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Introduction of Rising Researchers

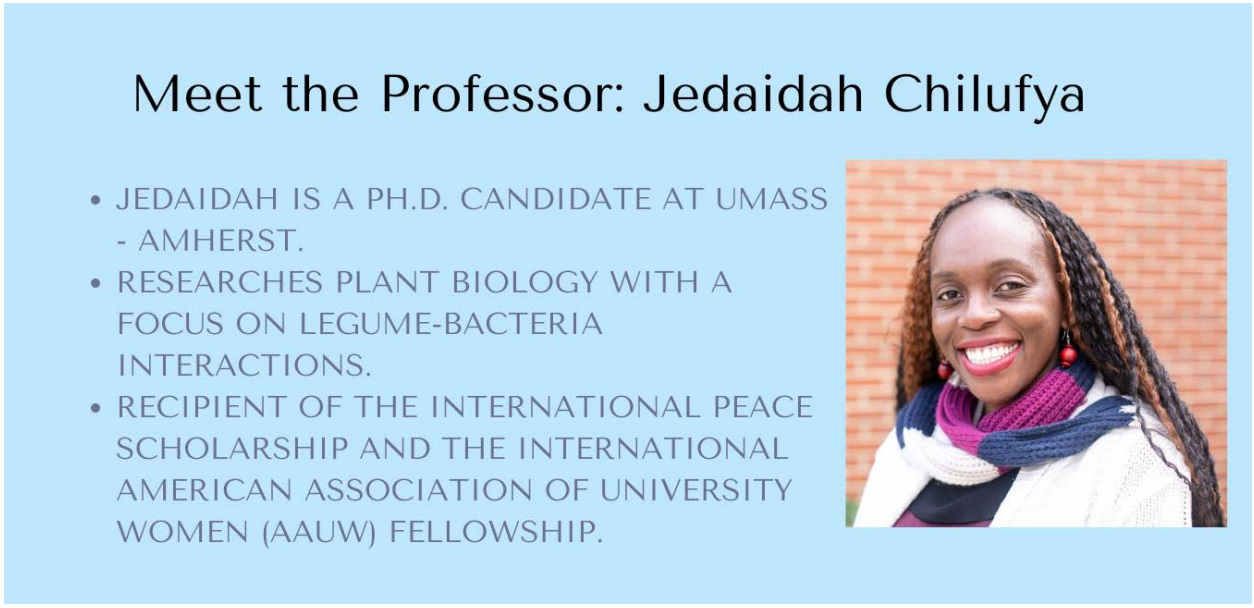
This edition highlights students who participated in the four-week virtual research intensive. The course was designed to provide students with an introduction to the principles of scientific and microbiology research. Through lectures, hands-on experiments at home, class, and small group discussions, students delved into the microbial world with an emphasis on the interactions of microorganisms with humans, plants and their impact on disease and agriculture.

The hands-on experiments emphasized the proper use of the scientific method to answer a research question, regarding host-microbe interactions, including Soil Microbiology, Rhizobia bacteria-legume symbiosis and Microbial genetics and identification of bacteria from host tissue.

Learning Units:

- Scientific method and introduction to general microbiology
- Host-microbe interactions, including Soil Microbiology
- Rhizobia bacteria-legume symbiosis
- Microbial genetics and microbiological identification of bacteria from host tissue

Academic Credit: 2 College Credits + Certification of Completion Issued by University of Massachusetts Amherst (UMass)



Rising Researchers' Students
Aareev Panda

To Determine Microbiome Effects on Legume Plant Growth in California

ABSTRACT:

My research project surrounds determining the microbiome effects on legume plant (scientific name crotalaria juncea) growth. This is important since this information can be used for people who want to help their plants grow beyond normal expectations. The purpose of my research was to figure out the answer to the main prompt. There isn't much info in my state about this specific subject and the type of soil I used so it may be useful for other researchers. The plant microbiome plays a major role in determining plant health and overall growth, it can also affect productivity. The plant microbiome has miniscule bacteria that can change these things. This information was crucial to my experiment because I could use it to come up with a hypothesis. The aim of this project is to answer the main question and determine how the microbiome can affect legume plant growth. I used legume plants specifically since they have a symbiotic relationship with a bacteria called rhizobia bacteria which provides nitrogen nutrient to the plant for its growth. This bacteria has been shown to effectively colonize root surfaces. This happens in the rhizosphere, which is a small area in soil where microorganisms have interactions with plant roots. The general methods for conducting the experiment were to plant legume plants, observe the root nodule formation, isolate the bacteria on it after sterilizing, and watch it grow for 2 days. My hypothesis was that the positive control would have the most bacteria and nodule turnout. The final results for the experiment showed how bacteria interacts with roots in a controlled setting, with sterilized roots.

INTRODUCTION:

My research project surrounds determining the microbiome effects on legume plant (scientific name *Crotalaria juncea*) growth. The aim of this project is to answer the main question and determine how the microbiome can affect legume plant growth. The specific aim for this project was to collect soil from two locations at my house in Palo Alto, California. After getting the soil, the plan was to plant the legume seeds and observe the growth of these seeds in different types of soil. The root nodules were to be microscopically observed and written down in the E-lab notebook. Lastly, I followed the protocol to isolate rhizobia bacteria from the roots of the legume plants. The point of getting all of this data was to determine the answer to the main question: How does the microbiome affect legume plant growth?
I hypothesized that the backyard soil would end up with the least nodules and rhizobia, just because it was kind of normal soil with nothing special. The positive control contains known rhizobia bacteria that interacts with several legume plants. The front yard/compost will have a decent amount of nodules and bacteria, but not the most because the soil was better for plant growth. I am focusing on the microbiome, and getting involved with microscopes and bacteria. I predicted that bacteria would help the plants grow and overall improve the health of the plant by providing them with nitrogen nutrients.

MATERIALS AND METHODS:

For this project, the methodology for conducting research was to observe each of the pots and write down data in the e-lab notebook. The materials used were: Planting pots and trays, digging tools, spade and fork, soil/dirt, glass of water, wooden tweezer, legume seeds. First, I gathered soil from 2 different locations with different types of soil. I then planted legume plants in each of the pots of soil. I watered them for two weeks every other day and once the legume plants started to grow, I marked down all the information about my experiment such as the amount of water given per day, the amount of seeds planted, size of plants, etc. Throughout the experiment, I tracked the grown plants and in-progress plants, and microscopically observed the root nodules. I noted down how many root nodules were found at the root of the plant by using a microscope to zoom in on it. I also collected data about rhizobial bacteria isolated from root nodules. Lastly, I isolated the bacteria from the root nodules by using sterilized water and sodium hypochlorite and cultured the bacteria on differential media for two days. All these experiments together were used to determine the answer to the main research question.

RESULTS:

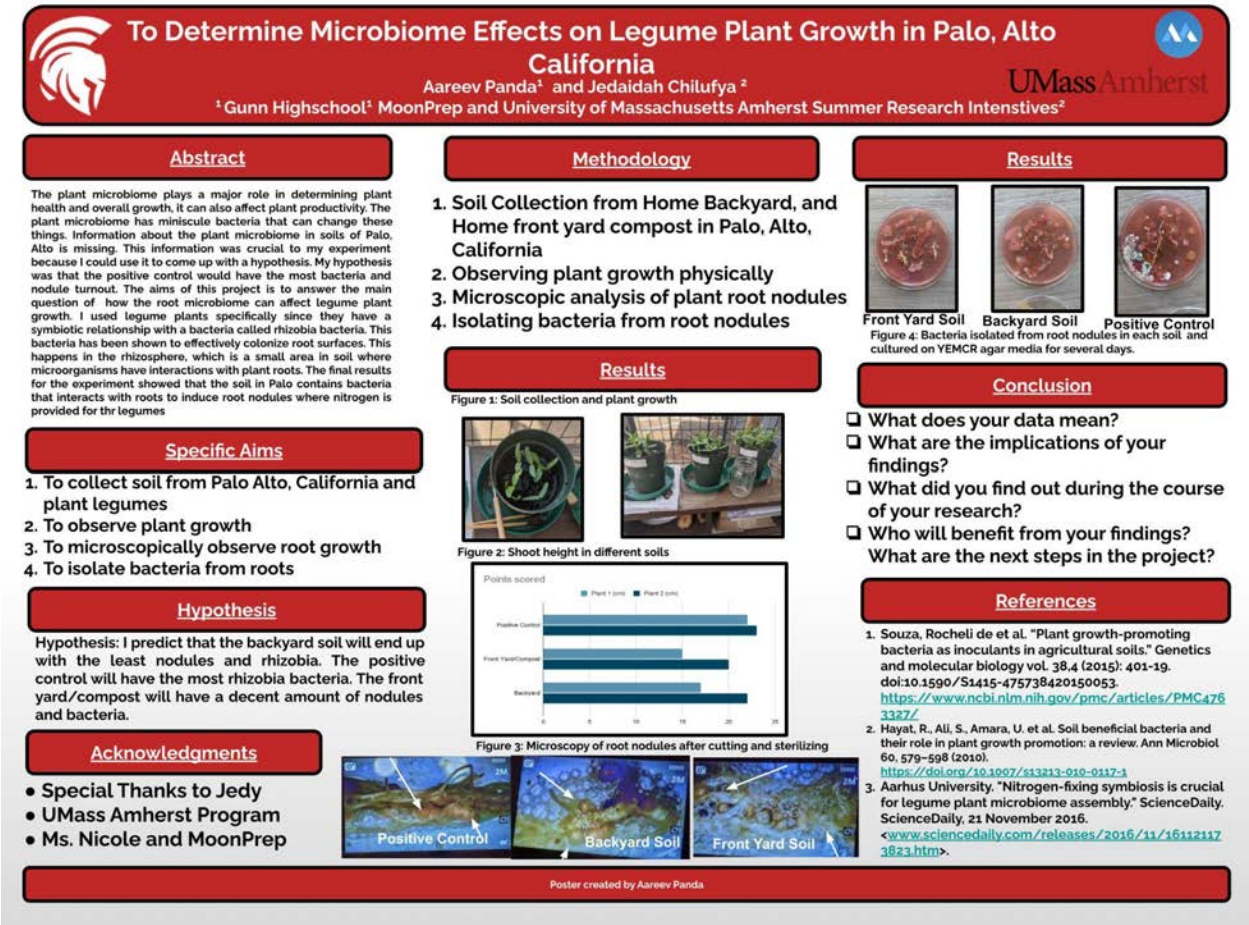
The results for this experiment are included in the photos here. In figure 1, you can see how I planted the legume seeds and their progress in growing. I watered them every other day for about 2 or 3 weeks. Figure 2 shows the difference in shoot heights of the plants in different soils. A ruler shows the height on the right side of the image. Figure 3 was where I used a microscope to zoom in on the root nodules of the different plants and counted how many were in each. I did this for different stages throughout the weeks, multiple times. The final results of this experiment in figure 4 shows how bacteria is related to the plant microbiome, and how root nodules play a major role in this. The root nodules are where bacteria reside. If we see pink nodules, then these bacteria are providing nitrogen for the plant. There are a lot of bacteria-like figures surrounding the original root nodules placed in the agar media plates. This is especially apparent in the positive control because there are even some green/white figures in the agar media plate

DISCUSSION:

This data gives us a lot of insight into how bacteria works and how they can help plants too. The rhizobia bacteria interacts with legume plants by entering the legume roots and induces a special root structure called nodules. Root nodules are formed when the legume plant allows rhizobia bacteria to infect their roots. The nodules are used to convert nitrogen in the air into ammonia for the plant to grow healthier. This is the symbiotic relationship between the legume plants and rhizobia bacteria. In the end, my hypothesis was partially supported, even though a few things went differently. Some things I found out during the course of my research are about plant care, and tips on how to improve the health of plants. I learned that different types of soil can affect how a plant grows so it's important to think about this before planting. People who can benefit from these findings are botanists, who work in the field of agriculture and plant science. This information may be useful in the future, to utilize it for healthier and stronger plants. The next steps in this project would ideally be to try this same project with different plants and soils to see how bacteria can affect this as well. I would like to continue future research in this field by experimenting with what are the infections and side effects of this method. This will avoid causes of

deceased plants and improve plant growth.

Acknowledgements:
I would like to acknowledge Jedy because she helped me a lot with my poster and my project overall. I had scheduling issues with the class so she really helped me catch up with the class and finish my experiment. I want to thank the UMass Amherst program too for the opportunity to learn this much about a subject I've never really considered learning about. Also Ms. Nicole helped me with my resume and taught us about college opportunities and information about that so thank you.



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Aditi Katragunta

To Determine How Microbes Interact With Legume Seeds In Ellicott City, Maryland

Abstract

In order for plants to survive, you must rely on the sun and soil. For plants, nitrogen found in soil is very important for growth and productivity, so there are bacteria such as Rhizobia Bacteria that help plants such as legume plants absorb the nitrogen in the soil. In this research project, legume seeds are planted in three types of soil, coming from two locations. The positive control used soil that had not been planted in from the garden, mixed with rhizobia bacteria known to aid legume plants in gro. The other two soils came from the backyard and garden in Ellicott City. The legume seeds were planted, monitored and inspected for a length of 4 weeks, where procedures were done to examine the roots and nodules of the plants in each soil type. The observations consisted of recording the length of the roots, phenotyping the plants and examining the nodules on the roots under a microscope from weeks 2.5 to 4. The results displayed that the backyard soil was ideal soil for the legume plants, with that soil type having the most plants per pot, best health per plant and overall more nodules on the roots. After 4 weeks, the positive control had the most nodules and the garden soil was unsuited for the legume plants.

Introduction

Nitrogen is an essential element for plant growth. There is an abundance of nitrogen in the atmosphere, however plants cannot obtain nitrogen in this form. Rhizobia bacteria is one type of bacteria that aids legume plants to absorb nitrogen. There is no information about the presence of rhizobia bacteria in Ellicott City, Maryland that interact with *Crotalaria juncea* legumes. Therefore, in this research, soil from two different locations in Ellicott City, Maryland were collected, along with a positive control containing bacteria known to aid legume growth. The question asked in this experiment was which soil in Ellicott City would be best suited for the growth of *Crotalaria juncea* legume?. To effectively answer this question, procedures were done by observing the nodules of the *Crotalaria Juncea* legume plants to understand if the nutrients, especially nitrogen, is abundant in a certain soil.

Materials and Methods

To approach this question, materials and methods were needed to understand and

articulate. The methods used were soil collection, legume planting, shoot and root observation, microscopy of nodules, isolation of Rhizobia bacteria, and Rhizobia identification. The first week, the soil collection and planting legume seeds, the materials needed were 3 planting pots and trays, digging tools, spade and fork, 3 soil types, bottle of water, wooden tweezer and legume seeds. The methods used were for the pots to be labeled and filled with their respective soils, the 15-20 one-inch holes made and inside a legume seed was placed. After this, the pots were placed in an area of sunlight where they could be monitored. Going into week 2, plant monitoring, all the materials used were a bottle of water to water the plants and a device to record the plant growth. Every two days, the plants were watered around 1⁄3 cup each pot. In week 3, phenotyping plant shoots and roots, the materials used were gloves, safety goggles, legume plants, digging tools and a bottle of water. Between days 17-19, a total of 6 plants were taken out of the pots and their roots washed off any excess soil. Then the plants were photographed for record. The plants were placed under a microscope and again photographed. After this process, the plants were put back into their pots. In this process, two plants were too damaged to be placed into their respective pots. Also during week 3, another experiment was used to isolate bacteria from the roots. This procedure used nodules collected from the plants from each soil type, microcentrifuge tubes and plastic pestles to simulate a centrifuge and agar media plates/ petri dishes to isolate the bacteria. In week 4, the procedure of isolating the bacteria was completed and recorded. Also in the last week, the plants were phenotyped one last time and recorded, completing the procedures and giving enough information to answer the question above.

