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The Effects of Arbuscular Mycorrhizal Fungi on the Growth of *Apium graveolens*

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ABSTRACT

Arbuscular Mycorrhizal Fungi are obligate biotrophs, meaning they extract nutrients solely from living plant tissues and cannot grow apart from their hosts (Besemer, 2021). AMF also are symbionts; they are able to form a mutualistic relationship with plants (Tracey Besemer, 2021). To find the ideal amount of fungus that accelerated plant growth, research was conducted on Golden Self-Blanching Celery seeds, also known as *Apium graveolens*. The hypothesis states that the celery plants with AMF would exhibit more growth than the control because AMF can aid in reducing fertilizer amounts that cause pollution and conserve water during droughts in the future. The experiment was conducted in bins with equal amounts of soil and differing quantities of AMF. The results showed that the control group without any AMF grew the best due to a multitude of reasons such as the altering amounts of water during the experiment and a lack of natural sunlight. One goal for future experiments is to control confounding factors that can inhibit or improve the growth of the Golden Self-Blanching Celery plant.

Introduction

The world's population has recently hit eight billion and is estimated to reach 9.1 billion in 2050 (Ortez, 2022). More than one billion more people will be on Earth in less than 30 years. Research shows that food production must increase by 70% to meet the needs of the rapidly increasing population (Ortez, 2022). Production of common foods like fruits and vegetables must be escalated in order to meet the demands of the growing population. Among the various types of vegetables, celery is one of the top ten bought by consumers (Top 20 Fruits and Vegetables Sold in the U.S., 2021). Celery is a member of the parsley family and has many unique uses and benefits ("Celery", n.d.). Not only are the stalks and leaves of celery used for many popular foods such as sandwiches, soups, and tacos, but also, celery seeds and oil have numerous benefits as well. For example, celery seeds are used for flavoring, garnish herbs, and celery oil is used as a natural herbal medicine to prevent cancer, bladder infections, and lower blood pressure ("Celery", n.d.). California produces about 95% of the United States celery crop, and Ventura and Monterey produce about 80% of California's production of celery (Lazicki et al., 2016). Research indicates that celery production has many demanding qualities; it is a nutrient-hungry crop that receives one of the heaviest nitrogen fertilizations. Celery production also requires a large water supply to grow and needs extensive care to avoid stingy or dry celery stalks ("Celery", n.d.).

AMF's long evolution period resulted in its mutualistic relationship with plants which has led to numerous advantageous effects. The symbiosis of AMF was first observed 400 million years ago and is now found in 80% of vascular plant families today (Begum et al., 2019). AMF (arbuscular mycorrhizal fungi) form mutually beneficial partnerships with the roots of most land plants. These fungi play a vital role in assisting host plants in absorbing and transporting nutrients such as phosphorus and other immobile soil nutrients. These natural root symbionts also contribute to plant growth promotion and enhance the plants' ability to tolerate stressful conditions (Yurong Yang et al., 2016). AMF is a soil-borne fungus (transmitted by or in soil) which vastly improves a plant's nutrient uptake and resistance to stress factors ("Soilborne," 2023; Begum et al., 2019).

One benefit of AMF on plants is an increase in surface area of the plant, which in turn increases phosphorus, water, and nutrient uptake, enabling the plant to absorb the nutrients needed from the soil efficiently (Mohammadi et al., 2011). AMF's ability to take in more water, nutrients, and increase surface area also improves a plant's rate of photosynthesis (Yurong Yang et al., 2015). Additionally, AMF can help plants in changing or stressful conditions, such as droughts and wildfires (Mohammadi et al., 2011). This fungus can especially be beneficial to celery because California is the largest producer of celery in the United States (Lazicki et al., 2016). In California, droughts and wildfires are very prevalent. The most recent drought in California lasted five years from 2012-2016 (California Department of Water Resources, 2015). In 2021, 7,396 fires occurred in California ("Statistics", 2023). AMF increases the growth of crops and decreases the uptake of sodium chloride (Begum et al., 2019). Concentrations above three g·L−1 of sodium chloride can prevent plants from taking in other essential nutrients, such as phosphorus (Mandy Bayer et al., 2016). Large amounts of fertilizers are currently necessary in order to increase plant growth and provide food at a fast rate ("Celery", n.d.). Fertilizer causes absorption of heavy metals into the plants and soil, and fertilizer runoff can enter the water system causing pollution, so the replacement of fertilizer with AMF for accelerated celery growth can benefit the environmental issue of pollution levels (Serpil Savci, 2012). AMF may be ineffective based on the plant as ten percent of plant species cannot become interdependent with AMF (Begum et al., 2019). The goal of this experiment is to examine the effects of AMF on Golden Self-Blanching Celery, also known as *Apium graveolens*, to identify the beneficial and damaging effects of AMF on celery production. If ten grams of Arbuscular Mycorrhizal Fungi were added per celery plant, the growth of *Apium graveolens* will increase because the fungus will stimulate plant growth and increase nutrient uptake.

