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The Effects of Nitric Acid Rain on Sunflower Plants (Helianthus annuus)

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The Effects of Nitric Acid Rain on Growing Sunflower Plants (*Helianthus annuus*)

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Introduction & Hypotheses

Helianthus annuus, the common sunflower, is a rotational crop with winter wheat since it is heat and draught tolerant, but soil acidity has a negative affect on the plant's height, vigor, and survivability (Sutradhar et al., 2014). When grown in soils with a pH range between 4.7-5.3, sunflower yield was reduced equal to or greater than 10% (Sutradhar et al., 2014). That study used sulfuric acid rain conditions on its sunflowers while my study used nitric acid rain from nitrogen oxide. Nitrogen oxide is a common pollutant and greenhouse gas that creates nitric acid when it reacts with water in the atmosphere to produce a type of acid rain (Lal et al., 2016). Compared to clean rain, which has a pH of ~5.6, acid rain usually has a pH between 4.2-4.4 (*What is Acid Rain?* 2019). The greatest sources of nitrogen oxide in the atmosphere are from burning fossil fuels, and car engine exhaust (*What is Acid Rain?* 2019).

Hypotheses: Acid rain will decrease growth, increase water stress, and slow physiological processes in sunflower plants. Sunflowers exposed to acid rain conditions as they grow will display fewer true leaves, thinner stem diameter, shorter heights, lower specific leaf area, and lower stomatal density than sunflower plants not exposed to acid rain conditions. The treatment plants will be more water stressed (more negative water potential), lower overall biomass, and lower light reaction readings for midday photosynthesis, transpiration, and conductance.

Methods and Materials

Setup
- Twelve sunflower sprouts were potted in 3.8L pots with soil (Promix Mycorrhizae Organik Soil, Quikrete Premium Play Sand, Turface Athletics All Sport)
- Fertilizer was added week 6 (Osmocote Plus Premium Formula, Marysville, OH)
- Plants were put in random block arrangements using Excel
- Greenhouse conditions were 26.7˚C and 32% RH with sodium halide lights on 7am-6pm
- Plants watered to capacity M/W/F and as necessary week 8 onward

Acid Rain Solution
- 15M nitric acid was mixed with water to produce a solution with a pH between 3 and 4 and tested using pH strip paper and used to water 6 of the plants
- Acid rain solutions were stored in glass Erlenmeyer flasks covered with parafilm to prevent evaporation

Weekly Anatomical Measurements
- Stem diameter was measured just above the cotyledons
- True leaves were counted, leaves excised for other tests were counted, and dead leaves were also included
- Height was measured from the cotyledons up using a meter stick

Specific Leaf Area
- Mature leaves were removed at the petiole, scanned into a computer, and Image J software (NIH program) was used to determine surface area in cm^2
- The leaves were put in a drying oven for 48 hours then weighed
- SLA was calculated by dividing the surface area by the weight
- Leaves were kept for total biomass

Water Potential (Water Stress Level)
- Mature leaves at the 3rd node were excised at the basal end of the petiole and store in an air-tight bag and cooler for immediate transport
- In the lab, the leaves were taken out and put into a PMS bomb to test water potential (PMS Instrument, Co., Corvallis, OR)
- Leaves were dried and kept for total biomass

Stomatal Density
- Clear top-coat nail polish was applied to the adaxial and abaxial side of the same mature leaf and left to dry
- The nail polish layer was carefully removed with heavy duty packing tape and put onto a microscope slide
- The slide was viewed under the 40x objective and stomata were counted
- Total stomatal density was calculated by dividing observed stomata by 0.126 to get total stomatal density per square millimeter

Leaf Gas Exchange Measurements
- The most photosynthetically active mature leaves were chosen and were left attached to the plant for the whole test
- Analysis of the leaves were conducted midday during the most photosynthetically active time of the day (~10am to noon)
- Photosynthesis, transpiration, and conductance were measured using an LI-6400 (LI-COR Bioscience Inc., NE)

Shoot Biomass:
- Plants were cut at the very base of the shoot system, put into individual bags, and dried in a drying oven for 48 hours at 79˚C
- Once dried, the shoot system was weighed on a balance
- Leaves excised for earlier tests were saved, dried, and weight added to the shoot biomass