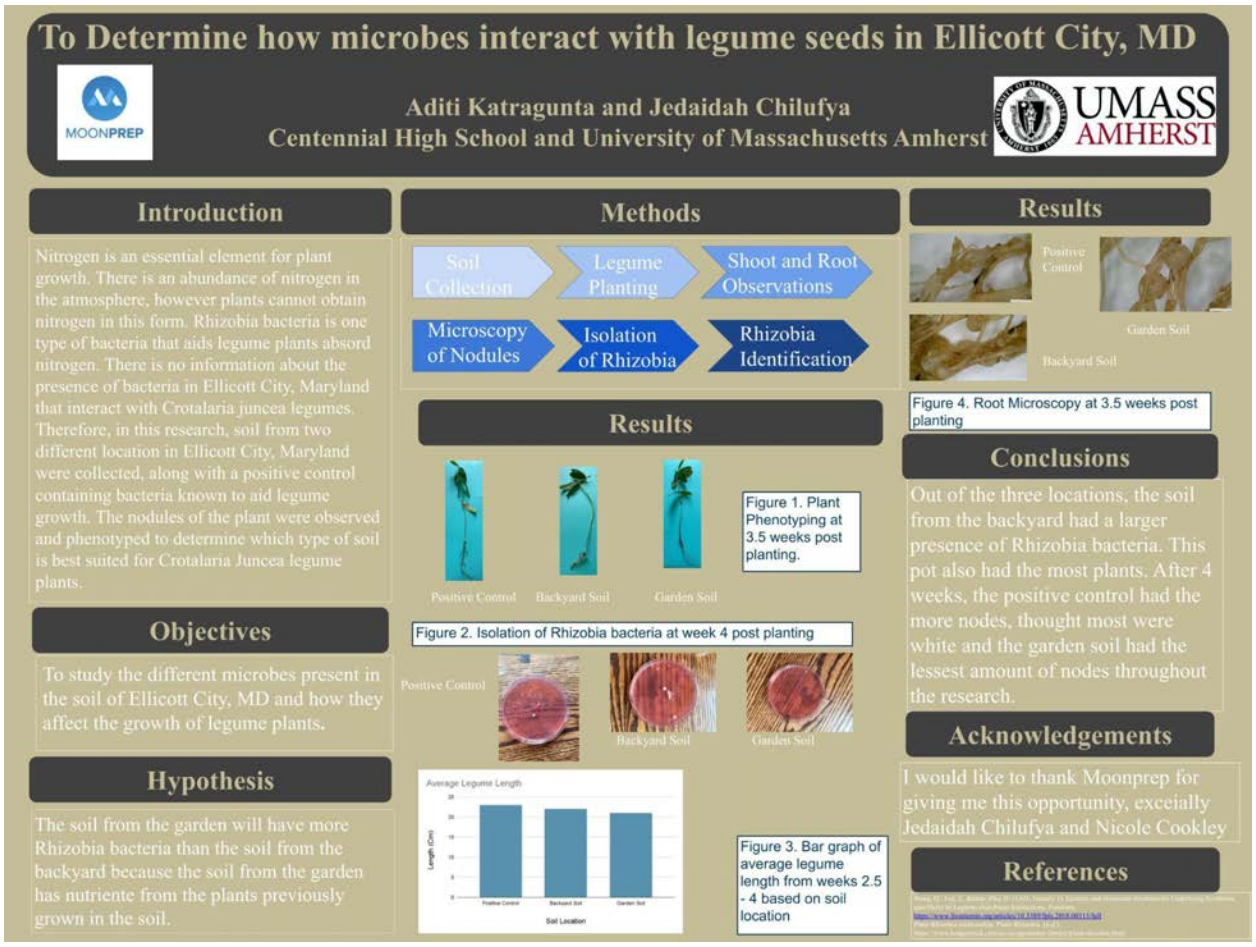
Results

The backyard soil did the best overall with most plant growth within the pot, stronger roots, shown in the isolation bacteria procedure and each plant had a good amount of healthy nodules. The garden soil was presented at second, with a slow start. The garden soil started weak, with fragile roots and very few to no nodules, but by the end of the 4 weeks, the garden plants became strong with healthy nodules. At last, the positive control had weak roots and few nodules, though the nodules were shown to be full of bacteria in the isolation procedure.

Discussion

The most significant results were that the backyard soil was best for sustaining legumes seeds. The procedures of isolating bacteria and phenotyping displayed that the backyard soil was strong in all aspects of the testing. This finding shows that the backyard soil in Ellicott City, Maryland is the best suited soil out of the three soil types to grow and germinate legume seeds. My hypothesis was that the garden soil would do the best because of the nutrients left by the previous plants used in the area, but thai was proved wrong. What proved to be a shock was the legume plants in the positive control. The soil in the positive control was mixed with fertilizer known to aid legumes and give them the minerals they need, but the positive control fared similar to the garden soil and when compared to the backyard soil, did not provide the legume plants with the best environment. Knowledge that the soil in our backyards

may have more minerals and nutrients than store-bought soil that are used in gardens can be used to learn how to better improve soil. The backyard soil may have nutrients that soils from other locations may be lacking. This experiment can open doors to finding out why the backyard soil is good and learning how to improve fertilizers to aid others in growing plants. This experiment shows that out of the soil types, the backyard soil proved to be best suitable for Crotalaria Juncea legume plants to thrive.



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Aditri Balaji

Identifying The Bacteria Present In Legume Plants

In order for plants to properly live and thrive they form beneficial relationships with bacteria in the soil. Specifically, legume plants require the presence of rhizobia bacteria in the soil they're growing in order to get the necessary nitrogen for plant growth (PubMed). Little is known about the presence of nitrogen fixing rhizobia in Boxborough that interact with sunn hemp legumes and affect their growth. Therefore, in this research, soil from two different locations in Boxborough, Massachusetts were compared to a positive control containing commercial inoculant that has known legume-benefiting bacteria. Plant growth and formation of root nodules were observed and studied in order to determine which soil had the best rhizobia bacteria to enhance the growth of the sunn hemp (*Crotalaria juncea)* legume plants in Boxborough, Massachusetts.

Methods

- Soil collection - about ¾ of the pot was filled with soil from the designated location (1 pot of Location 1 soil and 2 pots of Location 2 soil), commercial inoculant was mixed in with one of the pots containing Location 2 soil to create the positive control
- Legume planting - about 20 legume seeds were planted into each pot of soil in holes about 1 inch deep, the three pots were watered every other day and placed directly in front of a window
- Phenotyping shoot and root - the plants were dug out of the pots and the roots were washed, the plants were placed on a dark background next to a ruler to record their shoot heights and colors. The roots were then placed under a microscope to take a closer look at the roots and see if there were any nodules
- Microscopy of Nodules - legume roots were observed under a digital microscope to see the number of nodules present and what colors they were (pink nodules meant nitrogen fixing bacteria and white nodules meant not nitrogen fixing)
- Isolation of Rhizobia - nodules were sterilized and crushed then placed on agar media plates to grow at room temperature, 3-4 days after initial isolation bacteria colonies began to grow (white colonies meant nitrogen fixing bacteria and red colonies meant not nitrogen fixing)
- Rhizobia Identification - after taking the results from the phenotyping, microscopy, and isolation, I was able to determine if the bacteria present in the soil was nitrogen fixing or not
- PPE in the form of safety glasses and gloves was worn to ensure no contamination of the plants and that the plants only had contact with the microbes in the soil they were planted in

Main Materials

- Legume plants
- Microscope

- Agar media plates
- Sterilizing 70% ethanol
- Microcentrifuge tubes
- Plastic pestles
- Scalpel

Results

Phenotyping of plant shoots took place at 2.5 weeks post planting where both the positive control and the woods soil had much taller and a darker green shade shoot while the backyard soil had a shorter and more lightish green tint. The shoots were measured by placing a ruler next to a dug out plant and measuring the shoot from the bottom of the shoot, or the top of the roots, to the top of the shoot, not including the top of the leaves. In average, the positive control was 20.5 cm tall, the wood's soil was 24.5 cm tall, and the backyard soil was 18 cm tall. Later, at 3.5 weeks post planting the root nodules of the plants were observed under a digital microscope to observe the number and color of the root nodules present. All three of the legume plants had 2-3 nodules on the roots. At this time, the nodules on all of the plants were small and white. However, after many further microscope examinations, the positive control and the wood's soil were shown to have larger pink nodules while the backyard soils' nodules were still small and white at 4-5 weeks post planting. After the isolation of rhizobacteria from the root nodules and letting it grow for 3-4 days, the positive control only had white bacteria colonies, the wood soil had more white colonies than red, although red colonies were present, and the backyard soil only had red colonies.