Experimental Procedures

Paper Towel Method

Golden Self-Blanching Celery seeds were placed in paper towels to begin the growing process. This involved using a moist paper towel, Golden Self-Blanching Celery seeds, and Ziploc bag. Each Ziploc bag contained about 30 to 40 seeds spread apart from each other. Three milliliters of water were added to the Ziploc every two days. This process continued for one week until the seedlings began to sprout without growing through the paper towel.

Greenhouse Set-up

Four 40-quart bins were placed inside the greenhouse with a red and blue LED light above them. 1280 grams of soil were placed in each bin along with different concentrations of AMF referenced in Table 1. There were two Kollea automatic watering systems and each watered two of the bins (Kollea Automatic Watering System, Indoor Plant Self Watering System, n.d.). Water flowed into each bin from three different corners. Two rows with 5 divots each were made in every bin. Each divot was seven thirds inches apart and each of the rows was ten thirds inches apart. One seedling was placed in every divot.

Table 1. Concentrations of Arbuscular mycorrhizal fungi in control group, group one, group two, group three. The mass of Arbuscular mycorrhizal fungi in each bin was measured in grams and the number of grams of Arbuscular mycorrhizal fungi per plant was calculated.

Group	Mass of AMF (measured in grams)	Number of grams of AMF per plant
Control	0	0
#1	30	3
#2	100	10
#3	250	25

Seedling Transfer

After the paper towel method was used to sprout the seeds, the seedlings were placed into the soil. Each paper towel contained about 20 sprouted seeds. The seedlings were carefully selected and picked up to ensure the seed ends could be seen and were not damaged. One seed was placed in each of the ten divots in every bin, then each divot was covered with a thin layer of soil.

Data Collection

Data was collected for the height of plants in centimeters, the number of leaves, and the relative health of the plants based on leaf color.

Water Regulation

At the beginning of the experiment, the watering system for all the bins was set at 200 mL every 24 hours. After a week, the bins were flooded with water. Thus excess water from each bin was disposed of in order to return the plants to their normal state. The watering system was reduced to 150 mL per day for all plants. Two weeks later, water levels within the bins were rising again. To prevent the possibility of flooding, the watering system was turned off for 20 days. The plants remained brightly green, and the control bin still had the healthiest plants. The plants were watered at 100 mL every day because 200 mL and 150 mL resulted in overflowing of water in the bin. They were watered at this rate throughout the rest of the experiment because it prevented underwatering and overwatering.

Results

This experiment was conducted over the course of 78 days, and throughout this time period, all of the Self-Blanching Celery plants were kept under the same conditions. In the experiment, four different groups were created with 10 celery plants each as shown in Table 1. Each plant's height and number of leaves were measured about every 14 days.