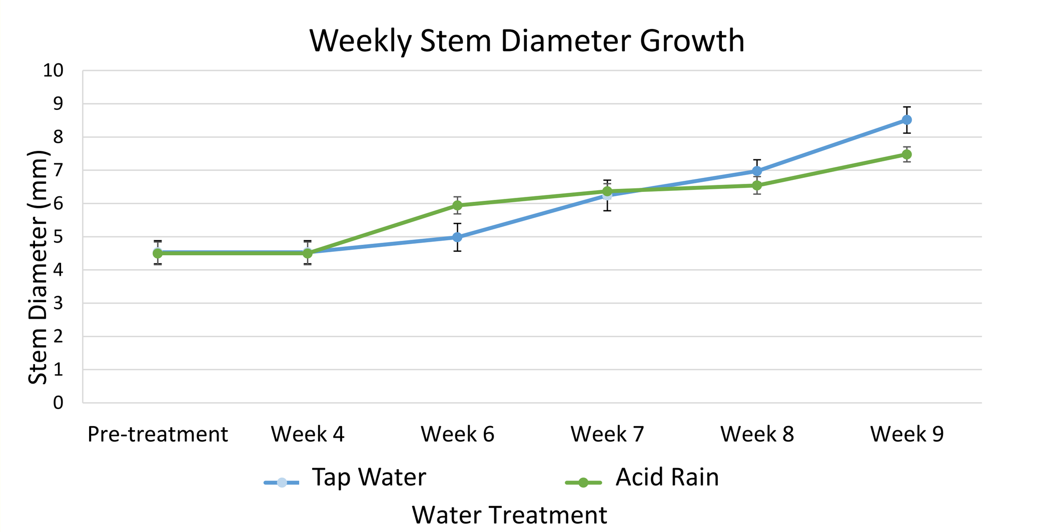

Figure 1. Weekly stem diameter growth of control (n=6) vs treatment (n=6) plants. Overall difference was not significant (t_{10}=2.08). Standard error bars shown.

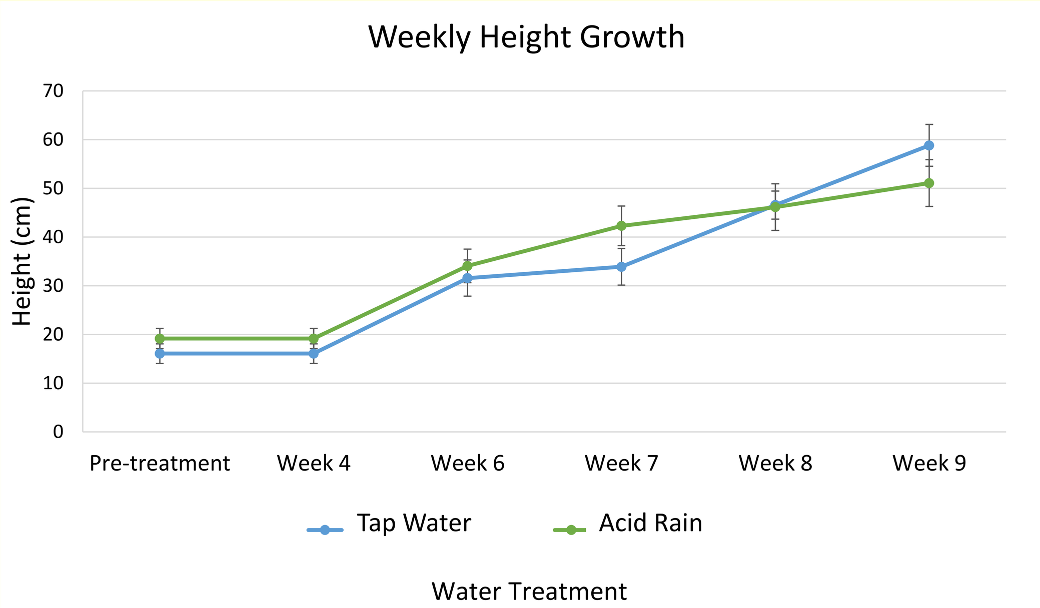

Figure 2. Weekly height growth of control (n=6) vs treatment (n=6) plants. Overall difference was significant (t_{10}=2.64, P<0.05). Standard error bars shown.

