The Effect of Various pH Levels on the Root Growth of *Arabidopsis thaliana* Seedlings

Tina T. Kang, Nadia A. Kari, Brittany H. Maxwell, Janelle D. Soquila

ABSTRACT

The detrimental effects of acid rain on plant growth are an environmental concern. We examined whether the pH of water affects the amount of root growth in *Arabidopsis thaliana* seedlings. We planted 192 *A. thaliana* seeds in Petri dishes and watered them at treatment levels of pH of 4.00, 5.50, 8.50, and a control at pH 7.13. We measured the root length, and on Day 14 recorded the mean root lengths at pH 4.00, 5.50, 7.13, and 8.50 to be 4.43 mm±0.39 mm, 7.07±0.33 mm, 9.82±0.39 mm, and 5.67±0.84 mm, respectively. Seedlings at pH 7.13 exhibited the greatest amount of growth and appeared healthier and more turgid on Day 14 than seedlings at all other treatments. Our results show a trend of decreasing root growth and health as pH diverges from a neutral pH of 7.13. Acidic water disrupts the Ca^{2+} processes, and basic water disassembles microfilaments, both of which inhibit cell growth (Hara *et al.* 2000, Guo *et al.* 2000). Results on Day 14 were statistically significant and indicate pH 7.13 as an optimal pH for seedling root growth. Thus, water at acidic and basic pH is detrimental to *A. thaliana* root growth.

INTRODUCTION

Arabidopsis thaliana is a multicellular, eukaryotic flowering plant. It plays a key role in plant research due to its short life cycle and small genome (Ridge 2002). This being said, through research conducted on *A. thaliana*, the scientific community is able to better understand the development and growth of angiosperms.

The burning of fossil fuels releases chemicals into the environment that react with atmospheric gases, forming nitric acid and sulfuric acid (Lane 2003). As precipitation occurs, these gases are drawn out of the atmosphere as acid rain, later reacting with chemicals in the soil. As a consequence, the chemical composition of the soil is altered and creates a harmful environment for plants (Frink and Krug 1983).

Soil pH affects plant growth because the solubility of minerals and nutrients differ depending on pH (Berg 2008). This is important as plants only absorb minerals in their soluble forms. For example, at acidic pH aluminum and manganese in soil water are more soluble and are sometimes absorbed by roots in toxic amounts (Berg 2008). Similarly, at basic pH, mineral salts essential for plant growth, such as calcium phosphate, become less soluble and therefore, are less available to plants and inhibit growth (Berg 2008). Overall, we see that environmental change affects soil pH, and in turn, soil pH affects plant growth.

With this in mind, pH plays an important role in the growth of plants in nature. The optimum soil pH for most plant growth is 6.5 to 7.5 because the essential elements needed by plants are most soluble in that range (Berg 2008). Soil is composed of inorganic minerals, organic matter, air, and, most notably, water (Berg 2008).

Furthermore, water is an essential requirement for germination and no seed germinates unless it has absorbed water (Fenner and Thompson 2005). Seeds uptake water during germination in three phases: (1) imbibition, in which the seed coat is penetrated and water is absorbed; (2) activation, in which developmental processes occur but little further water is absorbed; and (3) growth, in which the radicle, or embryonic root, elongates and breaks the seed coat (Berg 2008, Fenner and Thompson 2005, Mayer and Poljakoff-Mayber 1989).

Proteins in the plant are chiefly responsible for the imbibition of water (Mayer and Poljakoff-Mayber 1989). However, the subsequent growth stages are more dependent on various external factors, such as water (Mayer and Poljakoff-Mayber 1989). Water is needed for most life processes to take place as it transports nutrients within the plant. Water is first absorbed from the environment by the epidermal root hairs, then moves

across the root through living cells, and eventually enters the water-conducting cells of the xylem (Glimn-Lacy and Kaufman 2006, Lack and Evans 2005). Successful water transport results in healthy, turgid plant cells; whereas, poor water transport leads to plasmolysis and wilting (Glimn-Lacy and Kaufman 2006).

