10%-14%

Data reorganized from findings by Somerville and Velez

Hypotheses

(1) Growth of *Agave nevadensis* will remain constant between a control group and a group exposed to waste water, and

(2) Growth of *Agave nevadensis* will remain constant between a control group and a group exposed to drought conditions

Materials and Methods

Fifteen plants were separated into three groups and grown over a period of 8 weeks. To understand how *Agave nevadensis* (Figure 1) responds to industrial water, which may still contain microbes, run-off, and corroded materials, 5 plants were treated with industrial water available in at UNLV's SEB building. They were watered at the same time as the control group, which was about every 10-14 days with the same amount of water. Because the required amount of water needed for optimal plant growth was unknown, the amount watered began with 5 mL but gradually increased to 100 mL because peripheral leaves of plants from all groups seemed to shrivel and die. The drought-treated group was watered every 15-20 days with the same increasing volume of water as the other groups. They were kept in a constant greenhouse environment and repeatedly rotated to reduce discrepancies in microenvironments.

To better gauge the water needs of the plants, an apparatus called the HH2 Moisture Meter with a Theta Probe attachment was used to measure the amount of moisture found by the roots of the plant (Figure 2). The probe sends an electrical impulse down the stainless steel prongs, and the amount of time it takes for the impulse to jump between the prongs reflects the percentage of water in the soil available for the roots.

Figure 1: A set of 15 *A. nevadensis* specimens were divided into three groups: control, experimental, and drought stress.

Figure 2: HH2 Moisture Meter with a Theta Probe

Because the splay of the plants is not perfectly symmetrical, the circumferential growth was measured by taking two diagonal measurements. Growth in height was measured by monitoring the length of a marked leaf, as seen below (Figure 3). Measurements were taken every two weeks and recorded in millimeters. A sugar refractometer, which is an optical instrument capable of measuring dissolved solids, was used to gauge the sugar composition in *A. americana*. Crops with higher Brix will produce more alcohol from fermented sugars and be more resistant to insects.

Diagonal measurement 1 · Diagonal measurement 2 · Length measurement

Figure 3 – Measurement methods

Results and Conclusion

Growth Data - Height

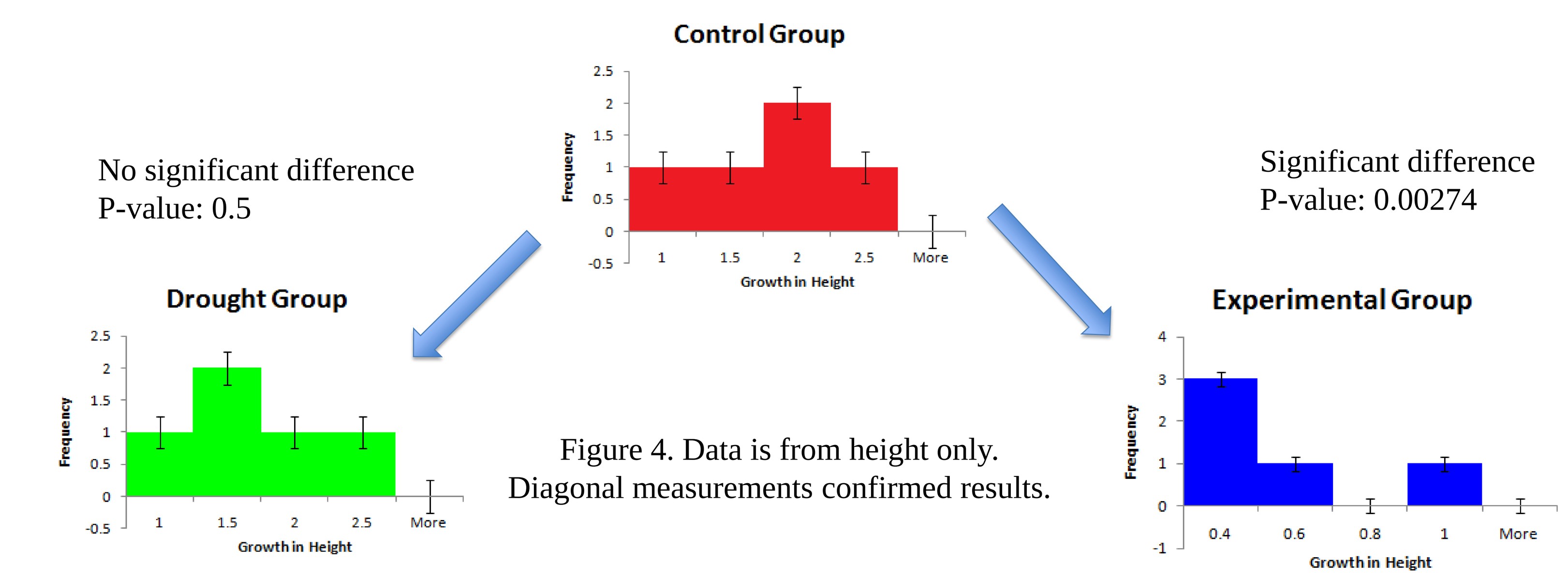


Figure 4. Data is from height only. Diagonal measurements confirmed results.

The results indicate that *A. nevadensis* does not grow as well under industrial waste water treatment, however this may be dependant on the specific species or the youth of the plants. Further research on the tolerance of waste water should be conducted on different agave species and on various stages of growth. The second hypothesis was confirmed, since the plants grew better when watered less frequently; the agave's ability to withstand drought makes it a viable biofuel crop, especially when potential climate change is considered.

Sugar Analysis

The Brix analysis found that *A. americana* has 10.6 Brix in its leaves. This would correspond to about 10.6% of its sap composition as sucrose.

Future Research

I. Flow Cytometry

Applications:
- Immunophenotyping assay
- DNA analysis
- Cell proliferation analysis
- Apoptosis

II. Chromosome Staining

Applications:
- Confirm ploidy from flow data
- Cross-breeding determination

References

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Acknowledgements

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