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The Plant and the Pathogen: Elucidating the Relationship between Root-Knot Nematodes, pH Levels, and Arabidopsis thaliana Development

Rachel Garnett
University of Lynchburg, garnetr947@lynchburg.edu

The Plant and the Pathogen: Elucidating the Relationship between Root-Knot Nematodes, pH Levels, and *Arabidopsis thaliana* Development

Rachel Crimora Garnett

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Erin Friedman, PhD

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Price Blair, PhD

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Stephen Burris, MEd

Abstract

Root-knot nematodes (RKNs) within the Meloidogyne genus are considered one of the largest threats to plant health and subsequent crop yield and profit (Forghani and Hajihassani 2020; Bernard et al. 2017). As a pest that presents global consequences, its mitigation through sustainable interventions may confer results for the treatment of similar plant pathogens. A member of the *Brassicaeae* family, *Arabidopsis thaliana*, was chosen for this study to determine how this model plant responds to RKN-standard pH media. pH was varied between control and experimental groups, and the phenotypic variables of primary root length and plant height were observed and analyzed. Wild-type Arabidopsis thaliana plants were grown hydroponically in order to monitor growth medium pH and primary root length. The difference in the average of plant root lengths for one of six trials was found to be statistically significant at 21 DAP following pH modifications, but plant height was not, which suggests this variable may be attributed to RKN presence rather than acidic pH. Concerning plant-pathogen relationships, these results contribute to the available data in order to better understand and potentially mitigate agricultural losses due to RKN infection.

Key Words

Root-Knot Nematodes; Hydroponics; *Arabidopsis thaliana*; Sustainable Agriculture; Phenotypes

Table of Contents

Introduction

Sustainable agriculture is becoming an increasingly salient topic as rising global temperatures threaten the availability of arable land and climate-dependent crops. A current threat to crop yield takes the form of parasitic nematodes, microscopic organisms that are considered the most active and biologically successful around temperatures of 25°C and above (Maleita et al. 2012). Up to 12% of global crop yield and 157 billion dollars were lost to these microscopic populations in 2015 (Singh et al. 2015), and these numbers may increase if rising temperatures coincide with continued use of synthetic treatments. One such obligate parasite, the microscopic root-knot nematode (RKN) of the genus *Meloidogyne,* poses threats to the economic, environmental, and agricultural facets of our planet. Cash crops such as banana, corn, and carrot, the leading crop exports for India, the United States, and China, respectively, are infected by RKNs every year (Lu et al. 2020; Windham and Williams 1994; Nagachandrabose 2018). As a parasitic organism, this pest infests and infects its host plant, contributing to losses in plant vigor and yield. The economic implications of this pest are enormous, as most cultivated crops are susceptible to infection (Torto et al. 2018). Of the seventeen Sustainable Development Goals proposed by the United Nations, three are related to the production and distribution of sustainable agriculture (UN n.d.). The RKN is an active and global threat to the production of sustainable food for all, which worsens as the planet warms. It may be possible to solve a piece of this puzzle by furthering our knowledge of the damage caused by the RKN and determining what solutions can be implemented to improve our crop yields. Increased stress on the planet would only hinder these efforts: solutions oriented towards the RKN may take the form of plant-derived agents, or methods of biocontrol, to effectively reduce or inhibit pest populations while avoiding the use of pollutants (Mulusa 2021).

Historically, RKN presence has been mitigated with synthetic compounds. These methods of control and treatment, such as methyl bromide and aldicarb, although effective, pose threats to our stratospheric ozone and water sources (Qiao et al. 2011; NRA 2001). As it is difficult to entirely remove synthetic compounds from the environment, the consequences of their implementation continue long after their intended purpose and affect both non-target organisms and humans (Sánchez-Moreno et al. 2009). The literature suggests that biocontrol may act as a suitable alternative to potentially hazardous compounds, where the presence of organisms and their exudates effectively ward off, damage, or inhibit RKN populations (Mulusa 2021). Biocontrol agents such as *trans-2-hexenal* produced by the *Solanum lycopersicum* tomato, coumarin produced by the *R. chalepensis* herb, and erucin, produced by *Eruca sativa* arugula, have demonstrated nematicidal effects, and suggest a promising future for plant-derived nematicidal agents (Lu et al. 2017; Ntalli et al. 2011; Aissani et al. 2015). Other biocontrol successes are found within the *Brassicacea* family, of which broccoli, rapeseed, radish, and the model plant, *Arabidopsis thaliana,* are members. Acting as natural biofumigants, glucosinolates released by *Brassicaceae* plants effectively reduce RKN populations (Monfort et al. 2007). Ultimately, a sustainable solution for the holistic damage RKNs have produced is not out of reach; plant-derived nematicides produced by cover crops help to mitigate RKN populations and subsequent crop loss.

As a parasitic organism, the RKN induces disease, which may lead to morphological and biochemical changes for its host plant (Mahapatra and Nayak 2019; Ibrahim and Massoud 2011). Phenotypic variations in crops are one indication of RKN presence. As microscopic organisms, RKNs travel to their host of choice by way of soil water, and enter through the host root as a second-stage juvenile (Miyashita et al. 2014). Thus, phenotypic changes most likely to take place

as a result of RKN infestation appear underground. The most common consequences of this pathogen are the formation of root galls, or knots, in the host root that limit their intake of water and nutrients, along with other variations in root development and structure (Mitkowski and Abawi 2003). However, above-ground consequences may result as well. Other indications of RKN presence include: chlorosis, or yellowing of plant leaves, which is indicative of nutrient stress, and overall reduced growth or changes in developmental patterns (DAF 2017).

Similarly, these phenotypes may manifest when a plant is exposed to inadequate pH levels. Plants tend to prefer a slightly alkaline environment, demonstrating optimal growth and reproductive outcomes when grown in soil with a pH range of 6-7 (NRCS 1998). Acidic environments (pH < 5.5) are associated with the leaching of essential nutrients, such as nitrogen, phosphorus, and potassium, that are integral to the production of chlorophyll and the development of leaf and stem, and root tissue (Haynes and Swift 1986; The Fertilizer Institute 2014). A spindly growth pattern is commonly observed in times of inadequate nutrition acquisition attributed to low-pH environments (Malone 2021). In alkaline conditions (pH >8), the plant's ability to uptake necessary micronutrients, such as zinc and iron, becomes limited due to a loss of water-solubility, which may interfere with the plant's ability to synthesize DNA and chlorophyll (Sharma 2006). Overall, the consequences of lost nutrients can contribute to altered color and developmental stages of a plant and inhibit its ability to conduct life-sustaining processes.

However, plants are equipped with multiple defense mechanisms to engage when faced with an abiotic or biotic hazard. As sessile organisms, plants do not have the luxury of travel to evade a threat, but are still capable of moving if needed. For example, plant stems shift in order to allow leaves access to light best-suited for their current life cycle stage, which may be found

in either blue or red wavelengths of the visible spectrum (Montgomery 2021). This phenotypic variation allows for the plant to acquire its Goldilocks-value of sunshine to induce the appropriate photosynthetic response. These responses allow plants to rearrange their ever-changing access to resources to create an environment that is best suited to their needs (Montgomery 2021). The same is true when a threat is realized; plants adapt, signal, and respond in order to protect themselves from future damage. In times of both nutrient stress and pathogen contact, plants express phenotypic adaptations to more appropriately and effectively adapt, signal, and respond in the future (Crisp et al. 2016). This working memory creates incredibly resilient and adaptable organisms. However, some stressors, pertaining to root health, temperature, or nutrient availability, may initiate plant bolting, a reproductive process that allows the plant to seed as quickly as possible in order to avoid its stressors and hopefully, provide a better outcome for their offspring (Gomez-Mena et al. 2001; Wu et al. 2016). Plants that have successfully bolted, or have flowered as a result of stress, will complete an early life cycle by acquiring height and flowers faster than unstressed plants (Wada and Takeno 2010).