With this brief overview of environmental change, soil pH, germination, and nutrient transport, it is obvious that water plays a key role in the early stages of plant life and growth. As such, we examined how the pH of water affects germination and growth of *A. thaliana* seedlings. Our null hypothesis states a decrease in the pH of water will have no effect or increase the length of the root of *A. thaliana* seedlings on Day 14. Whereas, our alternative hypothesis states a decrease in the pH of water will decrease the length of the root of *A. thaliana* seedlings on Day 14.

METHODS

We gathered 25 Petri dishes, 4 bottles of solutions with pH of 4.00, 5.50, 7.13, and 8.50 (as seen in Figure 1), 25 sheets of 70 mm diameter filter papers, 4 trays, and 192 *A. thaliana* seeds. The lab technician prepared the solutions using HCl and tap water for pH 4.00 and 5.50, only tap water for pH 7.13, and NaOH and tap water for pH 8.50. Each different pH represents a different treatment, with pH 7.13 being the control.

Figure 1. The 4 bottles contain the 4 treatment levels of pH 4.00, 5.50, 7.13, and 8.50.

We placed 1 piece of filter paper in each Petri dish, assigned 6 Petri dishes per treatment, and labelled the bottom of each dish with the treatment pH. We moistened the filter papers with the corresponding treatment, inverting each bottle several times to ensure the solutions were well mixed. We added 2.0mL of the treatment using a micropipette to each replicate before placing 8 seeds on each filter paper, to ensure that the seeds remained on the paper. We assured the filter paper was fully saturated by adding the treatment solution directly on the paper until a thin film of liquid formed at the bottom of the Petri dish. After watering each replicate, we covered the Petri dishes and placed them on 4 trays labelled with the different treatments and brought the trays into an incubation chamber set at 17°C, with 14 hours of light and 10 hours of dark (as seen in Figure 2).

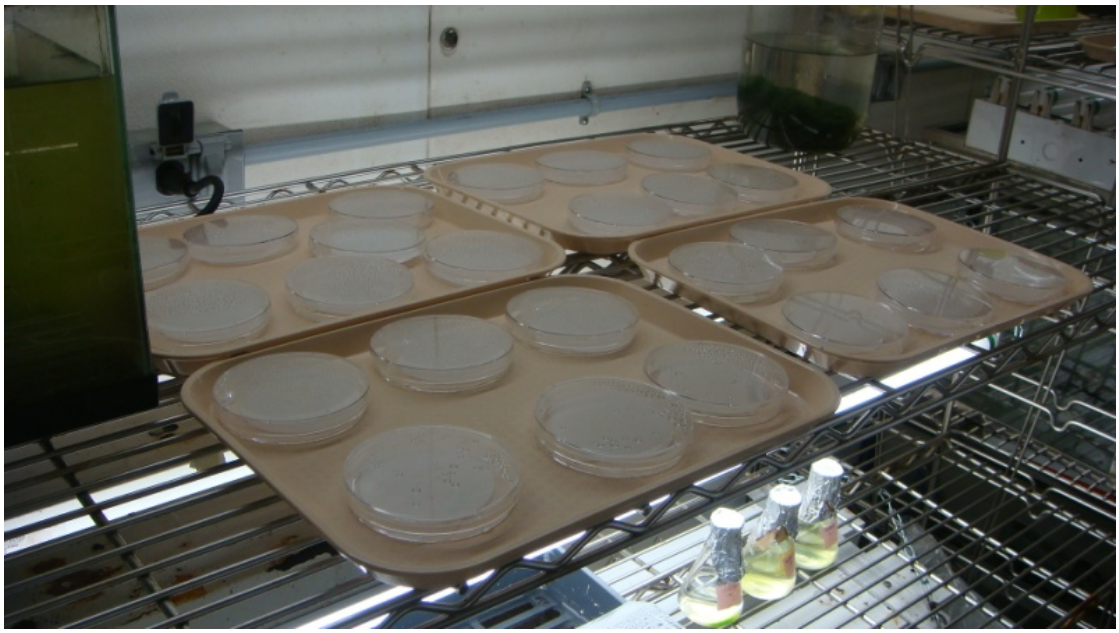

Figure 2. All the replicates of the 4 treatments in the incubation chamber.