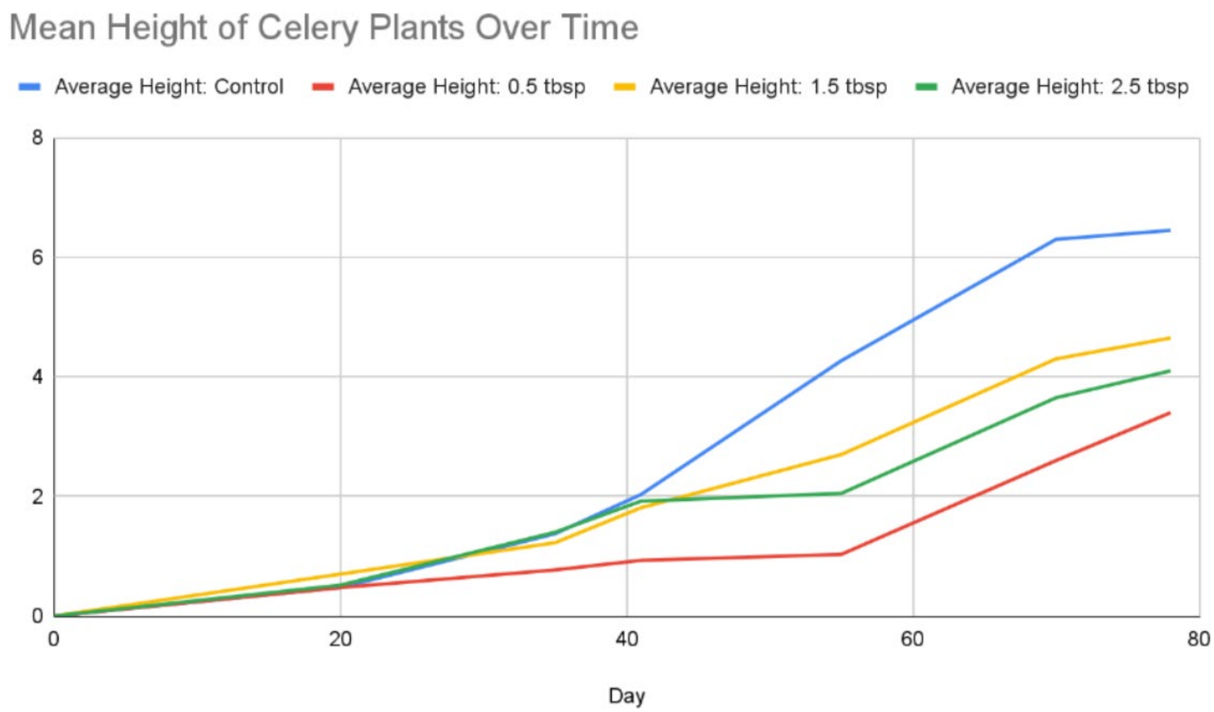


Figure 1. Mean plant height from each bin that contains different amounts of fungi

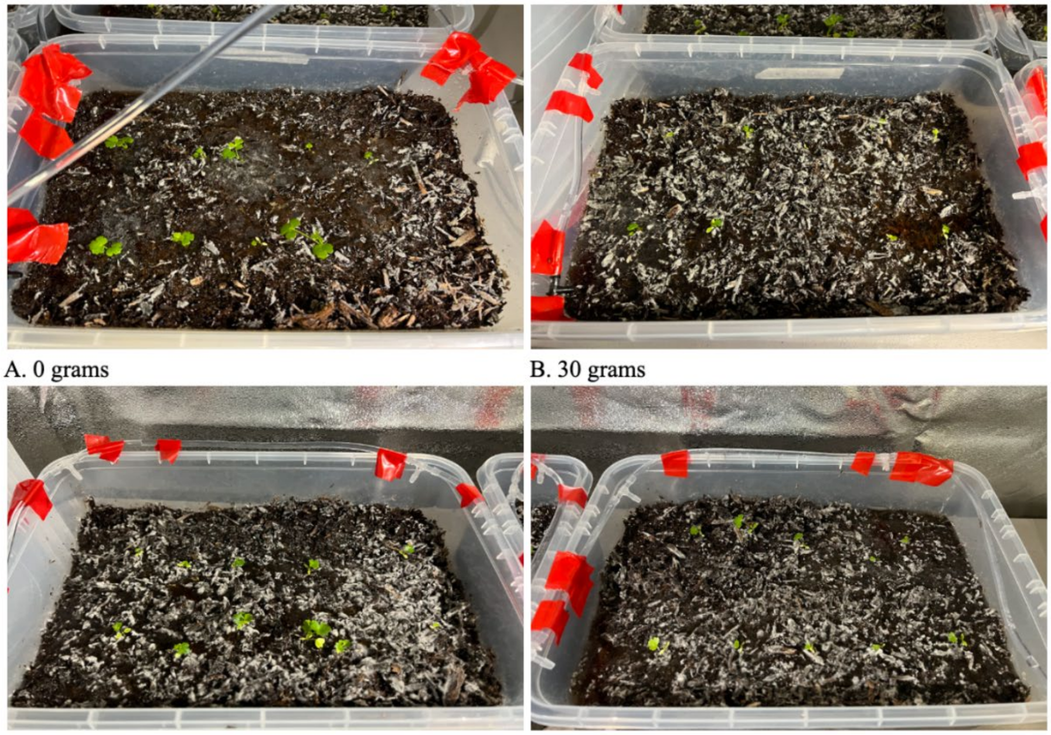
Based on the results, the control group had the greatest increase in height (Figure 1). The height and growth of plants is correlated with the absorbance of nutrients such as light and water that can alter the rate of photosynthesis and affect the height of plants (Proulx, 2021).