Abiotic factors present in the RKN's environment can influence its growth and reproductive success as well. The RKN achieves infestation underground, traveling, as second-stage juveniles, through soil water (Miyashita et al. 2014). Sandy soils promote greater root-knot nematode mobility as compared to soils composed of clay or silt, which feature small, tightly-packed particles. The movement of water through these large sand particles precipitates a loss of calcium and magnesium and the retention of phosphates, which creates an acidic environment (NRCS n.d.). Root-knot nematodes are more likely to be found in these acidic regions than neutral or alkaline conditions, demonstrating a preference for soil pH ranging from 4.5 to 5.4 (Wang et al. 2009). The acidity value at the base of the plant root, or the zone of

elongation, is particularly indicative of RKN response (Verbelen et al. 2006). Regardless of specific root morphology, acidity of the plant root is lowest within the apoplastic space, or the area contained within the cell wall, where the RKN penetrates and invades the host plant (Martinière et al. 2018). Consequently, it may be difficult to ascertain whether the effects of decreased plant vigor are consequences of low pH, and subsequently, a lack of nutrients, or the decreased capacity of roots to acquire nutrients due to the RKN. Thus, experiments may focus on the phenotypic outcomes of a plant grown in pH values indicative of either nutrient stress or the RKN.

pH may be altered in matrices of either soil or liquid media, but it is considerably easier to manipulate in the latter. A hydroponics system involves the use of liquid media, rather than soil, as the basis for plant germination and growth. Hydroponic cultures were first realized as a viable way to access and subsequently catalog plant tissues in the 1800s (Hershey 1994 & Conn et al. 2013). Like conventional methods of cultivation, hydroponics presents advantages and disadvantages. One advantage allowed by soilless growing methods is the quantification of the root system, which is more difficult to extract and analyze in soil (Conn et al 2013). Liquid media is also easier to manipulate, in terms of pH or nutrient availability, than soil, as the presence of soil ions contributes to a more complex and heterogeneous matrix (Zeng et al. 2018). A disadvantage of a soilless growing method takes the form of algal growth; thus, sterilization and opaque or covered growth chambers are an important aspect of any hydroponic culture (Schwarz and Gross 2004). The use of a hydroponics system allows for the quantification of variables that are difficult in soil-based mediums and provides applicable lessons from its study to soil-based organisms.

Model organisms are helpful in this regard, as they allow us to apply lessons learned from their study to biologically-similar plants: conclusions reached through the study of a model organism may be applicable to thousands of other organisms (Chang et al. 2016). *Arabidopsis thaliana* is one such model organism; its study provides information as to the genetic implications of plant stress and defense, both in terms of abiotic and biotic threats. *Arabidopsis thaliana*, a common weed, presents a genome composed of over 25,000 protein-encoding genes located in 11,000 different families (AGI 2000). Further, *Arabidopsis thaliana* presents a short propagation timespan and a large number of offspring, which allows for relatively quick trials and analysis of multiple, inexpensive samples (AGI 2000). The implications of *Arabidopsis thaliana* as a member of the *Brassicaceae* family are not well-studied, but it is possible that this plant could exude compounds that would inhibit the pathogenic RKN. By better understanding the consequences faced by plants grown in low pH mediums, we can determine what consequences may be attributed to the RKN, and what consequences may be attributed to low pH levels.

Methods

Creation of Hydroponic System

Wild-type Col-0 *Arabidopsis thaliana* seeds were sterilized in 70% ethanol, washed with three trials of sterile deionized water, and suspended in sterile deionized water and covered with aluminum foil to simulate dark conditions conducive to germination. Holes 2 mm in diameter were punctured into the top of 0.5 mL microcentrifuge tubes with a push pin and scissors (Fig.

1A). The bottom centimeter of the microcentrifuge tube was cut off with a razor (Fig. **1B)**. 20 mL of 1/2 strength Hoagland's nutrient solution (Caisson Laboratories IncSmithfield, UT), of the appropriate pH value, were added to 0.15 g of micropropagation type 1 agar powder (Caisson Laboratories Inc., Smithfield, UT) (Fig. **2B**, **2C**). The solution was microwaved until molten, pipetted into the base of the tube, and left to solidify for approximately five minutes. ½ Hoagland's solution filled the remainder of the tube. Inverted tubes were placed into the holes of the pipette tip box, and sterilized and stratified seeds were pipetted into the hole of the tube. The nutrient solution for the experimental group and its agar were adjusted to pH values of 4.9-5, and nutrient solution for the control group and its agar were adjusted to pH values of 5.6-5.7. Nutrient solution additions to the pipette tip boxes were kept consistent in order to maximize the amount of nutrients available to the seedlings.

Growing Conditions

After two weeks of growth in the pipette tip box, the roots of the seedlings extended past the bottom of the microcentrifuge tubes (Zeng et al. 2018). Holes the width of the microcentrifuge tubes were drilled into one gallon-buckets to facilitate transfer of the seedlings (Fig. **3A**). Air stones, air tubing, and a timer allowed the nutrient media to be oxygenated every 15 minutes, which ensured that seedlings would receive enough oxygen in a respectively larger environment. Five-gallon buckets stored large quantities of nutrient media for use with samples in gallon buckets (Fig. **2A**).

Six one gallon-buckets, each containing six holes for tube seedlings, allowed for thirty-six plants to inhabit the buckets at one time. Trials for the experiment were staggered in order to optimize the space needed to cultivate as many samples as possible for analysis. Trials 4, 5, and 6 were completed solely in pipette tip boxes, which allowed for the plants to complete

their life cycle (Zeng et al. 2018) while minimizing the amount of media required for growth and eliminating the aeration component that contributed to increased pH values.

Quantification and Analysis of Phenotypes

Measurement of plant roots and plant height took place one, two, and three weeks after germination for both control and experimental groups. A ruler was held at the base of the microcentrifuge cap to measure the length of the roots in (cm) (Fig. **2D)** and at the base of plant rosettes to measure plant height in (cm) (Fig. **4B**). Data obtained from measurements was analyzed using an unpaired Student's t test in order to determine significance of difference between the control and experimental groups.

pH Modifications

pH measurements following seedling growth in both pipette tip boxes and gallon buckets demonstrated a basic shift (Fig. **9**). Dilutions of HCl were created in order to return media to appropriate growing values of 5 and 5.7 for experimental and control groups, respectively, throughout the three-week long trials. Following Trial 3, control groups were subjected to media with a pH value of 7 achieved with additions of 0.1 M KOH.

Results

Hydroponic Seedling Growth

Our observations suggest that all seeds capable of doing so germinated, on average, by the fourth day after placement (Fig. **6**). The rosettes of our samples emerged at either seven or eight days after germination (Fig. **4AB**). As the rosettes developed, pronounced trichomes appeared on two of the four perpendicular leaves. Processes of secondary growth took place in the black buckets, where plants began to extend upwards. In the first three trials, control group

plants were more likely to begin processes of secondary growth than experimental group plants (Fig. **2D**).

Observational Effects of pH

Phenotypic differences between the control and experimental group were first observed in the pipette tip boxes, around 10 DAP (data not shown). The intensity of yellow present in the leaves of the experimental group was more pronounced than any yellow color presented by the control group, but control group plants were likely to have a larger number of light green or yellowish plants (Fig. **1EF**). These differences became more pronounced as the plants continued their growth in the pipette tip boxes. Any samples that exhibited signs of nutrient stress earlier in their life cycle were more likely to exhibit a more intense yellow, an indication of chlorosis, later on. On average, the trials that received a plant-standard pH presented fuller rosettes. The rosettes of the experimental group featured leaves that would curl downwards, as compared to the rosettes of the control group that would lay more flat atop the microcentrifuge tube (Fig. **4CD**). Both the control and experimental groups exhibited relatively darker, green plants. However, plants in the experimental group that did not acclimate well to nutrient stress were bright-yellow in color and exhibited smaller cotyledons and rosettes. This less-than-optimal growth followed any control and experimental samples that featured indications of stress once transferred into the black one-gallon buckets.