We observed the replicates under a light microscope 2 to 3 times a week. We added the treatment solutions when the filter papers were unsaturated (refer to Table 1: Watering Schedule). We used the DinoXcope to take pictures of 2 random seedlings from replicates 1 and 4 of all treatments.

Because the plants were tiny, we estimated the lengths of the roots and shoots by observing the amount of field of view covered, and multiplied this by the field diameter. For the plants with intact seed coats, we measured the shoots from the top of the stems to the center of the seeds, and the roots from the tips of the root to the center of the seeds. For those plants without their seed coats, we measured the lengths from the division between upper growth and lower growth, which we distinguished by a colour change from green to clear. We also recorded the general health of each treatment, as well as whether or not the filter papers were saturated.

The root length was not measured from Day 0 to Day 3 because the seeds had not yet germinated, and from Day 4 to Day 5 because the lab was closed. Thus, we did not record any data for Day 0 to Day 5. In addition, we did not measure the root length on Day 9 due to group member time constraints, and from Day 11 to Day 12 due to lab closure, again. Hence, we have no data points from Day 9, 11, and 12.

Day	**Amount of Treatment Solution Added (mL)**			
	4.00	5.50	7.13	8.50
0	2.0	2.0	2.0	2.0
3	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	1.0
8	1.0	1.0	1.0	1.0
13	1.5	1.0	1.0	1.5

Table 1. Watering Schedule

For our data analysis, in order to see the differences in the root lengths between the treatments, we plotted the mean root length of each treatment from Day 0 to Day 14, including the 95% confidence intervals of the means. We also created a bar graph of the root lengths of each treatment on Day 14, along with the 95% confidence intervals.

RESULTS

Over a period of fourteen days, seedling root growth was measured during various observation sessions. At these times, the following values of mean root length were plotted for each treatment level and are shown below with 95% Confidence Intervals in Figure 3.

