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Effects of drought conditions on water use efficiency, transpiration and photosynthesis in *Silphium integrifolium*

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ABSTRACT

Drought conditions are expected to increase as a side effect of climate change in the near future affecting crop production and, in turn, public health. To understand and develop plans for the future ahead it is important to understand the way different plants will react to more variable weather conditions that come along with changing climate. *Silphium integrifolium* is one of the many midwestern perennial prairie plants available for study. *Silphium integrifolium* Is a sunflower-like plant that is used in this study as a comparable subject for agriculture by The Land Institute in Salina, Kansas. The study is conducted from four genotypes and two different watering treatments in 2019, then four consistent watering treatments in 2020 during the second flowering. The experiment was conducted in a greenhouse setting under ambient light and CO_2 concentrations. Data was collected using a LICOR-6800 instrument during the mid-morning and early afternoon, allowing plants ambient lighting as mentioned before in order to ensure a consistent photosynthetic rate. Data collected was cross analyzed with one-way Analysis of Variance (ANOVA) to find significance in Water Use Efficiency (WUE) vs 2020 watering, WUE vs Genotype, A vs 2020 watering, A vs Genotype, E vs 2020 watering, and E vs genotype, These data sets showed no significance due to small sample size. Sample size necessary would be about nine times as large. This shows that watering patterns and genotype don't show a difference in WUE of *Silphium integrifolium* according to this sample size. With a larger sample size, it is possible for the data to show relevance to the study of drought effects on plant life.

Keywords: *drought, photosynthesis, Silphium integrifolium, transpiration, water use efficiency*

INTRODUCTION

Atmospheric CO_2 has been increasing substantially since the industrial revolution (Ainsworth and Rogers, 2007). There are already warnings of irreversible damage to Earth's major climate systems and ecosystems necessary to the survival of plants and animals on Earth. Emissions associated with burning fossil fuels, along with high and increasing levels of deforestation around the globe have led to this exponential increase in atmospheric CO_2 concentration. Without climate action, atmospheric [CO_2] is expected to reach 730-1020 ppm by the end of the century (Ainsworth and Rogers, 2007). This significant increase will affect many functions of climate including precipitation rates. Arid and semiarid ecosystems are likely to experience extended drought conditions (Liu et al., 2020). The stress this puts on plants has a variety of implications on the human species. Learning how plants will react to increased CO_2 and drought conditions, however, will help further understand how climate change will affect all ecosystems.

In order for the plant to survive in drought conditions, it will have to increase net water use efficiency. Water use efficiency is the ratio of the transpiration rate to the photosynthesis rate of a plant. With changing climate conditions, it is important to understand the way these processes will respond. For people to make a living on Earth, it is necessary that food is available and sustainable. With a growing human population, finding ways for food to be available is already a challenge. However, not only is the population size an issue, but climate change and its effects on crop management and agriculture is becoming a major area of concern. As the climate is changing more quickly than it has over the last hundreds of thousands of years, plants will have to adapt rapidly to survive.

The main concern facing agriculture is the effect of drought induced by climate change (de Sousa et al., 2020). Understanding the ways in which plants will respond to drought conditions will be key in creating a plan for the sustainability of food crops and allowing scientists to understand where and when these plants may run into problems under rapidly changing conditions.

Controlled environment and field studies have shown that elevated [CO_2] directly affects photosynthetic rates (Sage, Sharkey, and Seemann, 1989) and transpiration rates in various food crops, including wheat and sorghum (Conley et al., 2001). Free-air CO_2 enrichment (FACE) is used in experiments to measure water use efficiency over time (Taylor et al., 2003). However, both photosynthetic rates (A) and transpiration rates (E) can be measured using the LICOR-6800 instrument over a short period of time to determine how elevated [CO_2] affects a plant's water use efficiency (WUE). Similar studies have been done incorporating treatments of nitric oxide and sodium nitroprusside, along with drought conditions on soybean plants, looking for results on drought tolerance and stomatal conductance (de Sousa et al., 2020).