After two weeks in the pipette tip boxes, plant roots extended beyond the bottom of the pipette tube, and were ready for transfer into the one-gallon buckets. The control group plants tended to exhibit lighter shades of green compared to the experimental group plants (Fig. **2EF**). after transfer into the black buckets, some samples in both the control and experimental groups presented leaves beneath rosettes dark brown or purple in color. Differences found between

control and experimental groups in terms of rosette size became more pronounced at this time in the experiment. Experimental group plants were more likely to present large rosettes and thick, long roots (Fig. **2D**). Control group plants featured smaller, less brightly-colored rosettes.

Effects of pH on Root Length and Plant Height

Following a week of growth in the black buckets, the roots of experimental plants were clear in color and thick, as compared to the roots of the control group plants, which were darker in color, thin, and more coarse (Fig. **2D**). The roots of the experimental group plants had a higher standard deviation between measurements than the roots of the control group, presenting both the shortest and longest roots in all trials. Control group plants were likely to begin processes of secondary growth sooner than experimental group plants, but experimental group plants were more likely to present the highest average value of plant height. pH was measured once Trial 4 completed its final week in the gallon buckets in order to ensure that the appropriate pH values were introduced and kept consistent throughout the trial. Multiple measurements demonstrated large basic shifts from original pH values of 5 and 5.7 (Fig **6)**. pH was monitored and appropriately acidified for both control and experimental groups in subsequent trials with dilutions of HCl.

Observations and Phenotypic Effects following pH Modifications

Control group plants, subjected to a higher pH value of 7, were more likely to germinate and be lighter in color compared to the experimental group plants in Trials 4 and 5 (Figures **5** and **6**). Similarly to the first three trials, plants which exhibited signs of stress earlier on in the pipette tip boxes, such as smaller rosettes and a brighter, more yellow color, were likely to feature decreased values of plant root length.The rosettes of the control group plants appeared more circular than those of the experimental group plants; the latter were likely to be thinner and

extend downwards, rather than rest atop, or extend upwards, on their respective microcentrifuge tubes (Fig. **5**). Phenotypes present in the black bucket environment in regards to root color and width were not present in the pipette tip boxes, which suggests that plasticity did not take place as a result of an added stress element of too much oxygenation. Both roots of control and experimental groups remained clear in color and demonstrated similar lateral root growth (Fig. **7B**). Control group plants were less likely to vary in color as compared to the experimental group plants in Trial 4, but these differences were not observed for Trial 5.

Discussion

The root-knot nematode will continue to cause economic and agricultural damage as a global pest. In order to avoid both the infestation of cultivated crops and further agricultural degradation, sustainable solutions should be of focus. Methods of biocontrol feature naturally-occurring compounds that target pests or other plant-targeting organisms. As plants that release natural nematicidal compounds, members of the *Brassicacea* family may present a solution for RKN infestation that is both effective and sustainable. As a model organism and member of the *Brassicacea* family, lessons learned from the study of *Arabidopsis thaliana* may be applicable to thousands of other species and provide information about the interactions between the plant and the pathogen.

First Half of Experiment

A hydroponic system proposed by Zeng et al. (2018) was recreated at the University of Lynchburg for the quantification of *Arabidopsis thaliana* phenotypes. A ½ strength Hoagland's nutrient solution liquid media was created for both the control and experimental groups. In the absence of any modifications, the Hoagland's nutrient solution presented a pH level of 5, which served as the RKN-standard pH for the experimental group. Originally, a plant-standard pH of 5.7 (Weigel and Glazebrook 2020) was achieved with the addition of KOH, but was modified and monitored in subsequent trials to reflect indications of stress experienced by experimental and control groups. Due to the pathogenic mechanism of the RKN, root length was decided as an appropriate phenotype to quantify in the experimental and control groups, in addition to the visible growth pattern of the samples. Plant height was chosen as a variable in order to determine if an acidic environment is indicative of bolting in relation to nutrient stress.

The literature suggests that a time period of 2 to 3 weeks after germination is a sufficient length of time to begin phenotyping *Arabidopsis thaliana* (Kang et al. 2012 & Vello et al. 2015). Thus, seedlings were grown in pipette tip boxes for two weeks, and transferred into one-gallon buckets for an additional week. All samples were exposed to thirteen hours of fluorescent lighting. The literature suggests that plants more likely to undergo mechanisms of bolting are exposed to short-day photoperiods, or less than 12 hours of fluorescent lighting (Gómez-Mena et al. 2001); therefore, we can eliminate the growth chamber as a confounding variable when considering plant height plasticity.

In this experiment, wild-type *Arabidopsis thaliana* plants were subjected to two levels of pH for a duration of three weeks. Measurements of nutrient media, completed during and at the end of the completed second trial, suggested that seedlings in the pipette tip boxes and buckets

were capable of modifying their environment as a response to acidic pH conditions (Figure 9), demonstrated by the experimental group from 0 to 14 DAP. In the latter environment, pH levels greatly exceeded those originally set. It is likely that the aeration component of the black buckets, which oxygenated the media every fifteen minutes, led to a loss of H+ ions and subsequently, increased the pH values of the plant-standard and RKN-standard medias. These pH measurements suggest that the experimental group plants were more likely to modify their environment than the control group plants, especially when inhabiting pipette tip boxes. It is possible that this preliminary pH value of 5 necessitated plasticity as compared to 5.7, which was not considered as stressful.

The greatest shift in both basicity of media and plasticity took place in the black buckets, but this did not necessarily correlate with statistical significance. Significance, determined for each trial at 7, 14, and 21 DAP with the Student's t test, tended to decrease with time prior to pH modifications (Figure 8). In the pipette tip boxes, differences found between the control and experimental groups were limited to color and overall size of the plant's rosette. In the first three trials, root length of the control group exceeded that of the experimental group for the first two weeks following planting. Observational differences arose when the seedlings were transferred into the black buckets; here, control group roots were darker in color, coarser, and shorter than those presented by the experimental group plants.

Originally, our hypothesis was that those plants subjected to a lower pH would perform poorly in comparison to the plants receiving a more basic pH. Following the observations and measurements of three trials, the experimental plants appeared to be "outperforming" control

group plants. Distinct changes arose between the lateral roots of control and experimental groups following a week of growth in the black buckets. Dimitrov and Tax (2018) reported lateral root growth in *Arabidopsis thaliana* 16 days after planting, which appeared consistent with our observations. The lateral roots of control group plants were smaller in number, shorter, and less likely to extend as close to the primary root as the lateral roots of the experimental group plants. Although the latter appeared healthier due to a lighter color and longer length, their length, in comparison to the control group plants, suggested that these plants felt more pressure to uptake nutrients and send out longer roots. These longer roots, perhaps created out of need, allowed for these plant roots to have a greater surface area and subsequently greater ability to uptake nutrients (Barber and Silberbush 1984).

Deficiencies suggestive of increased stress exhibited by the experimental group plants were no longer present after a week of growth in the black buckets. Perhaps because these plants were already accustomed to changing the pH levels of their media they were better able to modify a relatively larger environment in the black buckets reminiscent of the pH value present in the pipette tip boxes. It is likely that this increased stress, a lower pH value, led to a more impactful memory imprint than the stress experienced by the control group plants.

Second Half of Experiment

Trials 4 and 5 control and experimental group plants were grown in media with a pH value of 7 and 5, respectively, in order to one, maximize the difference in pH values between these two levels and two, subject the control group to a more appropriate plant-standard value. Interestingly, control group plants were still likely to exhibit a brighter, more yellow color than the experimental group plants, which made distinguishing the trichomes of these plants more difficult. Apart from observational differences, control group plants did present a significant

difference in terms of root length compared to the experimental group plants. Plant height did not present significance, which suggests that this phenotype may be indicative of RKN presence as compared to acidic pH values.