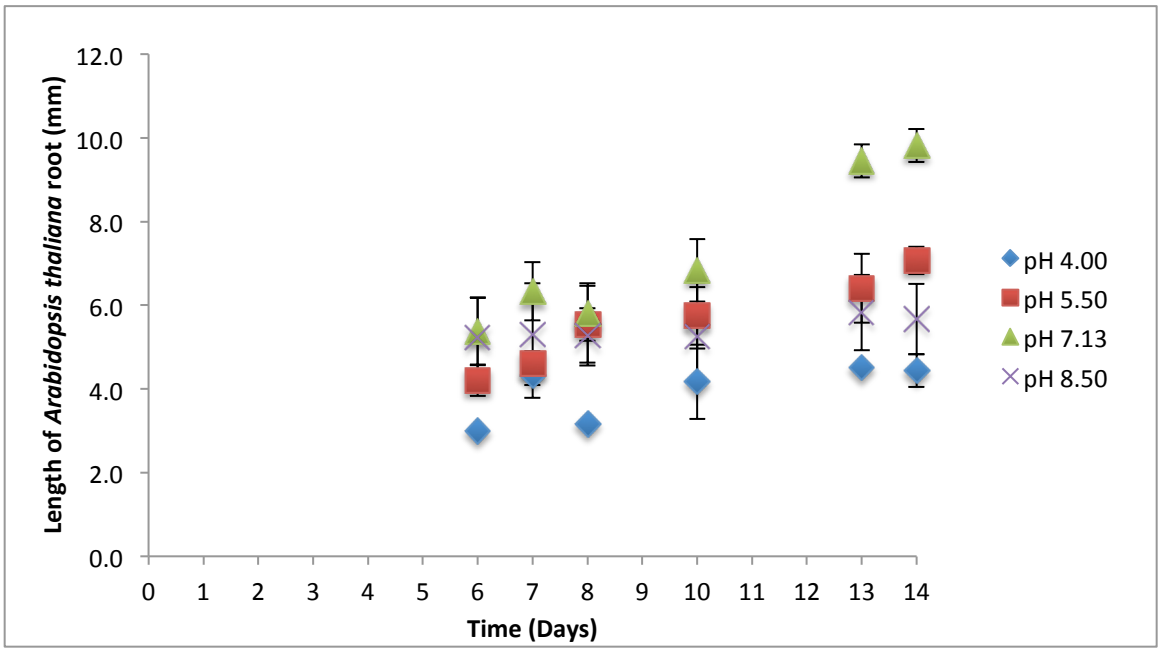

Figure 3. The length of the root of *Arabidopsis thaliana* seedlings (mm) over a period of 14 days at treatment levels of pH 4.00, 5.50, 7.13, 8.50 with 95% Confidence Intervals, n=6.

The seedlings at pH 7.13 underwent the most root growth over the fourteen day period, with a mean root length of 9.82±0.39 mm on Day 14. On the other hand, the seedlings at pH 4.00 underwent the least amount of root growth, with a mean root length of 4.43±0.39 mm on Day 14. In addition, the mean root length of seedlings on Day 14 watered at pH 5.50 was 7.07±0.33 mm and of seedlings watered at pH 8.50 was 5.67±0.84 mm.

To obtain the final mean root length and 95% Confidence Interval the following calculations were done:

1. Root Length (mm) = Fraction of Field of View * Field Diameter (mm)

   = (3/4)(9.5 mm) = 7.13mm

2. Mean Root Length (Calculated on Excel) = $\bar{x}$ = (Σx_i) / n

   = (6.33 + 7.13 + 7.13 + 7.13 + 7.60 + 7.13)/ 6 = 7.07 mm

3. Standard Deviation (Calculated on Excel) = s = sqrt[$\Sigma(x_i - \bar{x})^2$ / (n-1)]

   = 0.41

4. 95% Confidence Intervals (Calculated on Excel) = $\bar{x} \pm z * s/\text{sqrt}(n)$

   = 7.07 ± 1.96 * 0.41/sqrt(6)

   = 7.07 ± 0.33 mm

There are no data points for Day 0 to Day 5 in Figure 3 as no data was collected on these days; Likewise, on Days 9, 11, and 12.

In addition, Figure 3 shows seedlings at pH 7.13 experience a general increase in root growth from Day 6 to Day 14. Seedlings at pH 5.50 also experience a continual increase in root growth from Day 6 to Day 14, but the amount of growth is not as large as seedlings at pH 7.13. Seedlings at pH 4.00 and pH 8.50 experience little root growth after Day 6, resulting in a plateau in mean root growth, shown in Figure 3. Seedlings at pH 4.00 and pH 8.50 also experienced a slight decrease in root growth from Day 13 to Day 14.