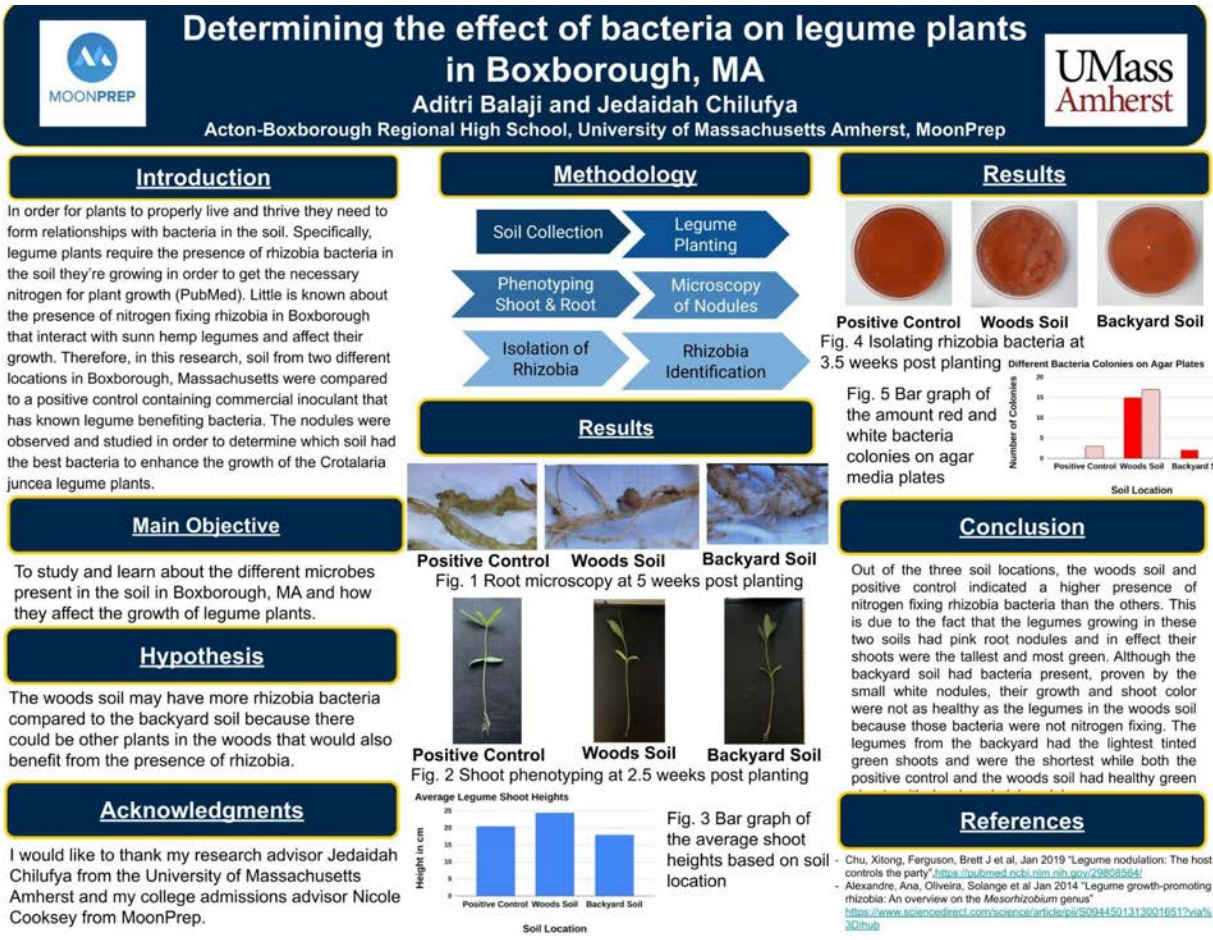
In a plant, chlorophyll is the site where photosynthesis takes place and it is also the substance that gives plants their green color. Since nitrogen is a key component of chlorophyll, photosynthesis can occur at higher rates if the plant has enough nitrogen, resulting in a taller shoot. A sufficient source of nitrogen will also allow the plant to develop a dark green color opposed to lightish yellow color if the plant is nitrogen deficient (University of Hawaii, Manoa). Since the results show that the positive control and the wood soil have taller, darker green shoots compared to the shorter lighter green shoots of the backyard soil, it is an indication that the positive control and wood soil have nitrogen fixing rhizobia in the soil compared to non-nitrogen fixing bacteria.

Nitrogen fixation is the process where an unusable form of nitrogen in the air changes into a form of nitrogen that plants can use by nitrogen fixing rhizobia bacteria (NMSU). Whether this bacteria is present or not in the soil can be determined by the color of nodules on the roots of the legume plant. If the nodules present are white, the rhizobia is not nitrogen fixing opposed to pink nodules indicating nitrogen fixing rhizobia. Although all of the nodules present were white at the beginning, after more weeks of growth the positive control and wood soil developed 1-2 pink nodules while the backyard soil still only had white nodules. This shows that the rhizobia present in the positive control and backyard soil was nitrogen fixing bacteria in accordance to the data from the phenotyping analysis of the legume shoots.

The last indicator done to determine which soils had nitrogen fixing bacteria present was to isolate the rhizobia from the root nodule. The bacteria was isolated from the root nodules and put on agar media plates to allow for the bacteria to grow. White colonies on the media plate would result from nitrogen fixing bacteria and red

colonies from non-nitrogen fixing bacteria. Since the positive control only had white colonies, the rhizobia present in the soil was nitrogen fixing. The wood's soil had both white and red colonies but more white than red, indicating that although there was non-nitrogen fixing bacteria in the soil, there was fixing rhizobacteria as well. However, the backyard soil only had red colonies, showing that all of the rhizobia in that soil was not nitrogen fixing.

After doing photyping analysis, microscopy, and isolating bacteria I was able to determine that, in accordance with my hypothesis, the woods soil had nitrogen fixing bacteria and the backyard soil did not. This was seen through the taller and darker green shoots, pink nodules, and white bacteria colonies. The initial hypothesis was created due the fact that it was possible for there to be other rhizobia needing plants in the woods, causing a presence in nitrogen fixing bacteria in the soil, compared to the backyard soil where no leguminous plants were present. Some further work that could be done is to study the other differences between the wood soil and backyard soil that may have also contributed to the large difference in shoot height between the plants.



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Akash Raman

Effects Of Pesticides And Fertilizer On Rhizobium Bacteria In The Soil In Michigan And Growth Of Legume Plants

Abstract

The use of pesticides and fertilizers in modern city parks is very common, even recommended. However, in addition to their benefits, there are some known harmful characteristics of these man-made chemicals that prevent the growth of plants. The purpose of this experiment was to demonstrate the effects of pesticides and fertilizers on the growth of the shoot of legume plants, and also the number of bacteria-containing nodules on the roots of the plants. There are 6 main steps taken to address this research topic: 1) Soil Collection, 2) Legume Planting, 3) Shoot and Root Observations, 4) Microscopy of root nodules, 5) Isolation of rhizobia and 6) Rhizobia identification.

Introduction

Nitrogen is an essential component, and it is necessary for all organisms to survive. This element plays a huge role in the construction of DNA and amino acid molecules. Specifically with respect to plants, they need to undergo a process called nitrogen fixation to obtain nitrogen (Walley 2013). Leguminous plants have evolved a unique and effective system employing symbiosis with rhizobia bacteria in order to obtain their nitrogen. These bacteria live in nodules on the roots of the plant. Therefore, the plants benefit the bacteria by giving them a place to live and grow in their root nodules, and the bacteria help the plant by sequestering and fixing nitrogen very efficiently (Mylona 2018). Although there is not much information present to clearly understand unique qualities of the rhizobia bacteria in Novi, Michigan, there is a harmful effect of pesticides on the symbiotic interaction of rhizobium bacteria with leguminous plant roots. These agrichemicals not only affect the growth and plant-beneficial physiological activities of rhizobia but also inhibit the molecular signaling between rhizobia and the host legume plants essential to establish symbiosis. (Ahemad 2013). The purpose of this research project is to further analyze the effect of pesticides and fertilizers on the symbiosis between the rhizobium bacteria and legume plants through observation of the number of nodules and growth of the

plants. The purpose of my experiment is to show the effects of pesticides and fertilizer on the growth of leguminous plants, using the species Crotalaria Juncea, or sunn hemp, to observe and measure these effects. I hypothesized that the plants grew in the soil at 41278 Scarborough Ln. will show more nodules in their roots than the plants in Novi Park because there is more bacteria allowed to connect with the plants in a symbiotic interaction due to a lack of excess organic chemicals.

Methods and Materials

- Gloves,
- 70% Alcohol,
- Black construction paper,
- Digital Microscope,
- Trowel,
- Spade,
- Phone
- Plants (one pot at a time)
- Digging tools : Trowel, Spade, Chopsticks
- Paper towels
- Cup/pot or beaker of water (get fresh water between pots)
- Flat surface with a dark background
- Ruler or measuring tape or print out of 15 cm ruler (from google search)
- Labels and a pen or sharpie
- Microcentrifuge tubes
- Plastic pestles (blue)
- Dropper
- Microscope slide
- Scalpel
- Sterile water in the falcon tube (or boil tap water to sterilize it)
- Household bleach (clorox or disinfectant with active ingredient sodium hypochlorite. Clorox wipes are good too.
- Agar media plates (1 agar media plate per pot) with YEMCR growth media
- Toluidine blue stain.