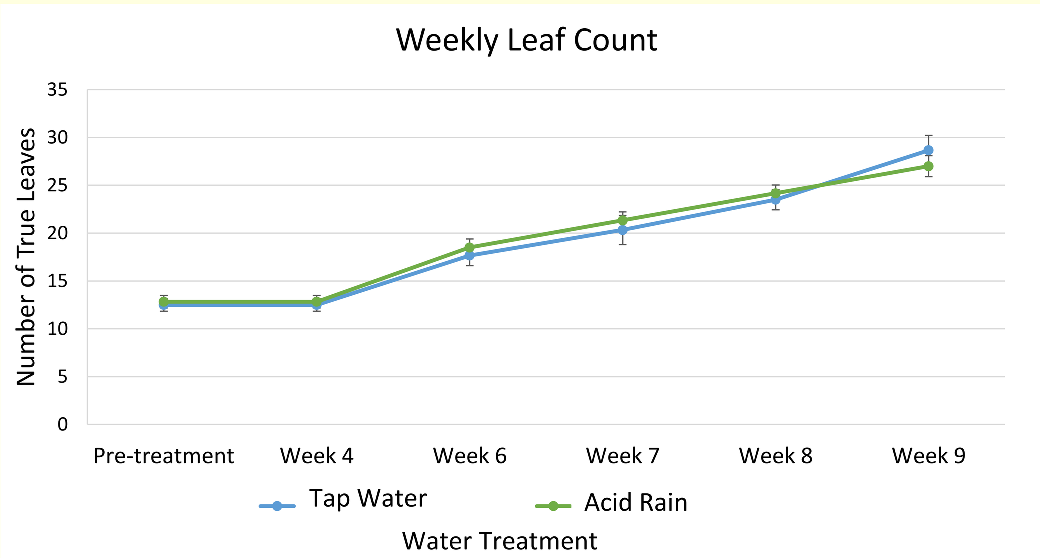

Figure 3. Weekly leaf count of control (n=6) vs treatment (n=6) plants. Overall difference was not significant (t_{10}=1.62). Standard error bars shown.

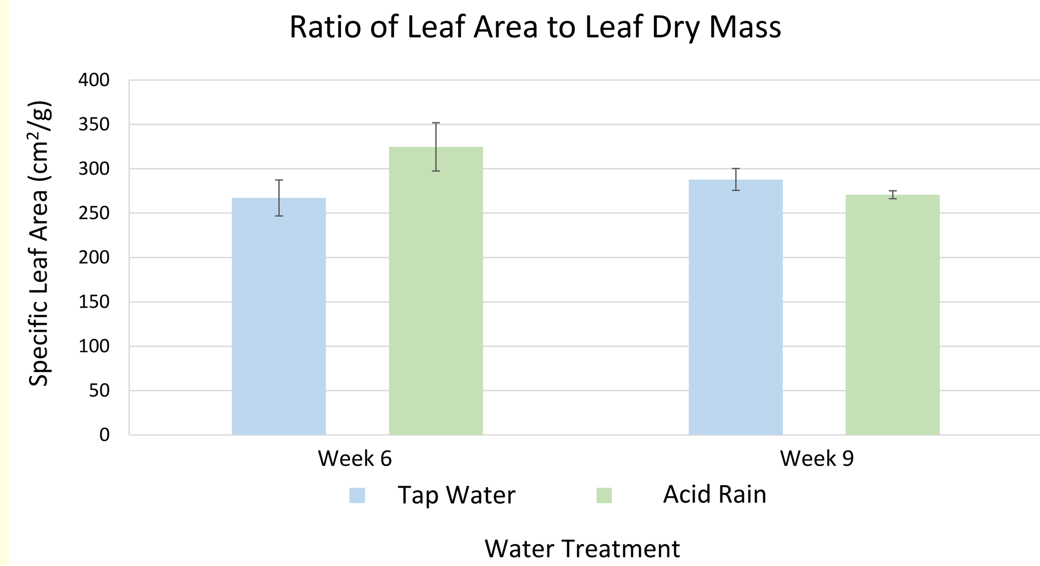

Figure 4. Specific leaf area of control (n=6) vs treatment (n=6) plants during week 6 and week 9. Week 6 data was not significant (t_{10}=1.69). Week 9 data was not significant (t_6=2.45). Standard error bars are shown.