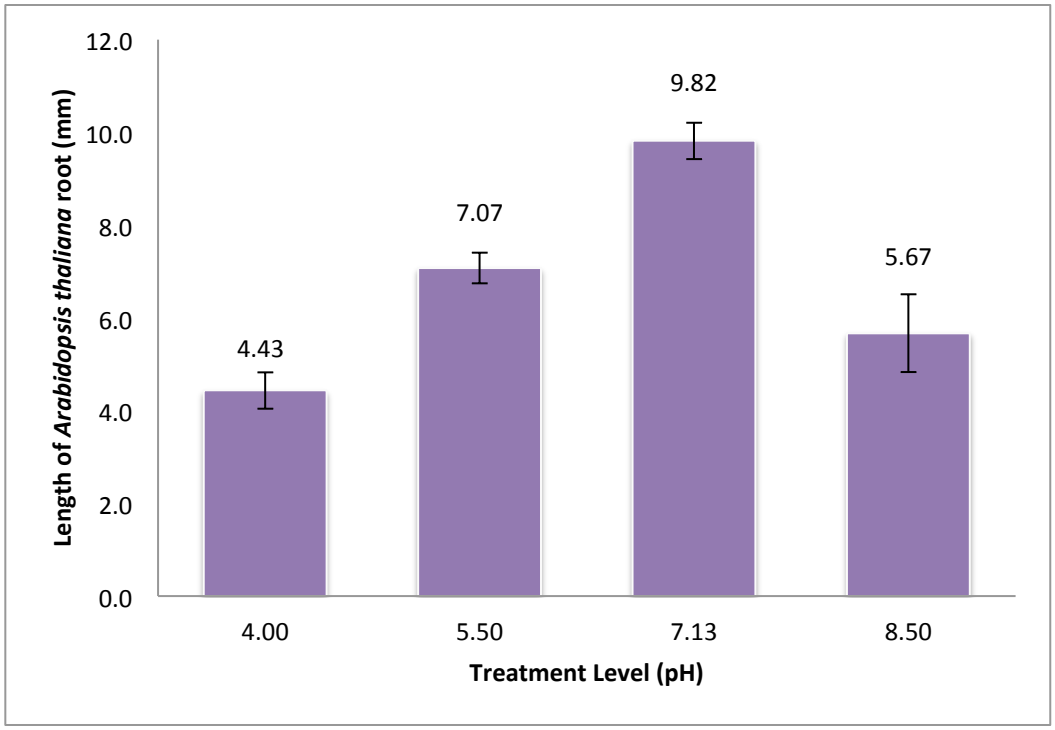

Figure 4. The mean length of the root of *Arabidopsis thaliana* seedlings (mm) on Day 14 at treatment levels of pH 4.00, 5.50, 7.13, 8.50 with 95% Confidence Intervals, n=6.

In order to better compare the mean root length of *Arabidopsis thaliana* on Day 14 at the different treatment levels, another graph was produced to reflect the final mean root length. Figure 4 shows a general trend of mean root length on Day 14 decreasing as pH moves away from pH 7.13.

Moreover, on Day 14 seedlings at pH 7.13 had a mean root length of 9.82±0.39 mm and a standard deviation of 0.49 mm. The mean root length and standard deviation of seedlings on Day 14 watered at pH 5.50 was 7.07±0.33 mm and 0.49 mm, at pH 8.50 was 5.67±0.84 mm and 0.41 mm, and at pH 4.00 was 4.43±0.39 mm and 1.05 mm. The standard deviation demonstrates the amount the data deviates from the mean and is observed within this data to be quite low; As a result, this indicates that the data does not

deviate much from the mean. This being said, Figure 4 shows there is a significant difference at all treatment levels as 95% Confidence Intervals do not overlap.

On Day 14, the following qualitative data was also taken:

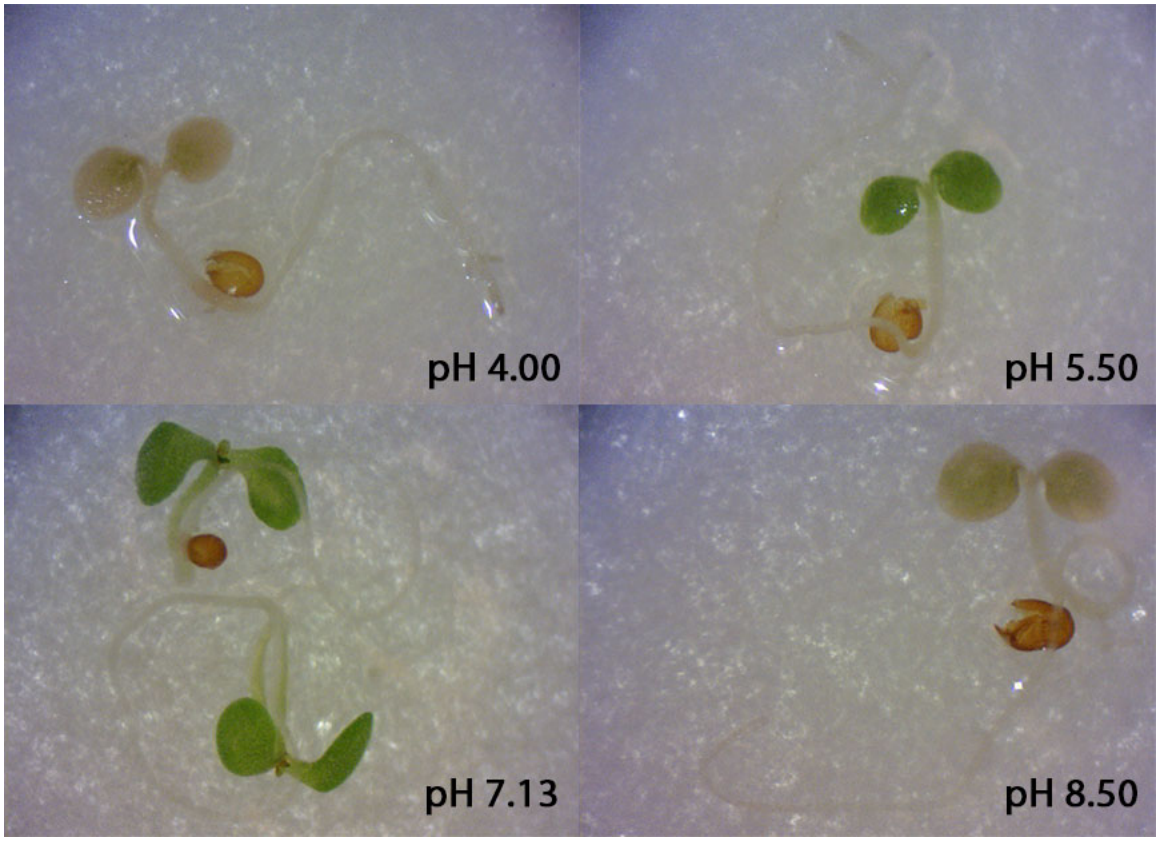

Figure 5. A visual depiction of *Arabidopsis thaliana* seedlings on Day 14 at treatment levels of pH 4.00, 5.50, 7.13, 8.50.

Figure 5 illustrates the overall appearance and health of seedlings observed on Day 14. The rosette leaves of seedlings at pH 4.00 and pH 8.50 had turned a yellow-brown colour, and brown patches were observed on both the leaves and the roots. Seedlings at pH 4.00 and pH 8.50 also appeared to be dead, as rosette leaves were wilted and roots were not as thick. The rosette leaves of seedlings at pH 5.50 and pH 7.13 were generally bright green and turgid, with thick, translucent roots; the rosette leaves of pH 7.13 appeared to be larger than those at pH 5.50, and more replicates at pH 7.13 exhibited these healthy plant

features, than those at pH 5.50. Furthermore, many seedlings at pH 7.13 were observed to have two more rosette leaves budding – as shown in Figure 5 – resulting in a total of four observed rosette leaves.

DISCUSSION

After thoroughly observing *Arabidopsis thaliana* germination over a fourteen-day period, the data indicate that on the fourteenth day the root length is affected by the pH used to water the plant. Figure 4 shows a significant difference between the watering treatment levels. However, the alternate hypothesis that a decrease in pH will cause a decrease in the root length cannot be supported. Day 14 results show that a decrease in the pH from the control treatment leads to a decrease in the root length; however, this is not the case when we decreased the pH from 8.50 to 7.13. As seen in Figure 4, there is an increase in the root length from a pH of 8.50 to 7.13. Rather, a trend of decreasing root length occurs as pH moves further away from the optimal growth pH of 7.13, which is within the optimal plant pH range of 6.50 to 7.50 (Berg 2008). Therefore, we fail to reject the null hypothesis.

