The effects of simulated acid rain on the growth of *Raphanus sativus* plants

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Abstract

Raphanus sativus plants were watered with various concentrations of simulated acid rain (SAR) made from a solution of sulfuric and nitric acid. The intent was to investigate the degree to which acidity affects plant growth, specifically in biomass, stem length, and leaf length, and to determine which acidity promoted the most plant growth. The results demonstrated that increased acidity in precipitation is detrimental to the growth of *Raphanus sativus* plants. The plants that experienced an environment with acid concentrations greater than 10^{-6} mol/L of H_3O^+ ions (a pH of less than 6.0), were smaller in all areas of measurement.

Keywords

Raphanus sativus, radish, simulated acid rain, sulfuric acid, nitric acid

Introduction

Acid rain is considered one of the most substantial long term environmental threats to many regions of the industrialized world. Acid rain is generically used to describe the phenomena in which air particulates oxidatively react with water (redox reactions) in the atmosphere producing acids and therefore decreasing the pH of subsequent precipitation below that of 5.6. (Baird & Cann, 2005; Ferenbaugh, 1976). A pH of 5.6 is considered to be a baseline value for normal healthy atmospheric water due to natural carbonic acid resulting from carbon dioxide in the atmosphere (Baird & Cann, 2005; Ferenbaugh, 1976). The compounds mostly responsible for the acidification of atmospheric waters are sulfur dioxide (SO_2) and nitrogen oxides (NOx), which react with atmospheric waters to produce sulfuric acid and nitric acid respectively (Baird and Cann 2005, Manahan 1994, Spiro and Stigliani 1996).

When analyzing plant growth affected by acid rain, it must be considered that acid rain is a mixture of acids, mostly consisting of the aforementioned nitric and sulfuric acids. In 2014, Lv et al. tested various ratios of the sulfuric and nitric acids in different solutions of simulated acid rain on the growth of subtropical forests in China. Their results showed that an increased concentration of nitric acid in solution produced greater inhibitory effects on subtropical plants. Currently, the concentration of sulfuric acid in acid rain is greater than that of nitric acid; however, it is predicted that this ratio will gradually change so that nitric acid will exist in a greater concentration relative to sulfuric acid. (Lv et al., 2014; Xu and Ji, 2001) The phenomenon of atmospheric acidification is known to cause a variety of repercussions on ecosystems, such as reductions in forest and especially crop growth, and the excess dissolution (process of dissolving ions) of various cations and nutrients from reactions with exposed buildings and heavy metals. Aluminum, a phytotoxic (toxic to plants) heavy metal which is known to detrimentally inhibit plant growth, is especially a concern. (Manahan 1994). Furthermore, acid rain can cause chlorotic and necrotic spots on leaves at certain concentrations. It is also known to flush off nutritive elements, which is responsible for the reduction in enzymatic activity and water balance. (Tong and Zhang, 2014; Chen et al., 2013)

The pH of acid rain can vary from different regions, and has a significant effect on the growth of plants. Acid rain with a pH less than that of 3.0 is known to be very detrimental to vegetation. (Wootton-Beard et al., 2011).

It should be considered that different plants have different sensitivities to acid rain. Tong and Zhang (2011) conducted an experiment which tested a controlled solution of simulated acid rain on five different plant species. Three different trends were found in their results where the simulated acid rain produced either an inhibitory, promotional, or hormetic (beneficial at low concentrations, detrimental at high) effect on their plant subject's growth. Their results were consistent with those of previous scientific studies (Huang et al., 2005; Cappellato, 1993; Lee et al., 2004).

Considering that plants can have different reactions, it should be realized that the reaction to acid rain exhibited by a certain species of plant cannot be assumed to be the same for any other species. Thus, each species of plant must be individually tested and indexed. Experiments conducted by Kostka-Rick and Manning (1993) determined *Raphanus sativus* (radish) plants to be an ideal model for studies on plant response to environmental stresses such as temperature, water levels, light supply and atmospheric carbon dioxide. The study did not, however, examine the sensitivity of R. sativus plants to acid rain. These experiments will test the response of R. sativus plants to varying controlled concentrations of acid rain. The results from these experiments will be used to determine the type of response R. sativus plants exhibit when exposed to different levels of acid rain, and will be used to determine the feasibility of R. sativus plants as models for acid-induced stress on crop plants. The results will be compared with those of similar experiments conducted on *R. sativus* plants.