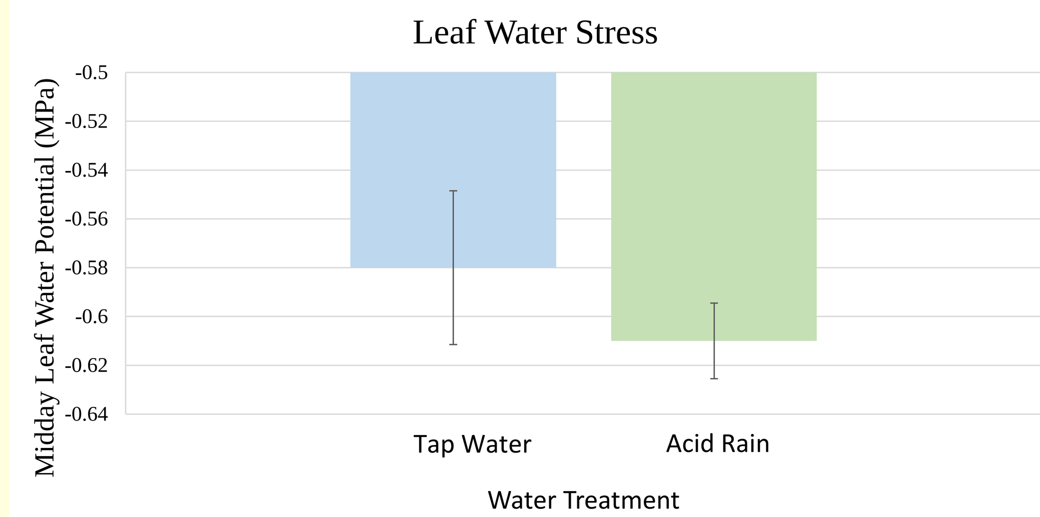

Figure 5. Leaf water potential of control (n=6) vs treatment (n=6) plants. Data was not significant (t_{10}=0.85). Standard error bars shown. More negative values correlate to a more water stressed plant.

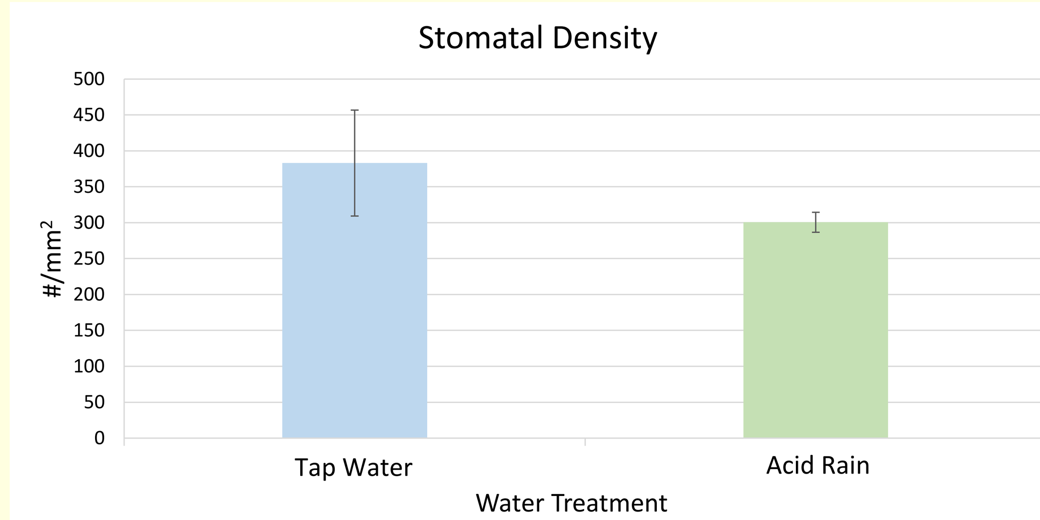

Figure 6. Stomatal density of control (n=6) vs treatment (n=6) plants. Data was not significant (t_5=1.10). Standard error bars shown.