Table 2. Mean number of leaves on day 78 in the control group, group one, group two, and group three. Each group contained ten self-blanching celery plants. The number of leaves in each of the ten plants in each group was counted on day 78 and the average of the number of leaves of each group was calculated.

Group	Mean number of leaves
Control	4
#1	2.4
#2	3
#3	3.1

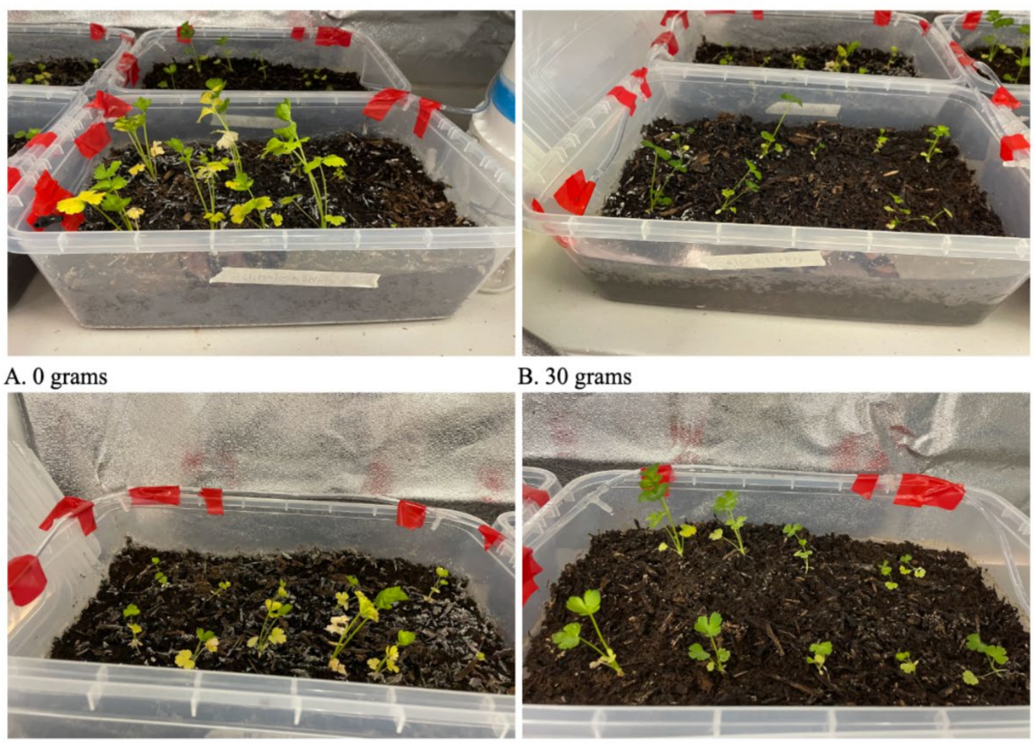
Average Number of Leaves Chart

The number of leaves are an indication of the amount a plant photosynthesizes, and the more leaves the plant contains the more nutrients and resources it has to grow and photosynthesize. A greater number of leaves correlates to healthier plants (Plant growth—patterns, limitations and models, n.d.). The control group (2A) contained the plant with the greatest average amount of leaves and group 2B contained the lowest mean number of leaves (Table 2).



A. 0 grams
B. 30 grams
C. 100 grams
D. 250 grams

Figure 2. Day 35



A. 0 grams
B. 30 grams
C. 100 grams
D. 250 grams

Figure 3. Day 78

Leaf Color

The color of leaves provides information on whether the plants have enough resources for photosynthesis. Photosynthesis is a process that transforms sunlight (and other components) into sugars for the plants as the autotroph's food source. If a plant isn't getting enough sunlight, it cannot convert the other components into food for energy, which can cause the plant's leaves to turn yellow ("When Plant Leaves Turn Yellow", n.d.). Photosynthesis uses chlorophyll, which does not absorb light energy from green light waves and instead reflects the green light waves (Photosynthesis, n.d.). Therefore, a higher rate of photosynthesis results in greener leaves. Group D contained the most green pigment within its plants on day 78 (Figure 3).