Observations of root phenotype, apart from root length, provided additional information about how the control and experimental plants adapted to the stress of their environment. A pH level of 5 was sufficient to produce plants with long, clear, healthy roots, similar to those produced by Zeng et al. (2018), who were less likely to prematurely flower than plants subjected to a higher pH. It is possible that calcium is more soluble at a pH of 5 than it is at a pH of 7, which explains why control group plants were less brightly colored than experimental group plants, which were a rich shade of green. As deionized water served as the matrix for nutrient media, it is unlikely that the addition of KOH to create a more basic media created any precipitates or caused nutrients to be leached out of the final media solution. However, it is possible that the addition of KOH led to a loss of solubility of some nutrients, such as calcium or nitrogen, which respectively, can contribute to short and thick roots and restricted lateral root growth (IPM 2011). It is possible that these nutrients become less water-soluble at this pH level, or that the addition of KOH increased the availability of phosphorus ions in solution, of which an excess can lead to chlorosis-like symptoms due to a loss of zinc and iron (IPM 2011).

Limitations

In future experiments, a smaller air stone may provide sufficient oxygenation for plants located in larger environments and mitigate root tangling. For oxygenation with a larger air stone, the prevalence of bubbling may be reduced from 15 minutes every hour to 15 minutes every two hours. Algal growth was observed in Trials 4 and 5, which suggests an increase in moisture, nutrients, or temperature present in these environments (Schwarz and Gross 2015).

Because the temperature of the growth chambers were kept consistent, and that both control and experimental groups featured algal growth, it is possible that the plants were provided too much media early on in their life cycles as necessary for growth. Schwarz and Gross (2015) determined that the formation of algae in their hydroponic systems led to decreased plant biomass. Leavitt et al. (1999) determined that the presence of some species of algae were higher in concentration in more acidic environments, and that those species who employed carbon sequestration mechanisms could increase the pH of their environment. There appeared to be little difference in concentration of algae in Trial 4 and 5 control and experimental groups, although the species of algae present in this system is unknown. Considering these limitations of algal growth, it is possible that the variable of plant height could have been considered significant were plants subjected to pH values of 5 and 7 in the absence of algae. However, this variable failed to indicate significance in earlier trials, so additional research is required to ensure validity of results.

Conclusions

The RKN, a parasite that infects its host by way of its root, is likely to cause morphological changes underground. The host plant can successfully defend itself against both the RKN and low-pH environments by employing defense strategies and modifying the pH of its rhizosphere, respectively, but may demonstrate phenotypes indicative of either RKN presence or the pH of the environment the parasite was attracted to.

The objective of this experiment was to determine what phenotypic effects could be attributed to a RKN, and what could be attributed to the acidic environment these microscopic pathogens are likely to be found in. The first half of our experiment focused on the root length of plants that were able to modify their environment. During the second half of the experiment, an

additional variable of plant height was tested, in order to determine how the plants were adapting to stress. A taller plant featuring signs of secondary growth, especially flowers or coarse and dark lateral roots, had likely completed their life cycle due to nutrient stress. This phenotypic adaptation takes place when a plant finds its environment too taxing, which is likely to take place in times of RKN presence, especially if these plants are grown in an acidic environment.

Future experiments could report on the lower half of pH preference for the RKN, 4.5, or could determine answers more directly with the use of the actual pathogen, which could be introduced to the test species through way of infested soil or groundwater. This study of *Arabidopsis thaliana,* and its responses to stress, may be helpful to consider when thinking of long-term, sustainable solutions for our global health. The future of sustainable agriculture may rely on the engineering of plants to produce crops more capable of enduring moderate, repeated stresses, employing aspects of memory to help them better cope with drought, nutrient-stress, or predation (Guzmán et al. 2021). Warmerdam et al. suggest that *Arabidopsis thaliana* genes be engineered in order to avoid the formation of root galls and inhibit reproduction of the RKN. It is unknown whether or not this engineering would pose deleterious effects for the organisms, or if these results would be applicable to more economically viable *Brassicaceae* plants, such as broccoli, cabbage, and mustard.

Figures

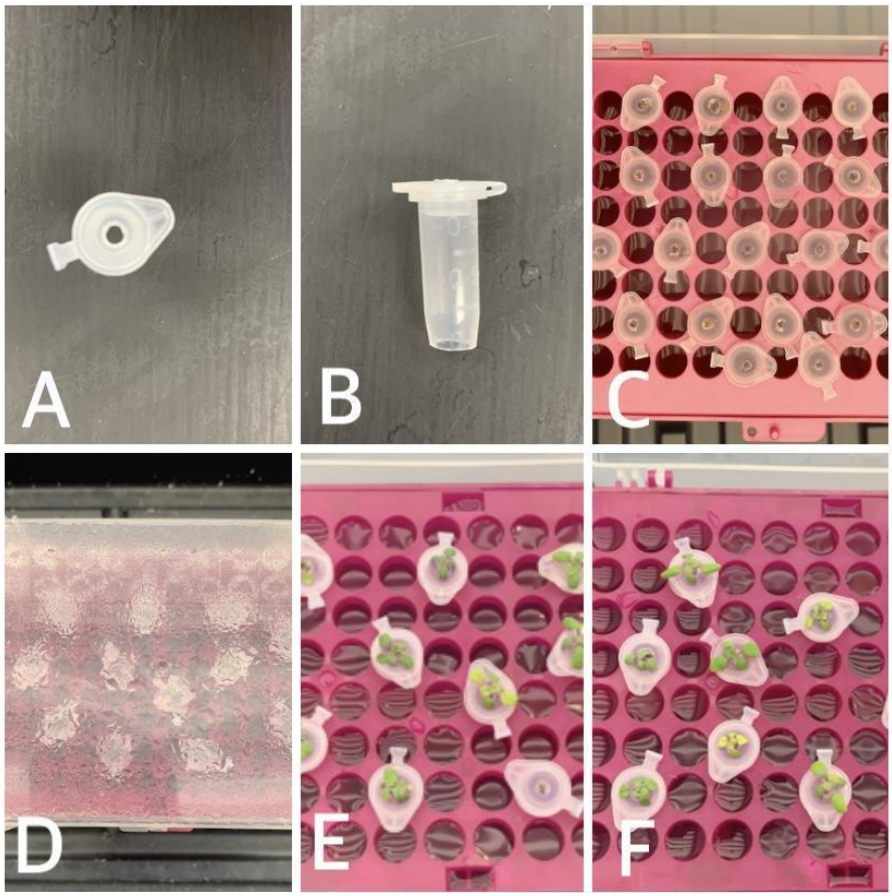

Figure 1. A: 0.5 mL centrifuge tube with 2 mm hole drilled within the cap. Wildtype *Arabidopsis thaliana* seedlings were placed inside this hole on top of solidified agar for germination. **B:** 0.5 mL centrifuge tube with bottom centimeter removed. **C:** Seeds placed atop microcentrifuge tubes in pipette tip box filled with nutrient solution. **D:** Pipette tip box cover

closed for the first 6 days of germination for increased humidity. **E:** Samples of Trial 1 control group, which received a nutrient solution with a pH of 5.7. **F**: Samples of Trial 1 experimental group, which received a nutrient solution with a pH of 5.