Figure 3 illustrates a trend that the control treatment (pH 7.13) is optimal for the growth of the *A. thaliana* seeds. This can be determined when compared to the other treatments, which underwent less growth. According to Figure 3**,** as time progressed, the roots of seeds in the pH 4.00 treatment displayed a trend of plateauing growth. The root growth is inhibited when low pH disrupts calcium processes (Hara *et al*. 2000). *A. thaliana* requires calcium for key biological functions, such as cell division and growth (Bibikova *et al*. 1997). For normal growth, plants require calcium fluxes to travel through Ca^{2+} channels in the cellular plasma membranes (Rowse *et al*. 1992). Therefore, a deficiency of calcium

outside the plasma membrane, specifically in the apoplast, damages the roots of *A. thaliana* and slows growth (Hara *et al*. 2000). In addition, there are calcium storages within the cells that allow root growth to continue until that source is depleted (Hara *et al*. 2000). As a result, the roots in all the treatments were able to develop before plateauing, as seen in Figure 3. Dawair *et al.* (1995) also found similar results where seedlings that had been saturated in a pH solution of 5.0 and 5.5 grew much better than the seedlings of pH 4.8. Non-growing roots, on the other hand, were not affected by the presence of low pH (Hara *et al*. 2000). We can also see from Figure 4 that a higher pH can also inhibit growth. Microfilaments are crucial in plants because they are involved in general growth, nuclear division, as well as cytokinesis (Guo *et al*. 2010). When high pH is introduced externally to *Arabidopsis thaliana,* the microfilaments begin to depolymerize, which renders them ineffective and leads to less root growth (Guo *et al*. 2010).

In addition to the biological reasoning, there may have been sources of error and variation in the execution of our experiment to produce these results. Such as contamination of the pH stock solutions due to improper sterile techniques and failure to change micropipette tips for the various pH solutions. Additional inaccuracies that could influence our results are the subjective root length measurements and the fluctuation of temperature in the incubation chamber due to the constant opening and closing of the door. Furthermore, different individuals took observations and data measurements at varying times by observing the amount of field of view covered; this produced a potential difficulty in our measurements as length can be interpreted differently. We attempted to reduce this by setting a guideline to define the possible lengths from 1/6 of coverage to 11/10 coverage, and by having multiple group members observe the root length if there

were uncertainties. On Day 13, the watering amount was inconsistent with the previous watering schedule, as seen in Table 1 However, we deemed this insignificant as *Arabidopsis thaliana* roots had already reached their plateaued final lengths.

Variation could have also influenced our results. Sources of variation include seedlings' resistance to varying pH, the thickness of the seed coats, and the initial health of the seeds. For example, the seed coat is crucial for the seedling's health, and any damages or differences could possibly affect root growth, which would alter our results (Debaujon *et al.* 2000).

Acidic and basic pH levels have detrimental effects on *Arabidopsis thaliana* by affecting its biomass, root growth, shoot height and rosettes (Im *et al.* 2006, Fenner and Thompson 2005). By observing the effects of varying water pH conditions, we are able to gain insight into how environmental change affects plant life.

Overall, although we failed to reject the null hypothesis, there is an obvious trend that the further the pH is from a neutral pH the less the root will grow. As shown in Figure 4, the Day 14 data is statistically significant, meaning that the root growth trend is viable. In other words, although our experiment is not conclusive in what the optimal growth pH of *Arabidopsis thaliana* is, we observed that a neutral pH is best suited to promote healthy growth. In the future, we could modify our experiment by examining the effect of neutral pH treatments on root growth in order to determine if there is an optimal neutral pH at which to grow *Arabidopsis thaliana*.

CONCLUSION

Overall, on Day 14 *Arabidopsis thaliana* seedlings treated at pH 7.13 had the greatest mean root length of 9.82±0.39 mm, and growth decreased as pH moved further away from

the optimal pH. The least amount of growth occurred at an acidic pH of 4.00 and a basic pH of 8.50, with a mean root length of 4.43±0.39 mm and 5.67±0.84 mm, respectively. This indicates that *A. thaliana* growth is negatively impacted by both acidic and basic conditions, but has optimal growth at a neutral pH. Thus, we failed to reject our null hypothesis that stated that a decrease in pH would have no effect or increase the length of the root at Day 14.