Discussion

Watering Fluctuations

In order to prevent the celery plants from overwatering, the amount of water was reduced from 200 mL every day to 100 mL every day starting from day 55 of the experiment. Research proves that overwatered celery droops and wilts due to a burst of cell walls ("How Much Water Does Celery Need", 2023). AMF cannot fix its own carbon since it does not photosynthesize, so it receives its carbohydrates from the host plant and absorbs nutrients from the soil which are passed along to the plant (Wyatt, Arnall, et. al, 2019). Emptying the excess water out of the bins may have extracted some nutrients from the soil. These water fluctuations may have altered the results discovered once the experiment was complete.

Saprophytic Fungus

After 20 days of watering the plants, a powdery white film appeared over the soil (Figure 4). This white mold is saprophytic fungi, a common fungus that grows on the surface of soil, usually on plants growing indoors (Frąc et al., 2019). Research indicates that this fungus is harmless to the plant (Lucca, 2007). Since the celery plants were grown inside a greenhouse with no direct sunlight, this fungus layered over the soil (Frąc et al., 2019). Another cause for the growth of this fungi was overwatering (Knodel et al., 2009). Overwatering at the start of the experiment ended up flooding the plants, which created moist conditions that were suitable for the growth of saprophytic fungus. In order to eliminate the fungi, the top layer of soil was removed from each bin and watering levels were reduced.

Figure 4. Saprophytic Fungus

Mutualistic Relationships

In the case of this experiment, the project was controlled in an indoor environment where water was provided to the plants daily. Usually, plants and fungi have a mutualistic relationship in which the fungi help the plants capture water and bring it closer to its roots (Mutualistic Relationships, n.d.). Given that the plants were provided enough water, the environment and conditions of the plants were not able to accurately represent the benefits of utilizing AMF with plants. In addition to capturing water and nutrients for the plants, AMF also fights against pathogens to keep the plant healthy (García-Garrido et al., 2002). However, since this was a controlled experiment, the soil was not exposed to any pathogens for the AMF to fight against. Recent studies reveal that AMF activates defense regulatory genes to fight off diseases; however, since the plants were indoors and protected throughout the duration of the experiment, there were no environmental stresses for the fungi to protect against, thus defeating its purpose (García-Garrido et al., 2002). In addition, a reduced or limited amount of sunlight such as the light in this experiment impairs plant growth. In one study, the findings suggest that microbial root symbionts create additive costs resulting in decreased plant fitness under light-limited conditions (Yang et al., 2020). Additionally, a study observed that both the quality and quantity of light can affect colonization by mycorrhizal fungi. Root colonization is negatively impacted by light deprivation. This can alter the effect of AMF in a favorable, unfavorable, or neutral way (Dunn et al., 2017).

Change in Experimentation

By day 78, the original hypothesis that AMF would increase celery plant growth was not supported due to an ineffective mutualistic relationship between the AMF and plant. In order to see the effects of the fungus in realistic conditions, the watering system was set to water the plants once a week with 150 mL instead of every day with 100 mL and the plants were monitored for an additional 34 days. This stimulated a more realistic, dry environment for celery plants. With the reduction in water, there were drastic changes. The control group lost the most leaves while the plants with fungi were able to sustain the dry conditions (Figure 5). The fungi were able to store excess water during drought conditions and release it back to the plant when it needed it to increase the survival chance of the plant (Begum et al., 2019). The AMF captured water from the soil and brought it closer to the roots while the plants provided the necessary resources to keep the fungus alive. This mutualistic relationship kept both the plants and fungus alive in the dry soil. Overall, the original hypothesis was better supported in drier conditions with significantly less water.