The objective of my study is to better understand how drought doubled atmospheric CO2 levels will affect soybeans' water use efficiency, photosynthesis and transpiration water stress levels in a perennial plant *Silphium integrifolium*. *Silphium integrifolium* is a flowering, perennial plant that grows in the Eastern region of North America and in the central United States as far west as New Mexico. The Land Institute in Salina, Kansas selected this plant based on four different genotypes in order to study how drought will affect the transpiration rate and photosynthetic rate. The Land Institute breeds and grows plants to study for agriculture, along with the *Silphium integrifolium.* The flower of *Silphium integrifolium* resembles a sunflower, with bright yellow petals atop a long prickly stem. It grows 1-2 meters tall and blooms July through September in grassland areas. In order to understand the plant, the LICOR-6800 will be used to measure carbon assimilation along with transpiration rate under drought conditions and elevated [CO_2].

MATERIALS AND METHODS

A total of 120 perennial plants *Silphium integrifolium* from four different genotypes were selected for treatments. Genotypes were labeled R, P, W, and Y. *Silphium integrifolium* grows 1-2 meters tall and resembles a sunflower when in bloom with bright yellow flowers.

Silphium integrifolium was planted and grown from seed in 2019 at The Land Institute in Salina, Kansas. Plants were grown in a greenhouse with selected watering treatments every day, three days, five days and seven days. In 2020, *Silphium integrifolium*, measurements were taken around mid-day under ambient light in the greenhouse. Watering treatments in 2020 were sectioned to every two days and every seven days for selected genotype cross section.

The data was collected by The Land Institute in Salina, Kansas using a LICOR-6800 instrument. The LICOR-6800 clamps onto the leaf of a plant and records information about the leaf inside a closed chamber. In programming the machine for research there is the option of changing variables like light intensity, carbon dioxide concentration, etc. allowing replication of natural scenarios. In this case these variables were left ambient to assess the way the watering treatments affect the water use efficiency. Data collected includes transpiration rate and photosynthetic rate. These data points help calculate water use efficiency.

The data collected by The Land Institute was analyzed through the multiple treatments with an analysis of variance, in order to find any relevant difference between the genotype, watering treatments, and water use efficiency of *Silphium integrifolium.* The following one-way analyses of variance tests were done: WUE vs 2020 watering, WUE vs Genotype, A vs 2020 watering, A vs Genotype, E vs 2020 watering, and E vs genotype. These were selected to give a well-rounded understanding of the way the plants used water and how it was or was not affected by watering pattern, genotype, and 2019 watering schedule.

RESULTS

The Land Institute selects plants for breeding based on the number of seeds the plants produce. They specifically find genotypes with variation based on the seed count, which is what gives us the selection of four genotypes used in this study.

In the one-way Analysis of Variance (ANOVA), dependent variable WUE vs 2020 watering data failed the Shapiro-Wilk Normality test with $P < 0.050$. Following the One-Way ANOVA, a Kruskal-Wallis One Way Analysis of Variance on Ranks was conducted.

Table 1. Data Analysis for WUE vs 2020 Watering treatment.

Group	N	Median	25%	75%
2.000	61	25.783	12.912	39.918
7.000	57	26.931	9.988	47.824

H = 0.00142 with 1 degrees of freedom.

It was concluded that the differences in median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability. With $P = 0.970$, there is no statistically significant difference.

WUE vs Genotype failed the One-Way ANOVA Normality test with a P value <0.050. Followed by ANOVA on Ranks, which resulted in a $P = 0.090$.

Table 2. Data Analysis for WUE vs Genotype.

Group	N	Median	25%	75%
R	25	17.917	8.210	33.886
P	31	22.954	9.760	39.212
W	32	29.894	13.763	53.590
Y	30	37.377	11.874	49.102

H = 6.502 with 3 degrees of freedom.

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference.

Using A as the dependent variable, data for A vs 2020 watering passed the Normality Test with a P value of 0.205. Following the Normality test was the Brown-Forsythe Equal Variance Test, which was passed with a $P = 0.692$.

Table 3. Data Analysis for A vs. 2020 Watering Treatment

Group	N	Mean	Std Dev	SEM
2.000	61	21.705	10.793	1.382
7.000	57	22.362	10.549	1.397
Source of Variation	DF	SS	MS	F
Between Groups	1	12.738	12.738	0.112
Residual	116	13220.871	113.973	
Total	117	13233.609		

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.739). The power of the performed test with alpha = 0.050:0.050 is below the desired power of 0.800. Less than desired power indicates that it is less likely to detect a difference when one exists.