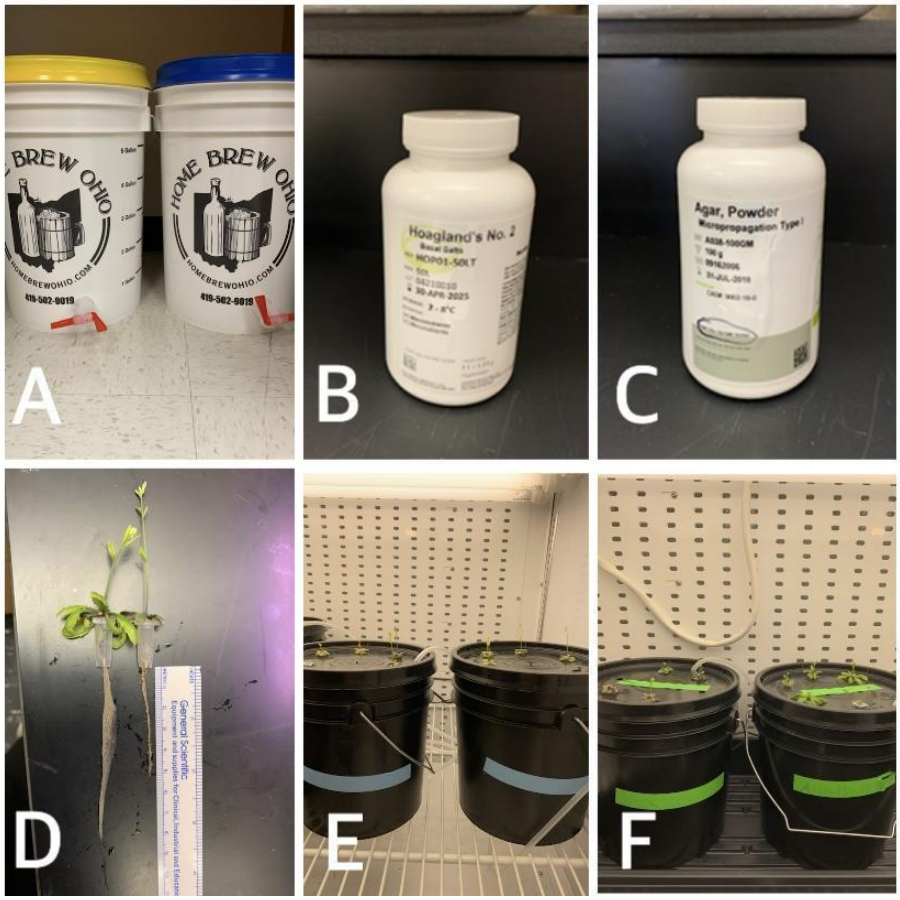

Figure 2: A: Five-gallon buckets used for storage of nutrient solution. **B:** Hoagland's No 2. Basal Salts used for creation of nutrient media. **C:** Agar powder used for creation of solidified agar in microcentrifuge tubes. **D:** Comparison of roots of experimental plant and control plant. **E:** Trial 1 control group plants in black buckets. **F:** Trial 1 experimental group plants in black buckets.

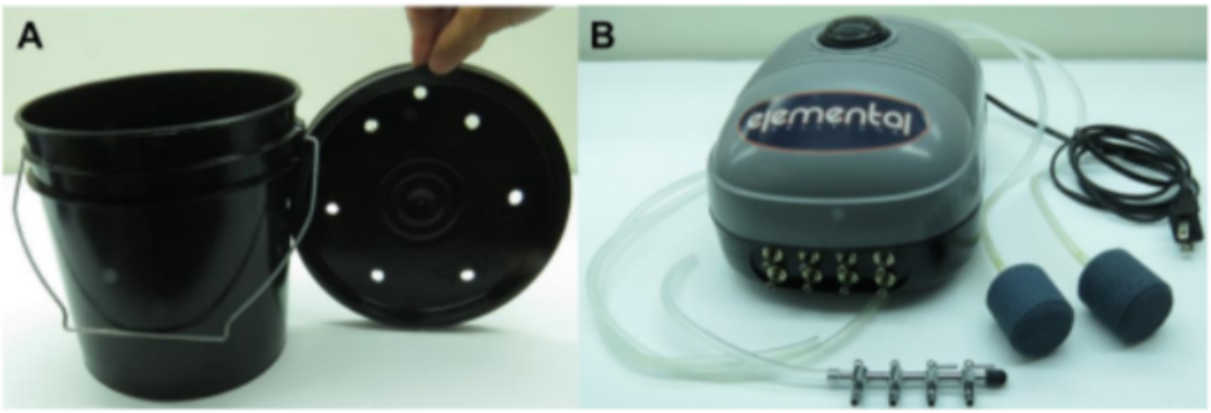

Figure 3: Hydroponic Set-up with Black Buckets (Zeng et al. 2018). A: One-gallon buckets were occupied by seedlings following two weeks of growth in pipette tip boxes. Features an additional drilled hole for air tubing. **B:** Air pump, air stones, air tubing, and 4-way outlet valve for media oxygenation.

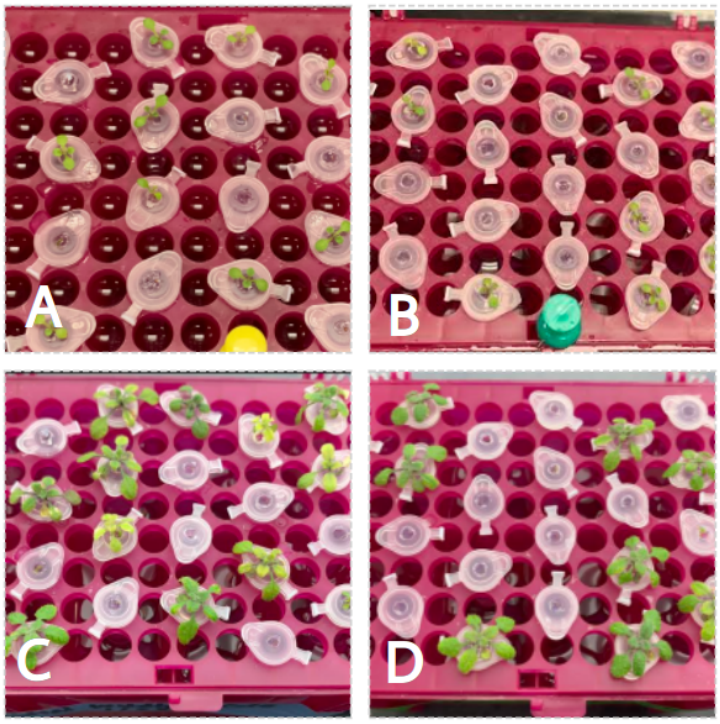

Figure 4: Trial 3 control (left) and experimental (right) plants. **A:** Control group plants 7 DAP. **B:** Experimental group plants 7 DAP. **C:** Control group plants 13 DAP. **D:** Experimental group plants 13 DAP.

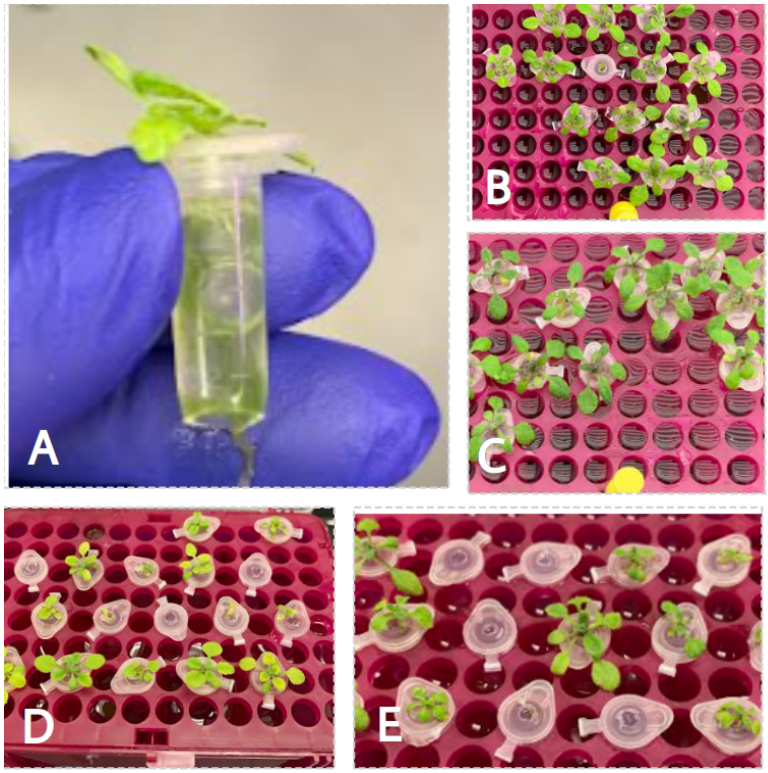

Figure 5: Images of Trials 4 and 5. **A:** Depiction of algal growth in microcentrifuge tube of Trial 4 plant. **B:** Trial 4 control group plants amidst algal growth. **C:** Trial 4 experimental group plants amidst algal growth. **C:** Trial 5 control group plants amidst algal growth. **D:** Trial 5 experimental group plants amidst algal growth.