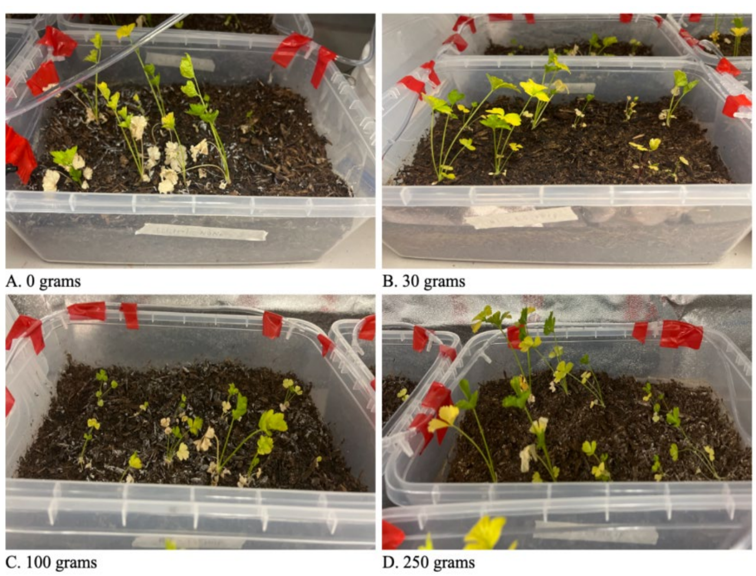


Figure 5. Day 112

Conclusion

The insight gained from this experiment is that the amount of water given to the plant and the effectiveness of AMF are greatly correlated. When the ideal amount of water is available, AMF is not effective. This experiment determined that AMF can damage the growth of Golden Self-Blanching Celery and limit the rate of photosynthesis if the plant is placed in optimal conditions with constant light and optimal amounts of water. Future research on this topic can be done by continuing the experiment with low amounts of water in each group to simulate celery plants growing in dry areas. This can provide more of an insight into how effective AMF is in realistic conditions where a majority of celery is grown, drought-stricken areas like California. In addition, it would be beneficial to conduct the same experiment and test whether or not the amount of sun exposure and placing plants in ground soil affects the way the fungus benefits the celery plants. Such conditions would emulate a more authentic environment conducive to the potential growth of plants.

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References

Arnall, Brian, et al. “Nutrient Loss and Water Quality - Oklahoma State University.” Nutrient Loss and Water Quality | Oklahoma State University, 1 July 2019, https://extension.okstate.edu/fact-sheets/nutrient-loss-and-water-quality.html#:~:text=Drainage%20of%20soil%20water%20can,move%20beyond%20the%20root%20zone.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N and Zhang L (2019) Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. Front. Plant Sci. 10:1068. doi: 10.3389/fpls.2019.01068

Besemer, Tracey. “Why Is There White Mold on My Houseplant Soil & How Do I Fix It?” Rural Sprout, 3 Jan. 2022, https://www.ruralsprout.com/houseplant-white-mold/#:~:text=The%20fuzzy%20growth%20of%20white,Mushrooms%20are%20a%20great%20example.

Briggs, G. M. (n.d.). *Chapter 25: Plant Growth-patterns, limitations and models*. Inanimate Life. https://milnepublishing.geneseo.edu/botany/chapter/plant-growth-patterns-limitations-and-model/#:~:text=As%20would%20be%20expected%2C%20plants,plants%20with%20less%20leaf%20area

Boeckmann, Catherine. “Celery.” Almanac.Com, 23 May 2023, www.almanac.com/plant/celery.

California Department of Water Resources. (2015). Drought. Ca.gov. https://water.ca.gov/Water-Basics/Drought

Cal Fire. (2019). *Welcome to Stats & Events*. Ca.gov. https://www.fire.ca.gov/stats-events/

Celery. (n.d.). Www.agmrc.org. Retrieved May 29, 2023, from https://www.agmrc.org/commodities-products/vegetables/celery#:~:text=During%202017%2C%20U.S.%20consumers%20used

Definition of SOILBORNE. (n.d.). Www.merriam-Webster.com. Retrieved May 30, 2023, from https://www.merriam-webster.com/dictionary/soilborne#:~:text=%3A%20transmitted%20by%20or%20in%20soil

De Lucca, A. J. (2007). Harmful fungi in both agriculture and medicine. Revista Iberoamericana de Micologia, 24(1), 3–13. https://pubmed.ncbi.nlm.nih.gov/17592884/