Methods

Growing conditions

Acid responses of 25 radish plants in groups of 5 exposed to simulated acid rain (sulfuric acid and nitric acid) at various pH levels were studied. All plants were grown in individual identical pots. Each pot was filled to the same level with pH neutral, nutrient enriched "Miracle Gro" potting soil. Every seed was planted approximately 1.5 cm deep in soil. The plants were kept under a light fixture which was designed to distribute 14 hours of light each day.

Experimental design

The artificial rain was distributed through a graduated cylinder at a rate of 10 mL/day on weekdays with every Monday having double the amount of simulated rain to cover the water not given over the weekend. This continued for the first two weeks until the third week when watering the plants was done regularly every day. The solution was slowly poured from the top of the plant onto the leaves to simulate the natural delivery of rain. All plants were subject to the same outside conditions and kept together to ensure environmental differences beyond the pH of the water were minimal. Each plant was exposed to 14 hours of light per day.

Preparation of simulated acid rain solutions

The simulated acid rain was composed of nitric acid (HNO_3) and sulfuric acid (H_2SO_4) at the manipulated pH level (3.0, 4.0, 5.0, or 6.0) and was mixed in a 30:70 ratio respectively (after Kelly and Strickland 1987). Carbonic acid was omitted due to its nature of reacting with the air and changing the acid concentration over time. The radishes marked for pH 7.0 were watered with distilled water, and the strong stock acid solutions were diluted to the appropriate pH values using distilled water. (Eq. 3) The concentration of hydronium ions (H_3O^+) determines the acidity in the solutions. For every molecule of HNO_3 in water, one molecule of H_3O^+ is produced. (Eq. 2) H_2SO_4 is diprotic, so hydronium production is slightly greater than a 1:1 relation, however this was considered negligible due to the accuracy of measuring tools. As pH increases by 1, the concentration of hydronium decreases by a factor of 10-1, so pH 3.0 is 10 times more acidic than pH 4.0. (Eq. 1) Therefore, 100 mL of a pH 3.0 solution was diluted with distilled water until there was 1000 mL of diluted pH 4.0 solution was produced. This process was repeated with until all desired solutions were produced. Various sizes of graduated cylinders (10 mL - 1000 mL) were needed in the process of preparing the acid solutions.

Recording Observations

On the final day of the lab, the radishes were carefully uprooted for various measurements. The biomass, stem length, and leaf length of each plant were recorded immediately after uprooting and being thoroughly washed with a spray bottle to remove excess dirt. Roots were cut off at the base of the stem prior to measurement as an effort to reduce error in measurement due to roots remaining in the soil upon uprooting. (Figures 13 and 14).

Solving for required $[H_3O^+]$ to achieve desired pH: (1)

$$[H_3O^+_{(aq)}] = 10^{-pH}$$

Finding $[HNO_{3(aq)}/H_2SO_{4(aq)}]$ required to achieve 0.001 mol/L $H_3O^+_{(aq)}$ in solution: (2)

$$1\ HNO_{3(aq)} + 1\ H_2O_{(aq)} \rightarrow 1\ NO_3^-{}_{(aq)} + 1\ H_3O^+_{(aq)}$$
$$[HNO_{3(aq)}] = [H_3O^+_{(aq)}] \times (1\ \text{mol}\ HNO_{3(aq)} \div 1\ \text{mol}\ H_3O^+_{(aq)})$$

Calculating Dilution: (3)

$$C_1V_1 = C_2V_2$$

Results and Discussion

These experiments tested the effects which different concentrations of simulated acid rain (SAR) had on the biomass, stem length, and leaf lengths of *Raphanus sativus* plants over a growth period of 39 days. In Table 1, the averaged biomass above roots, stem lengths, and leaf lengths of *Raphanus sativus* plants tested with increasing concentrations of simulated acid rain can be found. The data show that simulated acid rain affected the growth in an increasingly detrimental manner across all areas of research as the pH decreased.