A vs Genotype passed the Normality Test with a P value of 0.157 followed by the Equal Variance Test being passed with P=0.340.

Table 4. Data Analysis for A vs Genotype

Group	N	Mean	Std Dev	SEM
R	25	20.418	11.768	2.354
P	31	23.396	10.115	1.817
W	32	22.901	12.100	2.139
Y	30	21.003	8.556	1.562
Source of Variation	DF	SS	MS	F
Between Groups	3	178.682	59.561	0.520
Residual	114	13054.927	114.517	
Total	117	13233.609		

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability (P=0.669); there is not a statistically significant difference. The power of the performed test with alpha = 0.050:0.050 and is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists.

The next test uses E as the dependent variable and 2020 watering as the independent variable. E vs. 2020 watering passed the Normality Test with a P value of 0.362, and then passed the Equal Variance Test with a P value 0.735.

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference. The final P value is 0.426. The

Table 5. Data Analysis for E vs 2020 Watering Treatment

Group	N	Mean	Std Dev	SEM
2.000	61	0.0230	0.0110	0.00141
7.000	57	0.0214	0.0101	0.00133
Source of Variation	DF	SS	MS	F
Between Groups	1	0.0000715	0.0000715	0.639
Residual	116	0.0130	0.000112	
Total	117	0.0130		

power of the performed test = 0.050:0.050 (0.050) Is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists.

E vs Genotype passed the Normality Test with a P value of 0.258. Following the Normality Test, the data passed the Equal Variance Test with a P value of 0.152.

Table 6. Data Analysis for E vs Genotype

Group	N	Mean	Std Dev	SEM
R	25	0.0227	0.0113	0.00225
P	31	0.0240	0.00983	0.00177
W	32	0.0219	0.0123	0.00217
Y	30	0.0205	0.00880	0.00161
Source of Variation	DF	SS	MS	F
Between Groups	3	0.000204	0.000068	0.604
Residual	114	0.0128	0.00113	
Total	117	0.0130		

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference. The final P value = 0.614. The power of the test (0.050) is below the desired power of 0.800. Less than desired power indicates you are less likely to detect a difference when one actually exists.

In order to find significant data, based on data for ANOVA E vs. Genotype, a sample size of 476 plants would have been necessary along with two groups leading to a total of 952 specimen to complete the study. The calculation was done with a power of 0.800 and an alpha of 0.0500. The sample size required is large and nearly unattainable, needing 952 data points to find significance.

DISCUSSION

Silphium integrifolium is a prairie native plant in the American Midwest. Throughout the history of the Midwest there has been series of prolonged drought under shifting climate conditions. This could have potentially caused the adaptation of *Silphium integrifolium* allowing the plants to maintain their average WUE during the short-term response tests acted upon them. Due to *Silphium integrifolium* being a prairie native plant, it doesn't naturally receive the type of care that typical crop plants do. When crops like maize, wheat, and soybeans begin growing, farmers maintain their health with irrigation. *Silphium integrifolium* however does not naturally receive this type of watering, creating the evolutionary need to adapt. When measuring the WUE of crop plants like the soybean, there are genotypes with higher WUE that also have a higher yield (Fried, Narayanan, and Fallen, 2019.) This emphasizes the effects of genotype on yield and water use efficiency which directly affects seed production.

Along with the potential of *Silphium integrifolium* to adapt to different watering conditions, there is the issue of the sample size of the experiment. According to the statistical analysis there would have needed to be 952 specimens in the analysis to create more accurate statistical data. However, that is a high number of plants to maintain and collect data from, making it a somewhat irrational experiment to conduct. On a crop plant however, the sample size would be easier to attain due to crops being planted in higher volume already for food production. Most crop plants, however, are not perennial. The experiment would need to be altered to fit the time frame of the growing season. Along with the time period there is the issue of where to grow the crops and how to control various conditions that may alter results. Rainfall and other weather patterns would need to be recorded in order to account for the extra water or lack of water in selected plants and would create a less controlled variable.

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