Dunn, B., Leckie, R., & Singh, H. (2017, April 1). *Mycorrhizal fungi - Oklahoma State University*. Mycorrhizal Fungi | Oklahoma State University. https://extension.okstate.edu/fact-sheets/mycorrhizal-fungi.html#:~:text=Plants%20that%20do%20not%20respond,%2C%20rhododendron%2C%20sedge%20and%20spinach

Food Production and Population Growth: A Cautionary Tale. (n.d.). Center for Commercial Agriculture. https://ag.purdue.edu/commercialag/home/paer-article/food-production-and-population-growth-a-cautionary-tale/#:~:text=The%20Food%20and%20Agriculture%20Organization

Frąc, M., Hannula, S. E., Bełka, M., & Jędryczka, M. (2018). Fungal biodiversity and their role in Soil Health. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.00707

García-Garrido, José M, and Juan A Ocampo. “Regulation of the Plant Defence Response in Arbuscular Mycorrhizal Symbiosis.” Academic.Oup.Com, 2002, academic.oup.com/jxb/article/53/373/1377/457415.

Jdlanier. (2016, January 15). *The Impact of Salts on Plants and How to Reduce Plant Injury from Winter Salt Applications*. Center for Agriculture, Food and the Environment. https://ag.umass.edu/landscape/fact-sheets/impact-of-salts-on-plants-how-to-reduce-plant-injury-from-winter-salt#:~:text=Sodium%20and%20chloride%20ions%20separate

Kollea Automatic Watering System, Indoor Plant Self Watering System. (n.d.). Kollea. Retrieved June 1, 2023, from https://www.kollea.com/product-page/kollea-automatic-watering-system-indoor-plant-self-watering-system

Knodel, J. J., Kinzer, K., & Smith, R. (2009). Houseplants: Proper Care and Management of Pest Problems. *NDSU Extension Service*.

Lazicki, P., Geisseler, D., & Horwath, W. (n.d.). Celery Production in California Historic Background. Retrieved May 20, 2023, from https://apps1.cdfa.ca.gov/FertilizerResearch/docs/Celery_Production_CA.pdf

Mohammadi, K., Khalesro, S., Sohrabi, Y., & Heidari, G. (2011). A Review: Beneficial Effects of the Mycorrhizal Fungi for Plant Growth . *Journal of Applied Environmental and Biological Sciences*, *1*(9), 210–119.

Peppershomeandgarden. “How Much Water Does Celery Need?” Pepper’s Home & Garden, 11 Nov. 2022, peppershomeandgarden.com/how-much-water-celery-need/.

Photosynthesis. Education. (n.d.). https://education.nationalgeographic.org/resource/photosynthesis/

Proulx, R. (2021). On the general relationship between plant height and aboveground biomass of vegetation stands in contrasted ecosystems. *PLOS ONE*, *16*(5). https://doi.org/10.1371/journal.pone.0252080

Savci, S. (2012). An Agricultural Pollutant: Chemical Fertilizer. *International Journal of Environmental Science and Development*, *3*(1), 1–4.

Top 20 Fruits and Vegetables Sold in the U.S. 2020. (2021, November 12). International Fresh Produce Association. https://www.freshproduce.com/resources/consumer-trends/top-20/

Yang, X., Mariotte, P., Guo, J., Hautier, Y., & Zhang, T. (2021). Suppression of arbuscular mycorrhizal fungi decreases the temporal stability of community productivity under elevated temperature and nitrogen addition in a temperate meadow. *Science of The Total Environment*, *762*, 143137. https://doi.org/10.1016/j.scitotenv.2020.143137

Yang, Y., Han, X., Liang, Y., Ghosh, A., Chen, J., & Tang, M. (2015). The Combined Effects of Arbuscular Mycorrhizal Fungi (AMF) and Lead (Pb) Stress on Pb Accumulation, Plant Growth Parameters, Photosynthesis, and Antioxidant Enzymes in Robinia pseudoacacia L. *PLOS ONE*, *10*(12), e0145726. https://doi.org/10.1371/journal.pone.0145726

Yang, Y., Liang, Y., Han, X., Chiu, T.-Y., Ghosh, A., Chen, H., & Tang, M. (2016). The roles of arbuscular mycorrhizal fungi (AMF) in phytoremediation and tree-herb interactions in Pb contaminated soil. *Scientific Reports*, *6*(1), 1–14. https://doi.org/10.1038/srep20469