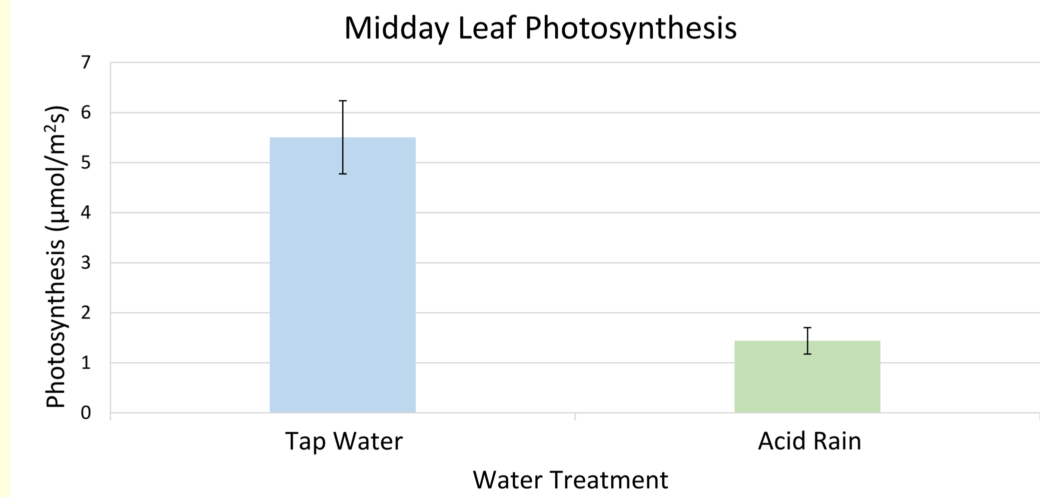

Figure 7. Midday leaf photosynthesis of control vs treatment plants. Data was significant (t_6=5.23, P<0.05). Standard error bars shown.

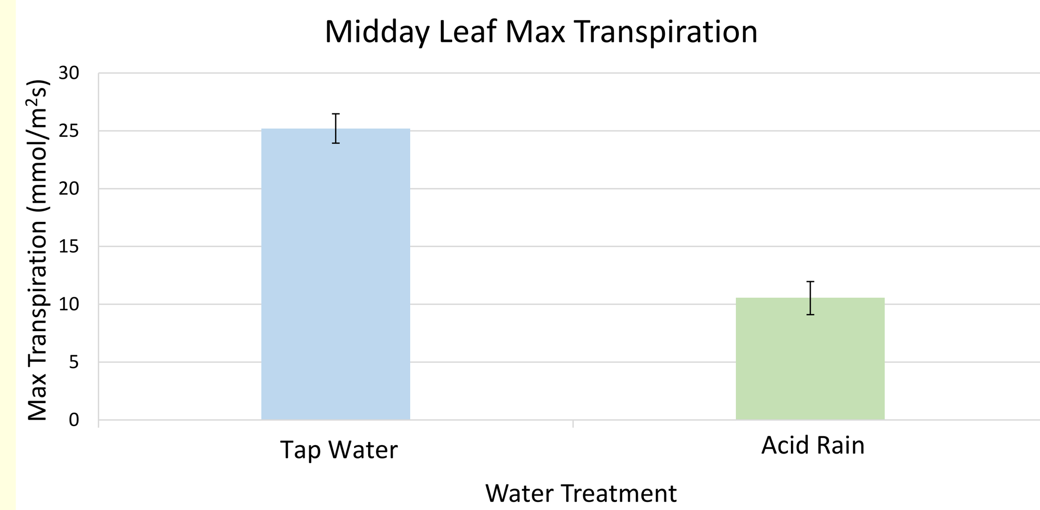

Figure 8. Midday leaf max transpiration of control (n=6) vs treatment (n=6) plants. Data was significant (t_{10}=7.68, P<0.05). Standard error bars shown.

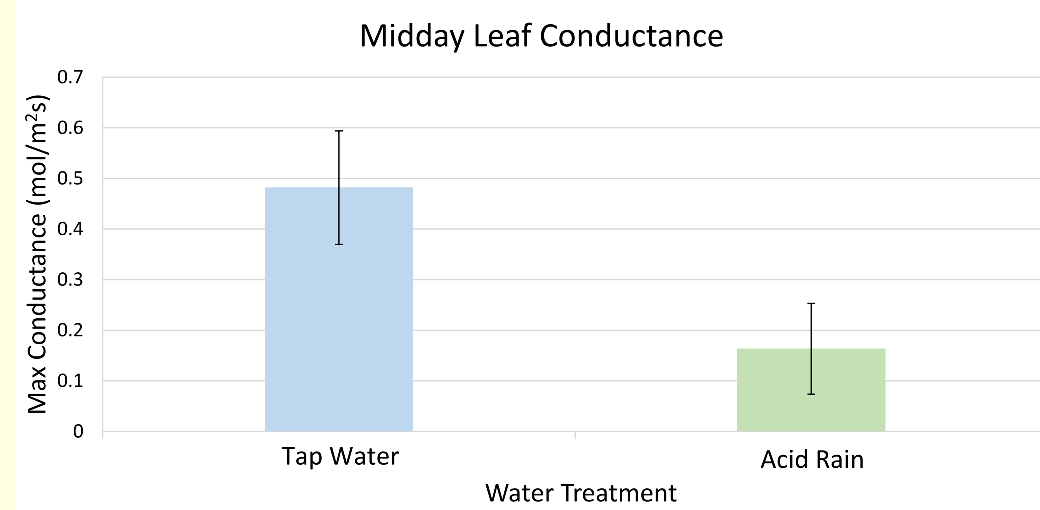

Figure 9. Midday leaf max conductance of control (n=6) vs treatment (n=6) plants. Data was marginally significant (t_{10}=2.22, P=0.051). Standard error bars shown.

