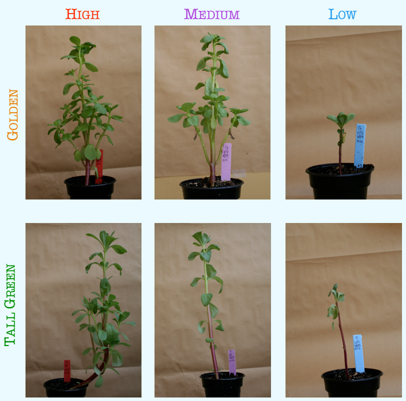
Effects of Water Deficit on Regulation of Phenolic Antioxidants and Betalain Pigments in *Portulaca oleracea (Portulaceae)*

Courtney Brimmer*, Tanner Miller, Kelly Summers, Dana Dudle, Bryan Hanson

DePauw University, Departments of Biology, Chemistry, &Biochemistry

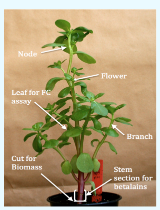
Introduction

As a result of global climate change, water availability may change dramatically, a shift that would undoubtedly impact plant life. The aim of this study was to investigate the response of two varieties of *Portulaca oleracea* to water deficit. Morphological data were collected as measures of fitness. Chemical analyses were conducted on phenolic antioxidants and betalain pigments.



Plants were assigned to one of three water treatments. Red tags indicate the high water treatment (300 mL/week). Plants with purple tags were put into the medium water treatment (150 mL/week). Blue tags represent the low water treatment (0 mL/week).

Portulaca oleracea (family *Portulacaceae*, common name: purslane) is a succulent and stress resistant plant that can survive at least 3 weeks without water. In some areas purslane is collected for medicinal purposes and as a food source. It has high concentrations of antioxidants and omega-3 fatty acids. For this study, two varieties of *P. oleracea* were used: Tall Green & Golden.



In order to collect data for each of the plants, the nodes, flowers, branches, and dropped leaves were counted. One leaf was removed for the FC antioxidant assay. A section of stem was removed for the betalain extraction. The plant was cut off at dirt level and dried for biomass.

Questions

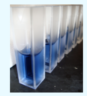
1. What is the effect of water deficit on morphological responses?
2. What is the effect of water deficit on antioxidant production?
3. What is the effect of water deficit on betalain production?
4. What is the effect of water deficit on the plants' cuticles?
5. How will the two genotypes differ in their responses to water deficit?

Design

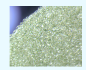
Seeds were germinated and established during a 2 week period with ample water. They were the assigned to one of three treatments (300 mL/week, 150 mL/week, and 0 mL/week) which lasted 3 weeks.

All plants were grown in the same greenhouse. Treatments and cultivars were randomized on the benches.

Data Collected

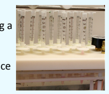


Folin-Ciocalteau (FC) antioxidant assay: The phenolic antioxidants from one leaf of each plant were extracted in water. Reactants were added which resulted in different shades of blue dependent upon antioxidant concentration. The extracts were put into a visible spectrometer to obtain their absorbance at 765 nm. This number was then used to calculate GAE (gallic acid eqivalents) for each sample.



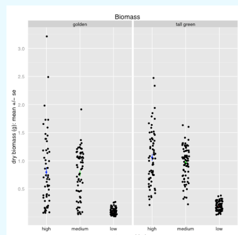
Infrared spectroscopy (IR) was used to determine a chemical fingerprint for the cuticle for one leaf of each plant.

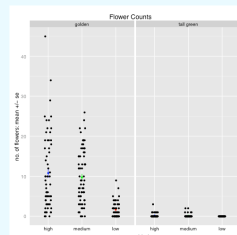
The betalain pigments found in the stems were extracted and filtered using a 60% methanol: water solution. The samples were then placed in a visible spectrometer to collect their absorbance values at 477 nm and 540 nm.