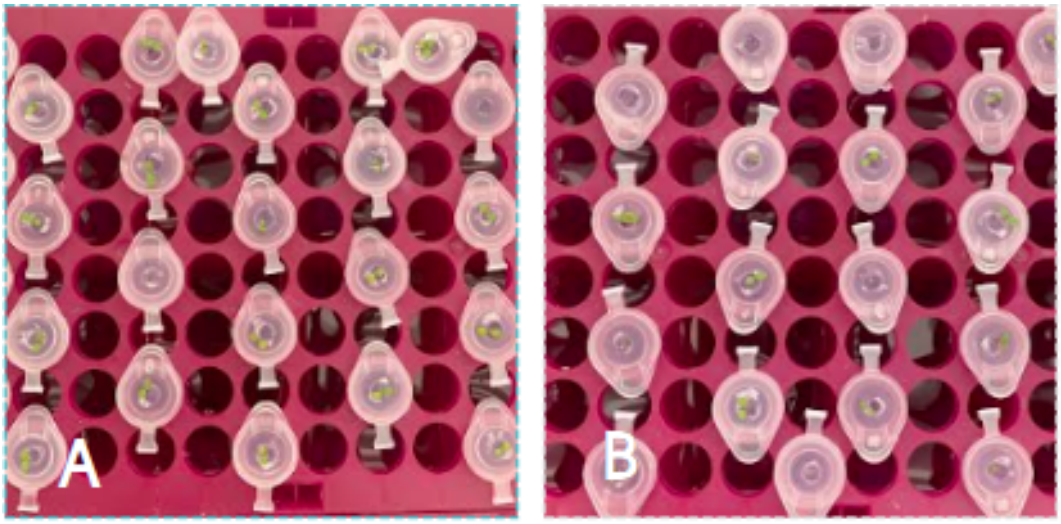

Figure 6: Trial 6 plants 4 DAP. **A:** Germinated control group plants. **B:** Germinated experimental group plants.

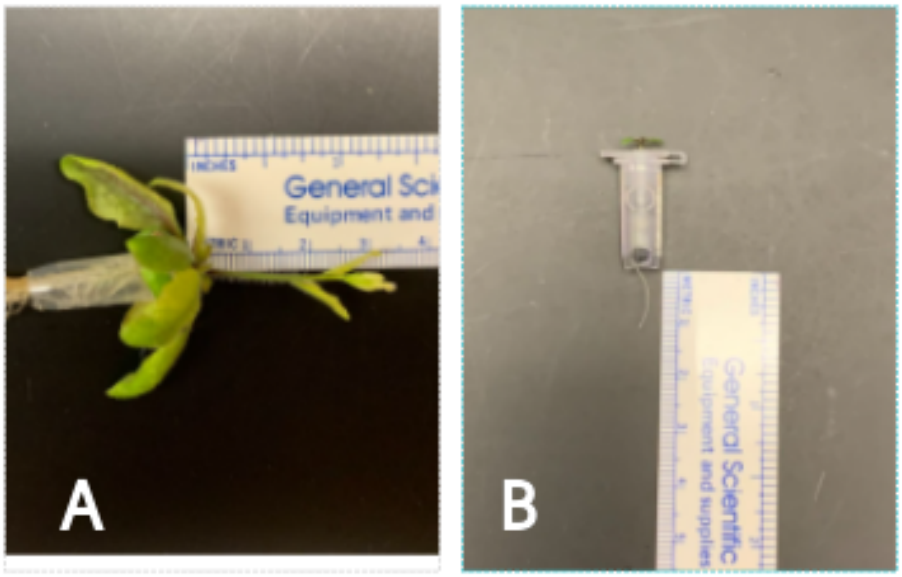

Figure 7: Depicts measurement of phenotypic variables of plant height and root length. **A:** A ruler held at the top of the microcentrifuge cap measures plant height. **B:** A ruler held at the base of the microcentrifuge cap measures root length.

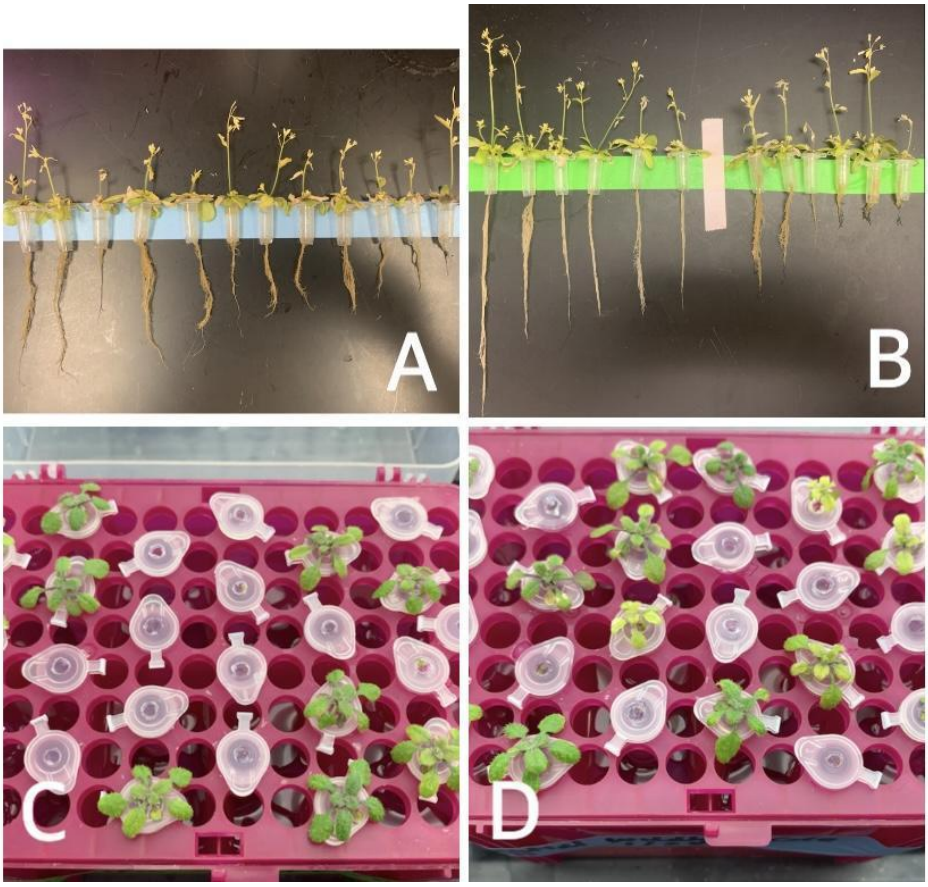

Figure 8: A: From left to right: Longest roots to shortest roots of Trial 2 control group plants. **B:** From left to right: Longest roots to shortest roots of Trial 2 experimental group plants. **C:** Experimental group seedlings after two weeks of growth in pipette tip boxes. **D:** Control group seedlings after two weeks of growth in pipette tip boxes.