The average masses ranged from 0.83 g in the pH 3.0 test to 1.94 g in the pH 7.0 test, the average stem lengths ranged from 5.5 cm in the pH 3.0 test to 6.5 cm in the pH 7.0 test, and the leaf lengths ranged from 7.7 cm in the pH 3.0 test to 10.3 cm in the pH 7.0 test.

Table 1: The average mass, stem length, and leaf length of *R. sativus* plants corresponding to each tested concentration of simulated acid rain.

Acidity (pH)	Mass Above Roots (g)	Stem Length (cm)	Leaf Length From Stem to Tip (cm)
7.0	1.94	6.5	10.3
6.0	1.81	6.3	8.7
5.0	1.21	5.0	8.6
4.0	1.32	5.1	9.6
3.0	0.83	5.5	7.7

Biomass

The plants watered with the two least acidic solutions (pH 7.0 and pH 6.0) had much more mass than those watered with more acidic solutions. The average biomass of the *Raphanus sativus* watered with pH 7 (1.94 g) is 1.11 g greater than that of pH 3 (0.83 g). The difference between the masses of pH 7.0 or 6.0 and the other acid concentrations pass the standard margin of error. (Figure 1)

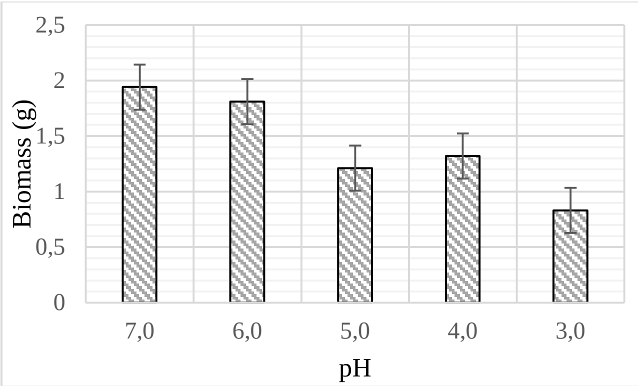

Figure 1: The relationship between the pH *Raphanus sativus* were watered with and their biomass

Stem Length

The stem lengths of the plants decreased significantly for any solution with a pH < 6.0. The greatest stem average length was watered with pH 7.0 with a length of 6.5 cm, and the shortest was pH 5.0 with an average length of 5.0 cm. The range that acid precipitation affected the plants was 1.5 cm. (Figure 2)

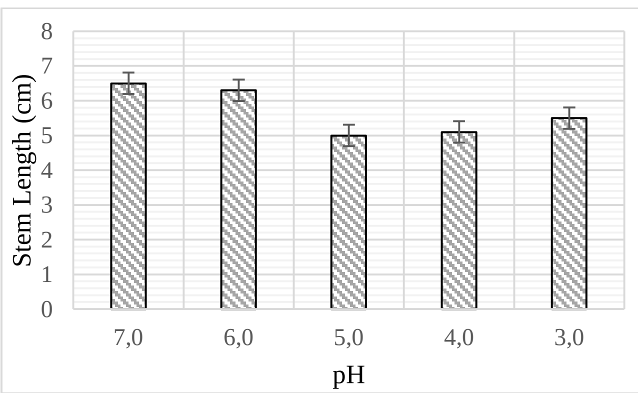

Figure 2: The relationship between the pH *Raphanus sativus* were watered with and their stem length

Leaf Length

The results for the average leaf length the plants at each pH had were mixed. The least acidic concentration: pH 7.0, resulted in the greatest leaf length, followed by pH 4.0. The aforementioned acid concentrations have overlapping margins of error. However, pH 7.0 and 4.0 can be completely distinguished from the leaf lengths of pH 3.0, 5.0, and 6.0. The disparity between the greatest average (pH 7.0) and the lowest average (pH 3.0) was 2.6 cm. (Figure 3)