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Assessments and Measures

In order to start this experiment, I first gathered the soil for the three different pots. The 3 pots were labeled with the soil location names - 41278 Scarborough Ln. behind the house under the coniferous trees, and the Novi Ella Mae Power Park Parking Lot. The scooper was then used to collect soil from the first location by first digging 3 or 4 inches into the soil. After this, water was only added to make the soil from the Novi Park wet before placing the soil in the pot by hand. This process of collecting soil was repeated until the soil was at a level of the first line in the pot. Once soil was collected from location 1, soil was then collected from location 2, Novi Park in the same manner. To collect soil for the positive control, soil was collected from location 2 and this was done a little differently. Mixed in 1-2 tsp of the rhizobia bacteria. Made sure that some decomposing leaves, some twigs and wood chips were mixed as well. I planted the seeds, making sure the soil is wet enough, then made 20 one-inch-wide holes in each pot for the placement of legume seeds. Once the seeds were planted, the pots were placed inside the house in a reasonably sunny area in front of a window.

To maintain the plants and grow them, I made sure that my plants got enough sunlight by relocating them to a sunnier venue, then I made sure to check the soil to see if it was dry, and watered when necessary, Sometimes, when it was sunny outside, I would set the plants outside in a sunny area. I kept doing this process for three whole weeks to ensure growth and health.

To phenotype, or in other words, to measure and keep record of the shoot height, I first sanitized my table and prepared all necessary equipment. Then, with my spade, I dug carefully in the soil of my positive control pot, digging up a plant with the roots intact. I then washed the soil particles away from the plant in a cup of water, let the plant sit for two minutes to dry and let the roots spread out, and laid the plant on a black sheet of construction paper

next to a ruler to examine phenotypic height and took a picture of that with my phone. Next, to monitor the number for root nodules on the positive control shoots, I laid my plant under a digital microscope with the background of black construction paper and took pictures using the microscope
Then I repeated this procedure for the two remaining pots of plants. I repeated this whole phenotyping procedure with all three pots every half week in order to track the growth and progress of the plants.

At the end of 3.5 weeks into the process, I prepared to isolate the rhizobia bacteria to grow in YEMCR growth media agar plates. I first made a diluted bleach sterilizing solution by diluting into the first microcentrifuge tube by adding sterile water in the tube according to the percentage of the active ingredient sodium hypochlorite in the bleach. The sodium hypochlorite was above 5% so I added 0.75ml sterile water in the microcentrifuge tube using the dropper. I then added 0.25 ml of bleach to the microcentrifuge tube. Starting with one pot at a time, I carefully dug out all the plants in the pot, washed off soil particles from the roots and checked for root nodules by observing under the microscope. After phenotyping those plants, using the scalpel, I placed the nodules in the tube with the bleach solution to sterilize. I then closed the lid and inverted the tube gently several times. Next I used the scalpel, removing the nodule or the root from the bleach tube and placing it on the microscope slide. I then washed off the bleach from the nodule by adding several drops of sterile water on top of the nodule/small root on the microscope slide using the dropper. I moved the nodule/root segment around using the dropper, sucking water several times to completely wash off the bleach. I then placed the sterilized nodule into a new microcentrifuge tube. I then labeled this tube accordingly for each pot with PC for Positive Control, Park for Novi Park, and 41278 for my backyard. I then added 0.25 ml of sterile water into the tube with the nodule/root. I then released the bacteria from the nodule by obtaining a plastic pestle and using it to crush the nodule/root. I kept crushing the nodule/nodule until the water turned a little cloudy and you see only small remnants of plant structures in the tube. I then got one agar media plate and sealed back the rest of the plates. I poured all the crushed nodule/root suspension onto the plate and gently swirled the plate around to spread the suspension. For complete spreading of the suspension on the plate, I used a new pestle to gently spread the suspension on the entire media surface. I then sealed the agar media plate with parafilm, placed it upside down (media on top) and wrapped it in aluminum foil. I placed it near a window in order to enable more bacteria growth at a warmer temperature.

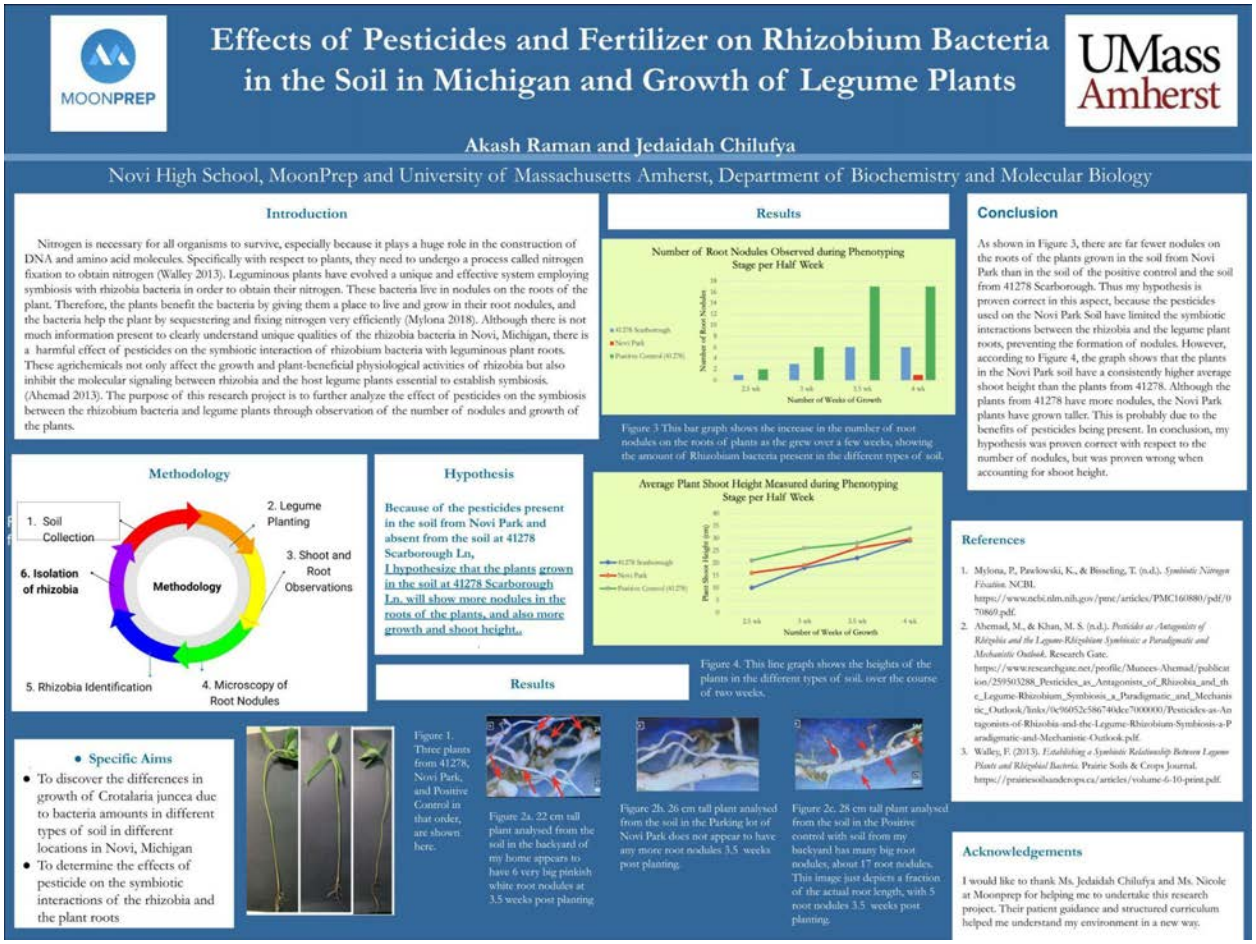
At the 4 and a half week mark, I completed the final step of my experiment: the identification of the rhizobia bacteria in the root nodules themselves. I carefully dug out all the plants from the first pot and carefully rinsed off soil particles from the roots. After phenotyping I got ready to prepare a microscope slide for one root nodule or root segment. For this I used the scalpel to cut off one root nodule/root segment onto the microscope slide. Then, I cut the root nodule in half and exposed the cut ends of the half nodules/root segments to face upwards. For observation I got a microcentrifuge tube and put about 0.75ml of water into it using the dropper. Back on the microscope, I placed a small drop of Toluidine blue stain on each cut end of the nodule. I then transferred the nodule into the microcentrifuge tube to cleanse it, and obtained a fresh microscope slide. Using the scalpel, I removed the nodule or root segment from the tube and placed it on the center of the clean microscope slide. I used the scalpel to orient or position the nodule with the cut (half) ends facing up. Then I carefully placed the cover slide/glass cover on top of the samples after having removed any excess water using a small piece of paper towel.