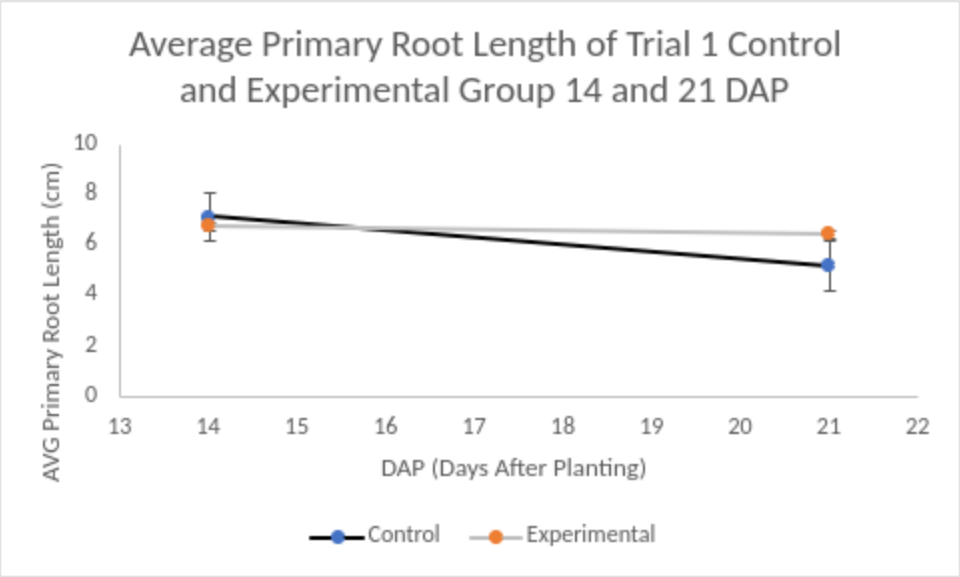

Figure 9: Average primary root length measured, in centimeters, 14 and 21 days after planting Trial 1 control and experimental groups. Seedlings were transferred from pipette tip boxes at 14 DAP. Final measurement retrieved following a week of growth in gallon buckets. Error bars represent SEM. (n = 9 at 21 DAP, p = 0.1497 at 21 DAP, ttest).

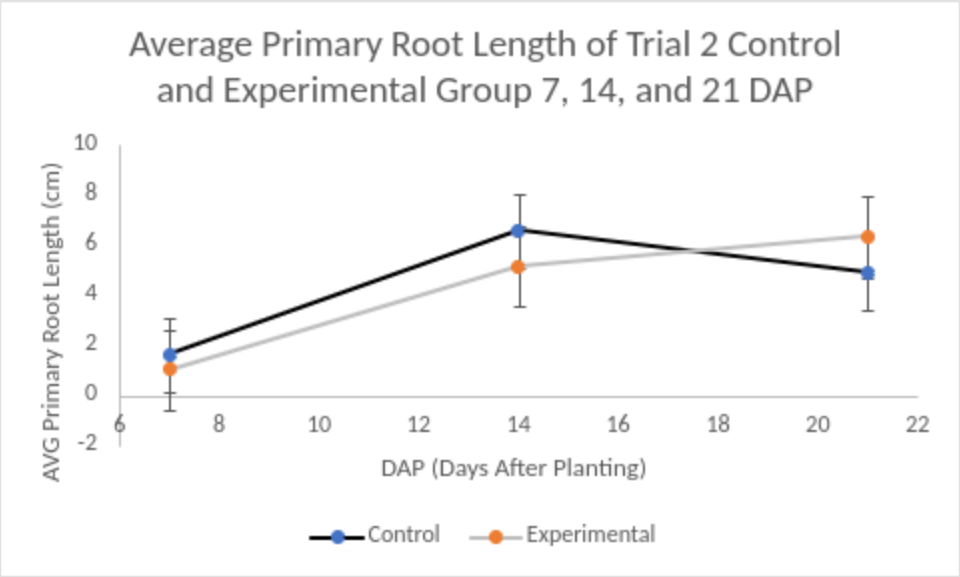

Figure 10: Measurements of primary root length for Trial 2 control and experimental groups at 7, 14, and 21 days after planting (DAP). Seedlings were transferred from pipette tip boxes at 14 DAP. Final measurement retrieved following a week of growth in gallon buckets. Error bars represent SEM. (n = 12 at 21 DAP, p = 0.1718 at 21 DAP, ttest).

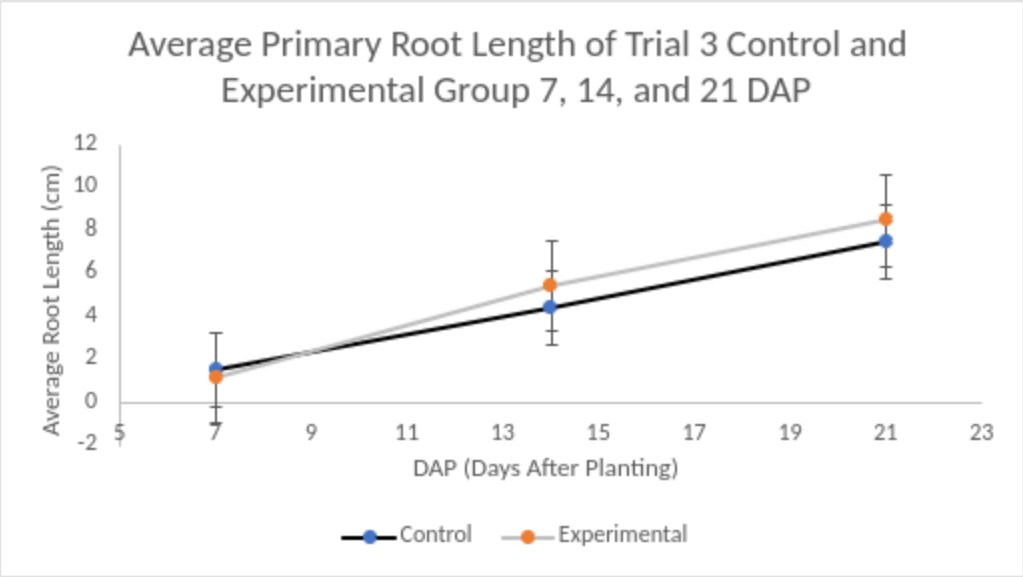

Figure 11: Average root lengths of Trial 3 control and experimental groups at 7, 14, and 21 DAP. Seedlings were transferred from pipette tip boxes at 14 DAP. Final measurement retrieved following a week of growth in gallon buckets. Error bars represent SEM. ($n = 10$ at 21 DAP, $p > 0.05$ at 21 DAP, ttest).

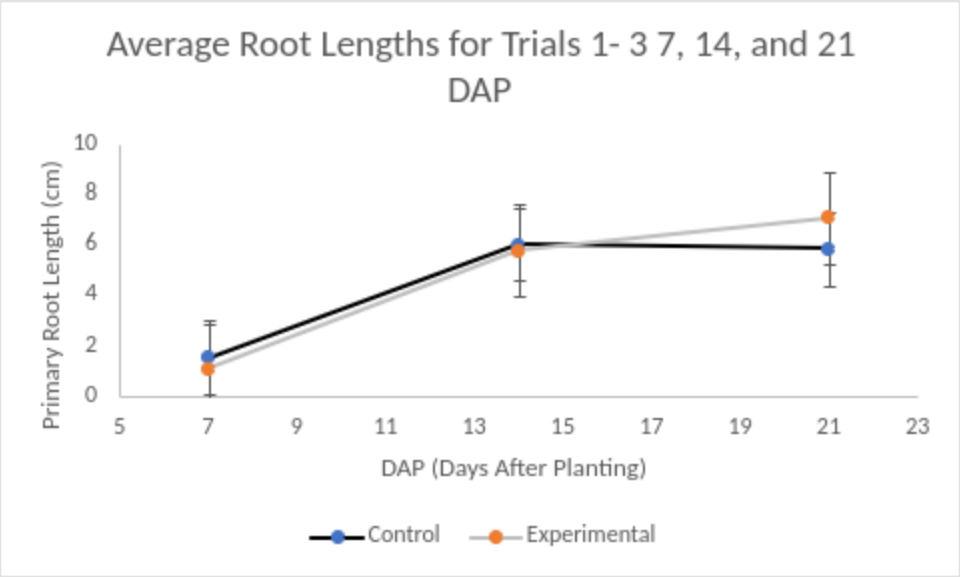

Figure 12: Averaged root lengths for first three trials 7, 14, and 21 DAP. Seedlings were transferred from pipette tip boxes at 14 DAP. Final measurement retrieved following a week of growth in gallon buckets. Error bars represent SEM. (n = 10 at 21 DAP, p = 0.0881 at 21 DAP, ttest).