ACKNOWLEDGEMENTS

We would like to thank Dr. Carol Pollock for her insight, expertise, and guidance throughout Biology 342. We would also like to thank Diana Rennison and Mindy Chow for their assistance in the laboratory during the experiment. We would like to specifically thank Mindy Chow for preparing our stock solutions. We also acknowledge The University of British Columbia for allowing us use of the Biological Sciences building.

LITERATURE CITED

Berg, L.R. 2008. Introductory Botany: Plants, People, and the Environment. Thomsom Brooks/Cole Corporation, Belmont, CA.

Bibikova, T.N., Gilroy, S., and Zhigilei, A. 1997. Root Hair Growth in *Arabidopsis thaliana* is Directed by Calcium and an Endogenous Polarity [online]. Planta, **203** (4): 495-505. doi: 10.1007/s004250050219.

Dawair, Z., Hara, T., Koyama, H., Toda, T., and Yokota, S. 1995. Effects of Aluminum and pH on Root-Growth and Cell Viability in *Arabidopsis thaliana* Strain Landsberg in Hydroponic Culture. Plant and Cell Physiology, **36** (1): 201-205.

Debaujon, I., Leon-Kloosterziel, K., and Koornneef, M. 2000. Influence of the Testa on Seed Dormancy, Germination, and Longevity in *Arabidopsis*. [online]. Plants Physiology (Bethesda), **122** (2): 403-414. doi: 10.1104/pp.122.2.403.

Fenner, M. and Thompson, K. 2005. The Ecology of Seeds. Cambridge University Press, New York, NY.

Frink, C.R. and Krug, E.C. 1983. Acid rain on acid soil: a new perspective. Science, **221**

(4610): 520-525.

Glimn-Lacy, J. and Kaufman, P.B. 2006. Botany Illustrated: Introduction to Plants, Major Groups, Flowering Plant Families. Springer Science+Business Media, Inc., New York, NY.

Guo, G., Guo, Y., Yang, Z., and Zhou, Y. 2010. Microfilament Dynamics is Required for Root Growth under Alkaline Stress in *Arabidopsis*. Journal of Integrative Plant Biology, **52** (11): 952-958.

Hara, T., Koyama, H., and Toda, T. 2000. Brief Exposure to Low-pH Stress Causes Irreversible Damage to the Growing Root in *Arabidopsis thaliana*: Pectin-Ca Interaction May Play an Important Role in Proton Rhizotoxicity [online]. Journal of Experimental Botany, **52** (355): 361-368.

Im, K., Kim, D., Kim, K., Lee, K., Lee, J., Lee, S., Lee, T., Lee, Y., Park, D., Park, J. Park, J., Yang, J. 2006. *Arabidopsis* leaf necrosis caused by simulated acid rain is related to the salicylic acid signaling pathway. [online]. Plant Physiology and Biochemistry, **44** (1): 38-42.

Lack, A. and Evans, D. 2005. Plant Biology. Taylor & Francis Group, New York, NY.

Lane, C.N. 2003. Acid rain: overview and abstracts. Nova Science Publishers, Inc, Hauppauge, NY.

Mayer, A.M. and Poljakoff-Mayber, A. 1989. The Germination of Seeds. Pergamon Press, Inc., Elmsford, NY.

Mouse-Eared Cress []. A website about Mouse-eared Cress. Available from http://www.illinoiswildflowers.info/weeds/plants/mouse_cress.htm [accessed 22 October 2012].

Ridge, I. 2002. Plants. Oxford University Press, Oxford, NY.

Rowse, P., Schiefelbein, J.W., and Shipley, A. 1992. Calcium Influx at the Tip of Growing Root-Hair Cells of *Arabidopsis thaliana*. Planta, **187** (4): 455-459.