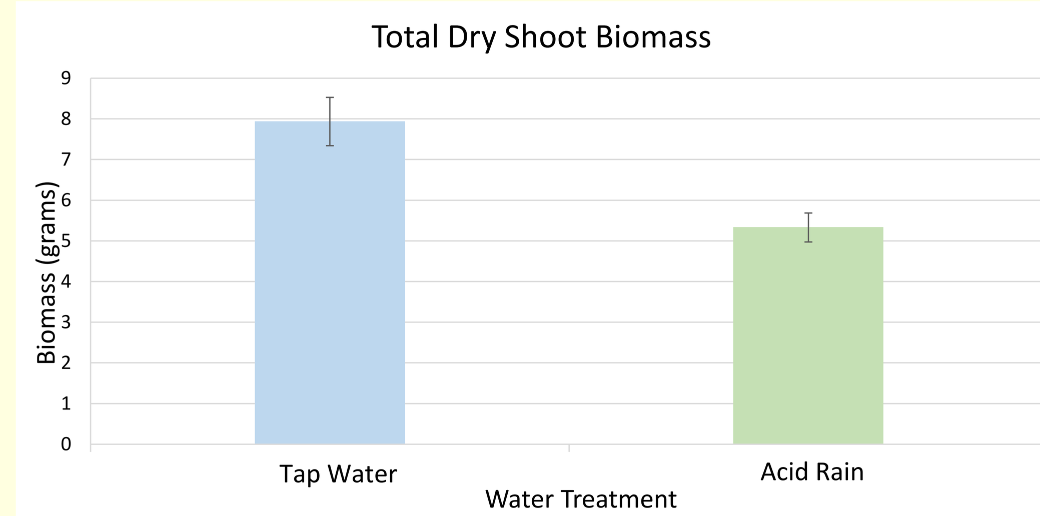

Figure 10. Total shoot biomass of control (n=6) vs treatment (n=6) plants (t_5=3.76, P<0.05). Standard error bars shown.

Results

Weekly Anatomical Measurements
- Overall stem diameter growth was not significant (Fig. 1)
- Overall height growth was significant (Fig. 2)
- Overall leaf count difference was not significant (Fig. 3)

Specific Leaf Area
- SLA was not significant (Fig. 4)

Water Potential
- Water potential was not significant (Fig. 5)

Stomatal Density
- Stomatal density was not significant (Fig. 6)

Light Reaction Measurements
- Photosynthesis was significant (Fig. 7)
- Transpiration was significant (Fig. 8)
- Conductance was marginally significant (Fig. 9)

Shoot Biomass
- Overall shoot biomass was significant (Fig. 10)

Other
- Some leaves exposed to acid rain showed topical damage over time on the adaxial side

Conclusions

The results support the hypothesis which stated there will be significant differences in height, photosynthesis, transpiration, and overall biomass between the sunflower plants exposed to acid rain conditions and plants not exposed to acid rain conditions.
- These significant results also support the findings of the study by Sutradhar et al. in 2014
- Perhaps the low pH made it more difficult for the plant to maintain a hydrogen ion gradient for its metabolic processes, guard cell function, and aquaporin function in its leaves
- Midday conductance was marginally significant and might've been truly significant had another leaf been chosen for testing or the plant allowed to grow a little longer
- Photosynthetic and transpiration rates were significant, so it makes sense that conductance would follow that trend as well since it is measured based on those other rates

The results do not support the hypothesis which stated there will be significant differences in the amount of true leaves, stem diameter, specific leaf area, stomatal density, and water potential between plants exposed to acid rain conditions and plants that were not.
- Over time these measurements would've become significant since the plant would first be impacted physiologically by the acid rain stress and then growth impacts would become more apparent
- Specific leaf area seems to trend toward less significant on the graph but was closer to being significant in week 9 than in week 6, so they were being impacted by the acid rain
- Specific leaf area, water potential, and stomatal density would have become significant had the study gone on for longer since the acid rain was effecting these aspects of the plants

Climate change and increased acid rain could impact the growth and survivability of crops like sunflowers. In the future, this could have an economic impact on the industries that rely on crop products.

References

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