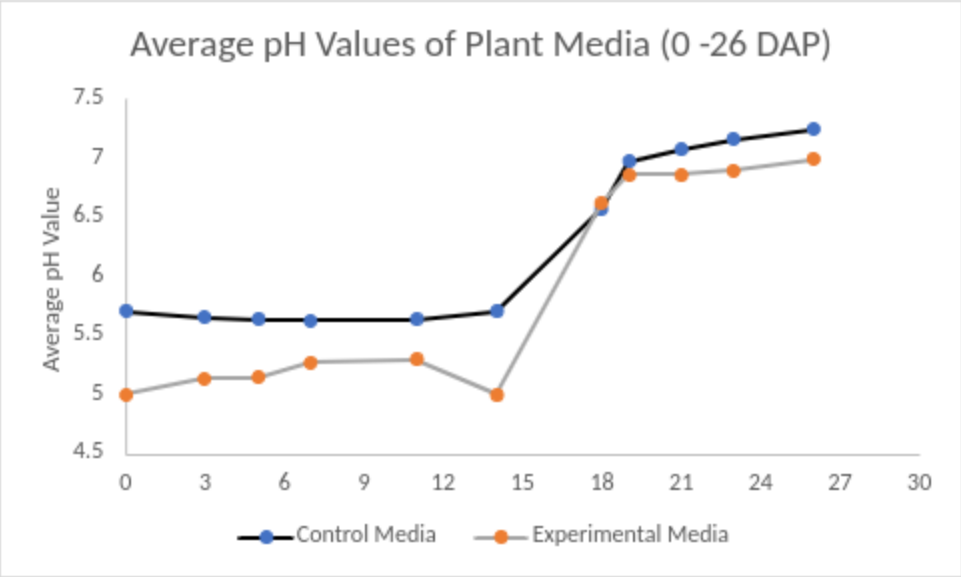

Figure 13: Average pH values recorded from 0 - 26 days after planting for Trials 2 and 3 control and experimental plants.

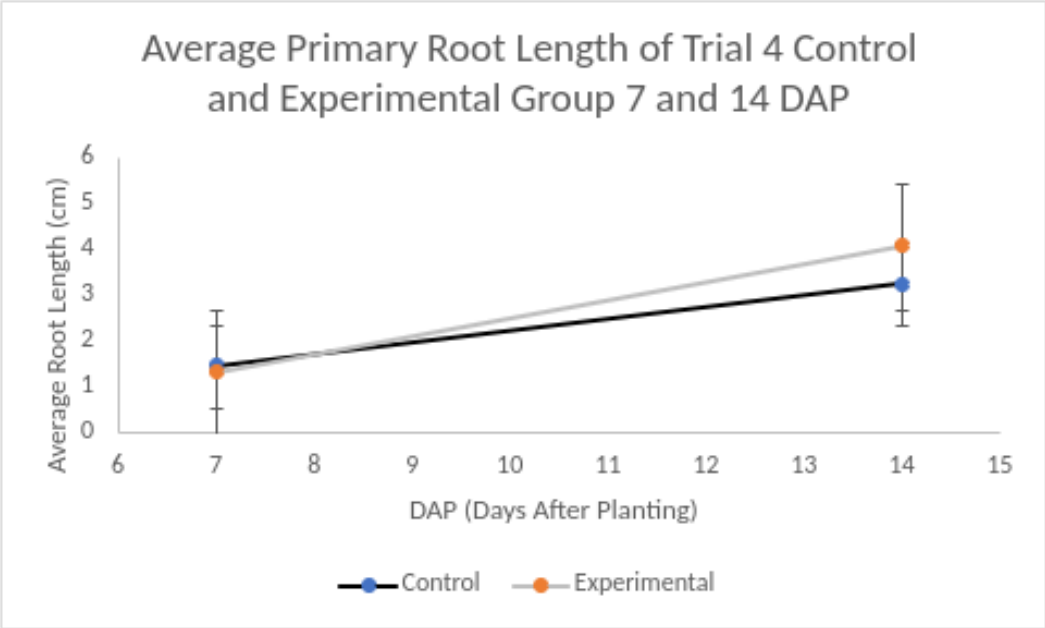

Figure 14: Averaged root lengths for Trial 4 control and experimental plants. Seedlings were not transferred from pipette tip boxes at 14 DAP due to algae presence. Final measurement retrieved after two weeks of growth in pipette tip boxes. Error bars represent SEM. (n = 12 at 14 DAP, p = 0.049 at 14 DAP, ttest).

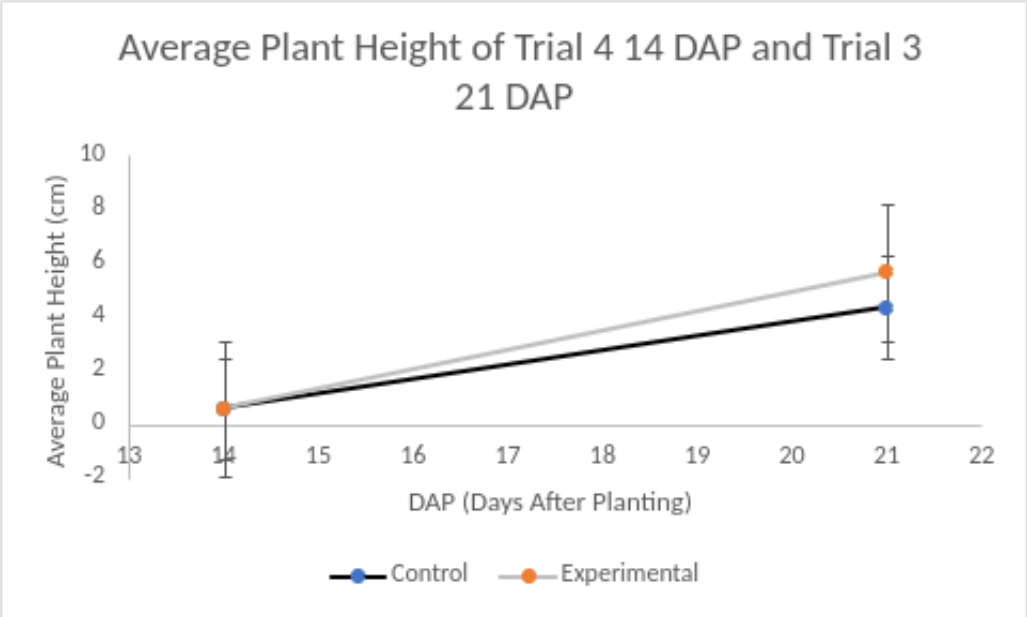

Figure 15: Averaged root lengths for Trial 3 plants 21 DAP and Trial 4 plants 14 DAP. Trial 4 seedlings were not transferred from pipette tip boxes at 14 DAP due to algae presence. Final measurement retrieved after two weeks of growth in pipette tip boxes and one week of growth in black buckets for Trial 4 and Trial 3 plants, respectively. Error bars represent SEM. (n = 10/12 at 14 and 21 DAP, p =0.3457 at 21 DAP, ttest).

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