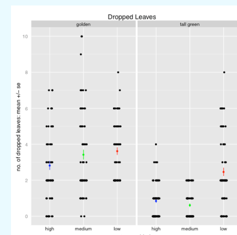


Results

Does water deficit result in morphological responses? YES

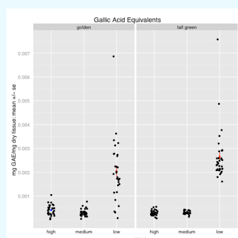






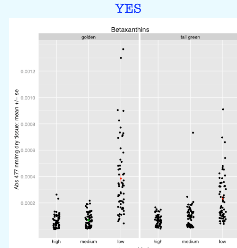
The treatments had a significant impact on the morphology of the plants in both cultivars. Plants in the low water treatment were smaller, produced fewer flowers and nodes, and they lost more leaves than the plants in the high and medium water treatments.

Does water deficit cause increased antioxidant production? YES



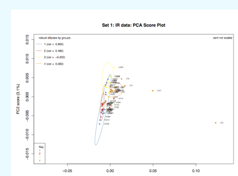
The treatments did cause the plants in the low water treatment to increase the amount of antioxidants produced. There is little difference between the production of antioxidants among the medium and high water treatments.

Does water deficit cause increased production of betalain pigments? YES



The plants in the low water treatment produced significantly more betalain pigments in comparison to plants in the other treatments. The two classes of betalains were highly correlated; only the absorbance for betaxanthins are shown above. The same factors were significant for both wavelengths.

Does water deficit cause a chemical change in a leaf's cuticle? NO



Using infrared spectroscopy, we did not detect significant differences in the cuticles. It is possible that changes in the cuticles did occur that we were not able to detect with our methods.

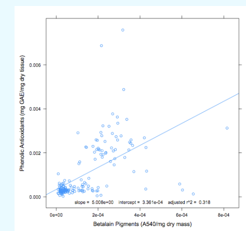
Discussion

Portulaca oleracea is a very drought resistant plant. Withholding water for 3 weeks did not cause the plants to die; however, they were obviously stressed by the end of the trial. An extreme water deficit was required to cause a response in the plants. The medium water treatment received half as much water as the high water treatment, but there was little difference between plants in those two treatments.

Inducing a water deficit in *Portulaca oleracea* caused a predictable but dramatic response in the plants' morphology. A variety of chemical responses were observed including: increased production of phenolic antioxidants and betalain pigments in the low water treatment. The plants from the low water treatment were smaller than their counterparts in the other two treatments. Significant changes in the plants' cuticles were not observed.

Plants experiencing drought usually close their stomata to reduce water loss, resulting in reduced photosynthesis. Because these plants continue to intercept a high volume of photons that aren't used in the photosynthetic process, leaves and photosynthetic stems of water-stressed plants may generate reactive oxygen species that can lead to photooxidative stress. Upregulation of phenolic antioxidants, including betalains, may mediate the effects of these damaging ROS, and allow plants such as purslane to withstand periods of water deficit.

Is there a correlation between the phenolic antioxdants in the leaves and the betalain pigments in the stems? POSSIBLY



An interesting positive correlation was found between the betalain pigments in the stems and the antioxidant correlations in the leaves. This may be an intriguing direction for a future project.

Future Directions

1. Is there increased antioxidant activity in the low water plants?
2. Detailed investigation of antioxidants using other antioxidant assays.
3. What is the benefit of producing more antioxidants during a drought?
4. What is the functional purpose of betalains?
5. Further investigate the trends observed in this study using NMR & mass spectroscopy.
6. Investigate the impact of water deficit on lipid composition.
7. Investigate correlations between betalain and antioxidant production.

Acknowledgments

I would like to thank Tanner Miller and Kelly Summers for their support and assistance in data collection. I would also like to thank Dana Dudle and Bryan Hanson for their guidance, support and advice. And thank you to the Science Research Fellows, DePauw University Nature Park, Manning Environmental Field Station, and the Departments of Biology, Chemistry & Biochemistry.