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

Hannah Moshinsky
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INTRODUCTION & HYPOTHESES
Helianthus annuus, the common sunflower, is a rotational crop with winter wheat since it is heat and drought tolerant, but soil acidity has a negative effect on the plant’s height, vigor, and survivability (Sutradhar et al., 2014). When grown in soils with a pH range between 4.7-5.5, sunflower yield was reduced to equal 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 (Lai 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 Organic Soil, Quikrete Premium Play Sand, Turface Athletics All Sport)
- Fertilizer was added week 6 (Osocomo Plus Premium Formula, Marysville, OH)
- Plants were in random block arrangements using Excel
- Greenhouse conditions were 26.7°C and 20% RH with sodium halide lights on 7am-6pm
- Plants watered to capacity M/W/F and as necessary week 8
- Plants were put in random block arrangements using Excel
- 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 cm²
- 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 stored 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-knot 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 number of leaves were counted midday during 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 70°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

RESULTS

Weekly Anatomical Measurements
- Overall stem diameter growth was not significant (Fig. 1)
- Overall height growth was significant (Fig. 2)
- Stomatal area difference was not significant (Fig. 2)

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

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. 10)
- Shoot Biomass
- Overall shoot biomass was marginally 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

REFERENCES

Other