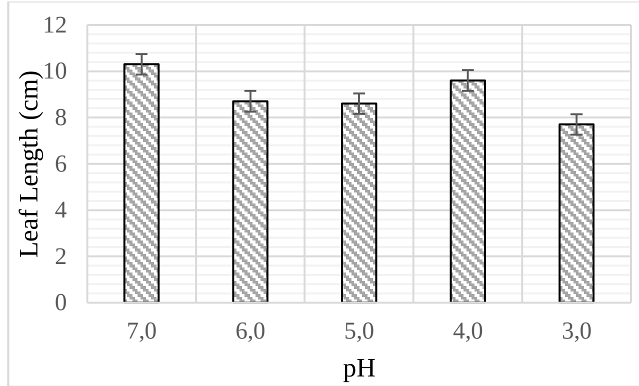

Figure 3: The relationship between the pH *Raphanus sativus* were watered with and their leaf length

Discussion

An anomaly was found in the pH 4.0 test which does not suit the trend suggested by the rest of the data. The plants in all replicates yielded results which were greater than the pH 5.0 plants exclusively, suggesting that the pH 4.0 test affected the plants in a manner which was less detrimental than that of the pH 5.0.This anomaly was not found to be consistent with the results of any publications researching the effects of acid rain on *Raphanus sativus*, which suggests that the pH 4.0 test was susceptible to scientific error. It should be considered, however, that despite the minor anomaly in the pH 4.0 test, which is within the bounds of the standard error, the trend found in these results are otherwise consistent with those of many publications, namely those of Rehman et al. (2009), Cohen et al. (1981) and Kostka-Rick and Manning (1991).

The method of the experiment ensured each of the plants were under the same conditions aside from being watered with different acid concentrations. Errors were minimized by keeping the plants together so they would all undergo the same conditions, despite variation. The specimens were kept under a full spectrum fluorescent light timed for 13 hours a day, however, they were not in an environment with constant, controlled heat. Despite this, due to the fact that the plants were kept together, results were consistent with other experiments involving acid rain and plants.

The experiments provided data that can be used to examine the implications of the continued release of sulfate, nitrate, and nitrites into the atmosphere. The results of this action will cause harmful effects to the plant ecosystem as demonstrated by the observations. The largest impact of this would be to the agricultural industry as the health of the crop yield is a crucial aspect to their success. As acidity of the rain increases, crop yield may become poorer, which could have a significant impact on the economy, especially those of developing nations. The observations from this experiment foreshadow the future impact of current industrial practices which can be avoided. The results found provide many opportunities for real world application as the whole experiment revolves around the real life significance of rain acidification.

Conclusion

This experiment outlined the effect of acid rain on *Raphanus sativus* plants. The results show that acid precipitation inhibits growth in the plant, and in most cases result in reduced plant growth with more acidic rain. This experiment explored the way acid precipitation impacted the growth of *Raphanus sativus*, however, it is unclear whether this effect will be consistent with those of other plant species. In order to determine the extent to which acid rain affects ecosystems, studies must be done with various plant worldwide. The acid levels used were very broad, and smaller increments could help find the ideal pH level for plants, if it is not 7.0. Further research into this topic will emphasize the significance of this issue and could bring awareness to acid rain and support work made towards suppressing it.