Results

What I found in my results was that there were significant differences between the plants grown in the soil from 41278 and the soil from Novi Park. As you can see in Figure 1, The roots from the first plant and third plant, which is the positive control, have roots that are very long and branched. They are more developed, and have many nodules, as shown in Figures 2a and 2c. The roots for the plant from Novi Park however are very small and do not have any root nodules, as shown in Figure 1 as the middle plant and Figure 2b. However, even though its roots are underdeveloped, the plant from Novi Park is 26 cm tall, while the plant from 41278 is only 22 cm tall, and the positive control is 28 cm tall. In other words, the roots are underdeveloped for the plant from Novi Park, but its average shoot height is taller than that of 41278. As shown in Figure 3, there are far fewer nodules on the roots of the plants grown in the soil from Novi Park than in the soil of the positive control and the soil from 41278 Scarborough. However, according to Figure 4, the graph shows that the plants in the Novi Park soil have a consistently higher average shoot height than the plants from 41278 Scarborough. In the YEMCR growth media plates, I observed lots of colonies grown in the positive control, a decent amount of colonies in the petri dish from 41278 Scarborough, and barely any colonies grown on the media plate for Novi Park. This further reinforces that there are more nodules with more bacteria present in the plants from 41278 and the Positive control.

Discussion

As my results show, the plants from 41278 have more nodules, but the Novi Park plants have grown taller, while the plants from the positive control have both the most nodules and the largest average shoot height. My hypothesis was proven correct with respect to the number of nodules, but was proven wrong when accounting for shoot height. I thought that since more nodules result in higher levels of nitrogen fixation, that would result in higher shoot height. Because the plants from 41278 have more nodules, I would have expected more shoot height in those plants as compared to the Novi Park plants. However, I believe that the extra fertilizer in the soil must have caused extra shoot height, even if the pesticides interfered with symbiotic activity between the rhizobium bacteria and the plant roots. This research provides many possibilities for future work because now we clearly know the effects of pesticides and fertilizer on the growth and development of the legume plants and we can conduct future research to develop pesticides that promote plant growth but do not prevent rhizobia from interacting with the plant roots. One particular potential application of this research is that it will be important to farmers who would want to understand and weigh the pros and cons of using these compounds on their leguminous plants, and even for their other crops as well. This research provides many possibilities for future work because now we clearly know the effects of pesticides and fertilizer on the growth and development of the legume plants and we can conduct future research to develop pesticides that promote plant growth but do not prevent rhizobia from interacting with the plant roots.



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Alexander Campos

Effects Of Microbes On Legumes

ABSTRACT:

The purpose of this experiment was to determine the different types of microbes in the soil in Norwich , Connecticut that interacted with sunn hemp legumes. Specifically to see if the different locations the soil was picked from had nitrogen fixing bacteria. In the end I determined that there was more nitrogen fixing bacteria in my backyard than in my front yard.

INTRODUCTION:

In my experiment I wanted to determine if my front yard and my backyard had nitrogen fixing bacteria in my soil. I initially hypothesized that my front yard would have more nitrogen fixing bacteria because earlier in the summer I had placed fertilizer in the soil to help my grass grow. I would later be proved that my initial hypothesis was incorrect.

MATERIALS AND METHODS:

To determine whether or not my front and backyard had nitrogen fixing bacteria I first obtained soil from the two locations as well as making a control group which I knew had nitrogen fixing bacteria. To do this I used a spade to dig up the soil and had a pot for each location. Once I had done that I planted 20 legume seeds in each pot and every day I would water the plants to make sure they grew. Once the shoots had sprouted I used my spade to dig out the legume plants and inspected the roots using a digital microscope to determine if there were any nodules in the roots. Afterwards I had cut off the nodule and cleaned it with the bleach. Then I washed the bleach off of the nodule using sterile water, placed the cleaned nodule into the microcentrifuge tube and added 0.25 mL of sterilized water to the tube. Then using a pestle, I crushed the nodule until the water turned a little cloudy and placed it into the agar plate to see if there were any colonies of bacteria

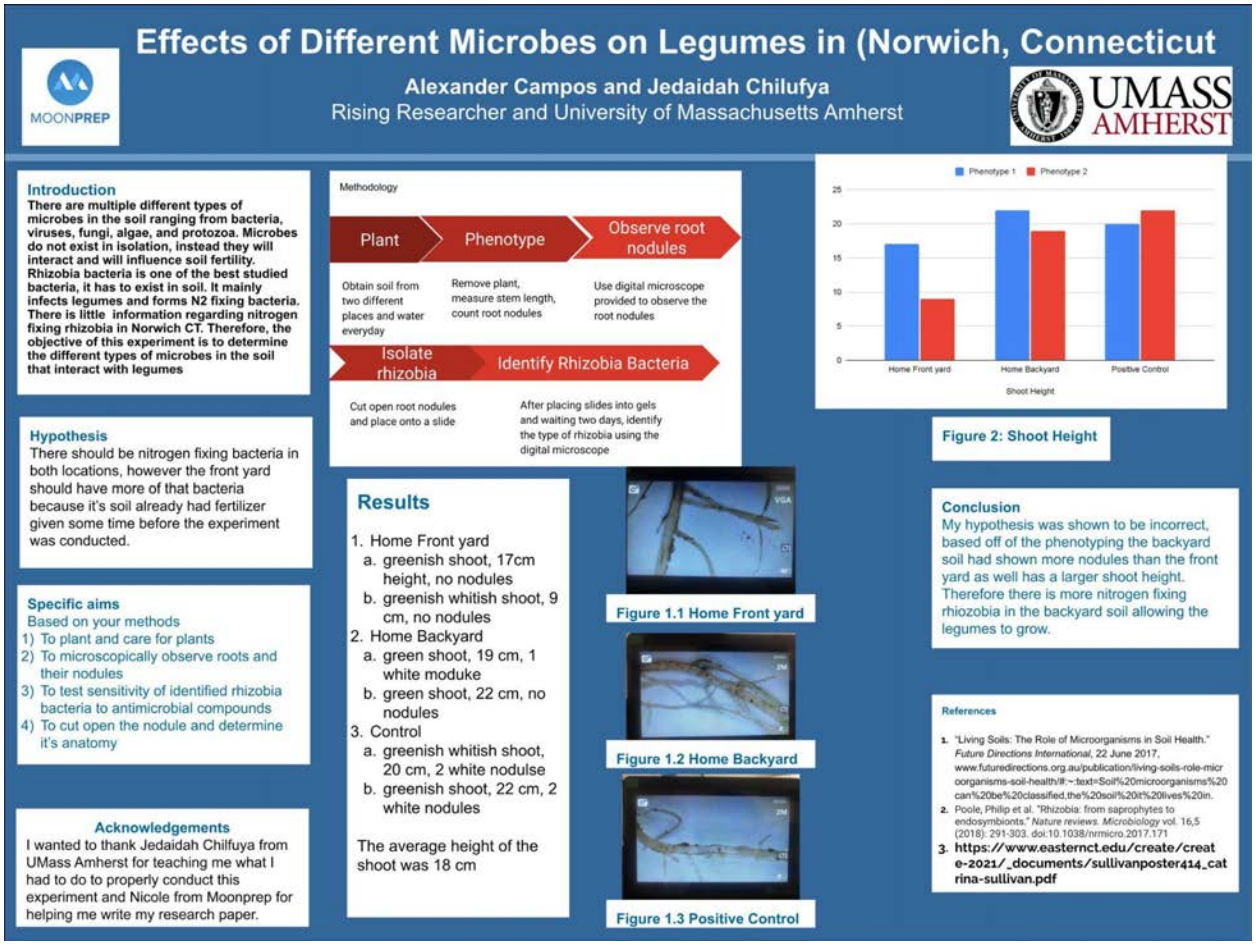
RESULTS:

When I removed the plants for the front yard I determined that the first shoot had a greenish color with an average height of 17 centimeters and no nodules present. The second planet from the front yard had a greenish whitish colored shoot with a height of 9 centimeters and no nodules. For the backyard the first plant had a green shoot with a height 19 centimeters and one white nodule. The second plant also had a green shoot with a height of 22 centimeters but no nodules. For the control group the first plant had a greenish whitish shoot with a height of 20 centimeters and two white nodules. The second plant had a greenish shoot with a height of 22 centimeters and two white nodules as well.