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Figure 4: Plants on day 1

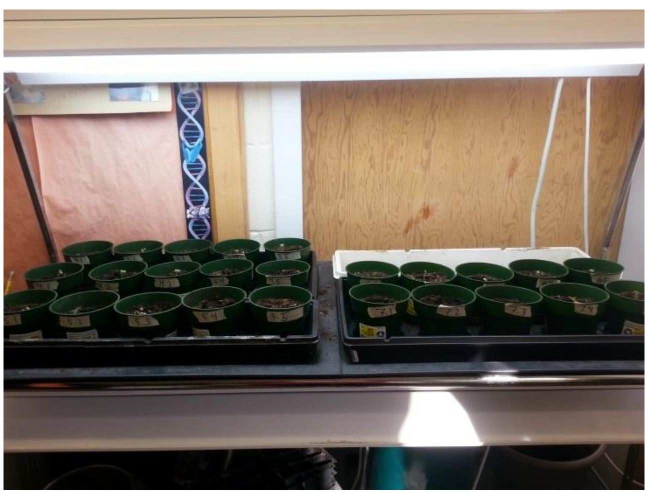
Figure 5: Plants on day 5

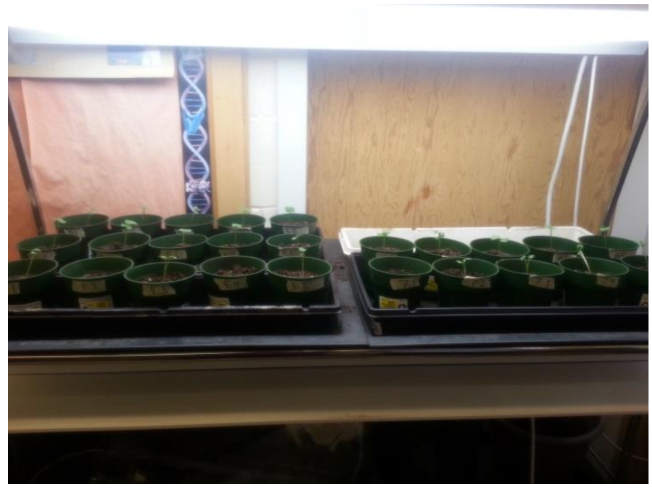
Figure 6: Plants on day 8

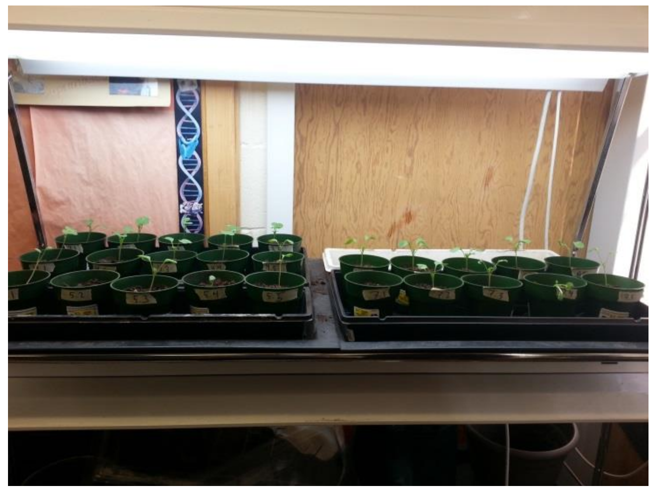
Figure 7: Plants on day 12

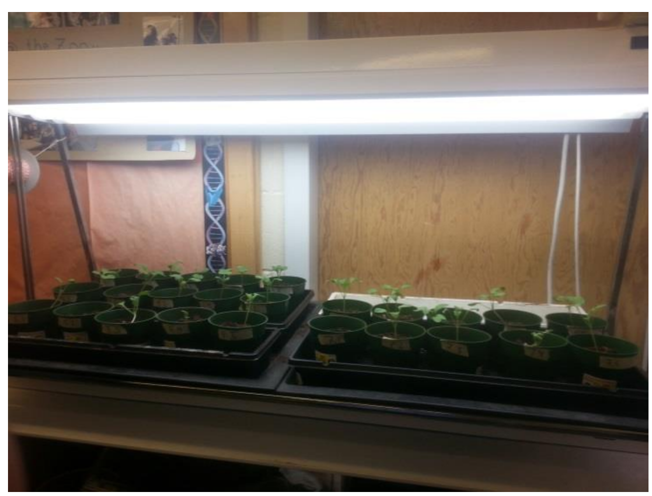
Figure 8: Plants on day 15

Figure 9: Plants with lab equipment and acids on day 18

Figure 10: Plants on day 26

Figure 11: Plants on day 28

Figure 12: Plants on day 42

Figure 13: Cutting off the roots on the final day of growth

Figure 14: Where the root cut was made to do the final measurements on day 44