DISCUSSION:

From the data I collected I determined that the backyard had more nitrogen fixing bacteria because it had more nodules than the front yard did. I was initially surprised by the data because I expected the front yard to have had the nitrogen fixing bacteria. I can repeat this experiment again in the future to determine if my initial results were outliers. A possible reason as to why the backyard had more nitrogen fixing bacteria

would be because of the fertilizer in the front yard. The presence of nitrogen fertilizers means the plant will have no need to interact with nitrogen fixing bacteria to obtain nitrogen. In the future I can conduct experiments to determine what types of fertilizer have a higher rate of nitrogen fixing bacteria to determine which one is best for use.



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Anika Roy

Determining Which Soil Characteristics In Illinois Induces Nitrogen Fixing Nodules In Legume Plants Through Symbiosis With Rhizobia Bacteria.

ABSTRACT:

The essential factors that induce legume germination and growth are its ability to photosynthesize and obtain nutrients and minerals from the soil. One of the key nutrients we are going to hone down in this study is Nitrogen, which is vital to the making of chlorophyll, amino acids, enzymes and ATP in plants. Nitrogen used by plants is mostly present in either mineral form in the soil or the most commonly abundant form being as gaseous N2 in the atmosphere. Legumes are unable to use this atmospheric N2 in its raw form so they are commonly known to form symbiotic relationships with rhizobia bacteria, a class of diazotroph bacteria that fix atmospheric bacteria into usable ammonia. This process occurs through legumes sending out flavonoids into the soil to attract compatible rhizobia and in return receive nod factors as confirmation of signals received that lead to the eventual growth of nodules on the roots of the legume plants. These nodules are the key determining factor in this study because, although there might be a successful symbiotic relationship established with rhizobia inhabiting nodules in the plants, successful nitrogen fixation does not always occur. Nitrogen fixation occurs when rhizobia successfully convert atmospheric N2 into usable ammonia for the aforementioned legumes and an exchange of materials is conducted with the plants providing essential supplies of energy to the bacteria in exchange for usable ammonia. Nodules either form to be pink or white, pink being the rhizobia conducting successful nitrogen fixation while white being the presence of rhizobia in nodules, but without successful fixation. In this study, we will be examining soil from 3 different locations and characteristics to identify which one induces successful nitrogen fixation in root nodules of legume plants.

INTRODUCTION:

The main purpose of legume-rhizobia symbiosis is to be able to create a successful exchange of usable N2 and supplies of energy between the legume and bacteria. Rhizobia strains are only effective if the creation of nitrogen compounds is successful and they are supplied to the legume plant, allowing for an increase of crop yields on one hand and decreased use of chemical fertilizers on the other hand. Consequently, the aforementioned benefits allow for a reduction of environmental pollution through the decreased need for chemical fertilizer. However, successful nitrogen fixation is rare and the symbiotic effectiveness of a rhizobium strain is an estimation of host growth promotion and is usually based on the enhancement of plant shoot dry weight upon inoculation. (A.H. Gibson). Considering these variables, we honed down on soil characteristics rather than microbiological processes during symbiosis to discover what specific locations and their qualities allowed for successful nitrogen fixation. There is known information about nitrogen fixation elements between the rhizobia and legumes, but a

lack of knowledge on soil characteristics that induce the same reactions in Illinois. We carefully choose 2 different soil locations in the near vicinity of Avalon drive, Buffalo Grove in Illinois with easily identifiable characteristics specific to each location to plant Crotalaria Juncea in and observe root nodulation for a duration of 4 weeks.

MATERIALS AND METHODS:

Two soil locations were chosen around the vicinity of Avalon Drive, Buffalo Grove in Illinois. They were - soil from a vegetable patch with crops growing in it previously, and the soil had added man-made fertilizer to aid the crop's growth; another location was the front yard of our house whose composition consisted of a lot of wood chips and loose, dry soil. The soil from location "Front yard" was filled into two pots of a depth of about 5.5 inches and labeled accordingly, while the same was repeated for location "Vegetable Garden". One of the pots for location "Front Yard" had 2 teaspoons of commercial inoculant mixed into it and labeled as positive control to act as a medium for comparison against nodule growth of other two pots as it had known rhizobial bacteria already present.

Next, to plant the Crotalaria Juncea seeds, the soil was wet with 12 ounces of water and mixed thoroughly. About 20 deep holes were made with the back of a pencil and the seeds were dropped into them individually and covered up back with soil. The pots were watered and monitored everyday for the duration of 4 weeks for which the study lasted.

At week 2, the first phenotyping of the plants was performed by carefully extracting one legume plant from each pot. Digging tools were used to dig around the soil of the chosen plant to loosen the surrounding soil around the roots, and then the plant was picked out of the pot using large wooden tweezers clamped at the roots of the plant. After the soil was washed off, the plant shoot height was measured using a ruler and their roots observed under a digital microscope for presence of nodules. Shoot height and number of nodules and their microbiological characteristics were recorded down at Week 2 and Week 3.

At around Week 3, rhizobia bacteria were isolated and placed into agar culture plates to look for culture growth. Each plant's rhizobia isolation was done individually, one at a time. First, three microcentrifuge tubes were prepared with a solution of 0.75 ml bleach and 0.25 ml sterile water and labeled according to the pot labels. Then, legume plants were extracted from all three pots as usual using the aforementioned steps.. Once Nodules were located under the digital microscope, they were cut off from specific locations of the roots carefully using a sterilized blade to be placed into their reserved microcentrifuge tubes. The tubes were gently inverted up and down to sterilize the nodules of the surrounding soil bacteria and retracted back to be placed onto a clean, sterilized microscope slide. To wash off the excess bleach solution, three or four drops of sterilized water was added to the slide using a clean dropper and the nodules were washed off. The clean nodules were then placed into new microcentrifuge tubes with 0.25 ml of sterilized water and mashed with a plastic pestle to release and isolate rhizobia from inside the nodules. After thorough mashing, the water turned cloudy with small plant particles floating in it and the crushed suspension was placed into red agar media plates for bacteria culturing. This process was repeated for all three pots.

On Week 4, for culturing agar media plates for pots with no nodules, a specific piece of root was extracted from one of the legume plants with the excess soil washed off and was directly smeared onto the agar plates using tweezers without prior sterilization. This was to induce growth of rhizobia possibly present outside the root but not interior enough that it created nodules. No

phenotyping for plant shoot and nodule growth was done on Week 4.

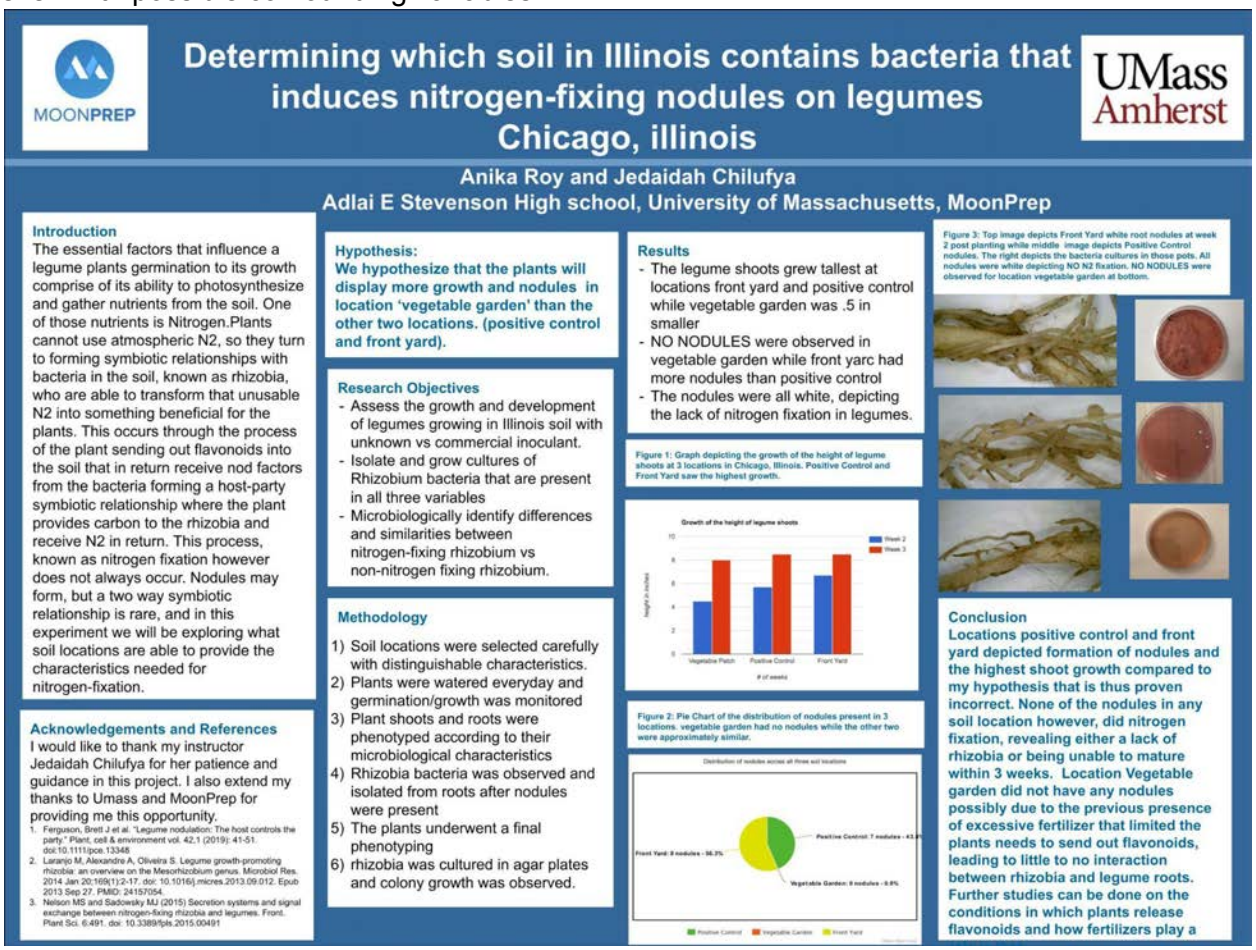
RESULTS:

Location	**Vegetable Patch, Buffalo Grove, Illinois**	**Front yard, Buffalo Grove, Illinois**	**Positive control**
2 weeks post planting 8/5/21	**Plant 1:** Shoot color: Green Height: 4.5 in Roots: Medium sized, Thin, Flimsy, White, NO NODULES	**Plant 1:** Shoot color: Green Height: 6.7 in Roots: Very Short, Rounded, Flimsy, White, NO NODULES	**Plant 1:** Shoot color: Green Height: 5.7 in Roots: Long, Stringy, Thin, Flimsy, White, NO NODULES
3 weeks post planting 8/10/21	**Plant 1:** Shoot color: Green Height: 8 in Roots: short, tapered, thin, white NO NODULES PRESENT	**Plant 1:** Shoot color: Green Height: 8.5 in Roots: long, stringy, bushy, NODULES PRESENT : 9, white, few solid round ones	**Plant 1:** Shoot color: Green Height: 8.5 in Roots: medium sized, bushy, stringy, thick near the start of the root NODULES PRESENT: 7, white, most of them are tiny

DISCUSSION:

The *Crotalaria Juncea* germinated successfully in all three pots and showed significant growth after the end of Week 2 only in soil locations - front yard and positive control. Regarding our investigation into root nodule formation and their microbiological characteristics, No nodules in any soil locations from the three chosen in the vicinity of Avalon drive, Buffalo Grove, Illinois produced any nitrogen fixing nodules in the planted crotalaria juncea. All nodules formed, if any, in Locations Front yard and positive control were all white, signifying the lack of nitrogen fixation by the rhizobia present. However, the Location Vegetable garden surprisingly produced no nodules at all when we initially assumed it would produce the highest yield of nitrogen fixing nodules. Our assumption was based on the presence of previously thriving crops such as pumpkin, spring onion and cantaloupe that had all produced fruit and were healthy. However, a variable that we had previously disregarded when making our assumption was the presence of man made fertilizer. We hypothesize that the presence of artificial fertilizer caused the decline of initiative from the crotalaria juncea to send out flavonoids to attract compatible rhizobia because they were provided with all the essential nutrients needed that were previously present due to the artificial fertilizer. The process of symbiosis itself is very expensive in terms of materials and energy the plant is spending, so if the crotalaria juncea already have all needed materials available to them, they will not expend their resources to create interaction with their surrounding compatible rhizobia to create unneeded nodulation. The results for

positive control was within expectations as the known commercial inoculant had a species of rhizobia compatible with that of crotalaria juncea and thus ended up forming successful symbios with 7 nodules created, but unfortunately no nitrogen fixation. This can also be attributed to the limited time of 4 weeks in which this study was conducted as the symbiotic relationship might not have progressed far enough for it to be able to perform successful nitrogen fixation yet. The results for location Front Yard was highly surprising as it produced the highest number of nodules out of all three locations even with possible confounding variables such as the presence of wood chips that might impede root growth of crotalaria juncea. We can conclude that locations Vegetable garden, front Yard and positive control with a commercial inoculant had no defining characteristics that induced the growth of nitrified nodules given the short time of 4 weeks that this study was conducted in. Further research and investigation can be done on the conditions in which plants release flavonoids and if the specific composition of artificial fertilize present in soil has the ability to affect nitrogen fixating nodule growth.



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Elisa Grillo

Determining the Bacteria That Interacts with Legumes in Cypress TX

ABSTRACT

The aim of this experiment was to determine the presence of nitrogen-fixing bacteria in Cypress, TX by collecting soil from two locations in Cypress and adding a positive control. Soil was gathered from two different gardens in Cypress. Garden 1, had soil that was denser and lighter in color when compared to Garden 2's soil. In order to create a control, more soil was collected from Garden 1, and an inoculant containing *Bradyrhizobium* sp. (Vigna), *Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli*, and *Rhizobium leguminosarum biovar viceae* were added to the soil. Fifteen (15) seeds of the legume, *crotalaria juncea* were planted into each pot of soil and the roots were observed for this experiment. After 4 weeks of growth, the plants in Garden 2 were found to have the most pink nodules indicating that these nodules were fixing nitrogen for the plant. In addition, These nodules had the most bacterial growth.

INTRODUCTION

The purpose for this project was to observe what microbes are in the soil of two different backyard gardens in Cypress, TX, and how they affect the growth of legumes. Legume plants interact with bacteria in several ways, one of them being through the roots where special root structures called nodules develop when the bacteria attach and enter the legume plants; bacteria can attach onto the roots to form nodules. Rhizobial bacteria, or rhizobia and legume plants have a symbiotic or two-way beneficial relationship with one another; the bacteria provides or fixes nitrogen for the plants which helps the plant to grow; When nitrogen is being fixed, the nodules appear pink. But when nitrogen is not being fixed, the nodules appear white. . Nitrogen is essential to plant growth, and plants cannot absorb it from the air, making Rhizobia vital. Bacteria on the other hand benefit from this symbiotic relationship by receiving carbon sugars from the plants as well as a conducive place to live in the nodules (Laranjo et al, 2014). There is not a lot of information on the types of bacteria in the soil of Cypress, TX, so this experiment set out to collect that information.

MATERIALS AND METHODS

To start this project, soil was collected with gardening tools from two different backyard gardens. The soil from Backyard Garden 1 was dense and light in color, and the soil from Backyard Garden 2 was loosely-packed and darker in color. A control was prepared by taking soil from Backyard Garden 1 and hand-mixing commercial bacteria microbes into the soil. The legume seeds were planted by poking 15 inch deep holes into each pot and placing a seed into each hole.

After waiting 19 days for the legume plants to grow, the plants were phenotyped. A plant from each pot was carefully removed and the height of the shoot was measured. After cleaning off the roots with a cup of water, the roots were observed under a microscope. The frequency, color, and size of the root nodules were recorded.

In the third week of growth, the bacteria on the root nodules were isolated to identify the bacteria. After a plant from each pot was phenotyped, several nodules were cut off using a blade. Then, the nodules were cleaned off using a solution containing 1% bleach to ensure that only the bacteria inside the nodules were isolated. The nodules were then placed into three microcentrifuges, one for each pot, with 0.25 mL of water. Afterwards, the nodules were crushed with a pestle and spread across three agar plates. The agar plates provide a place to culture the bacteria that allows for colonies of bacteria to form. The colonies help identify the bacteria inside the nodules. Each agar plate was labeled and sealed with wax to prevent contamination. After three days, the agar plates were observed.

The root nodules were also stained with Toluidine Blue for bacteria identification. A root nodule was cut off from a plant from each pot and placed on three microscope slides. Each nodule was cut in half and a drop of Toluidine Blue was used to stain the nodules. The remaining Toluidine Blue was washed off with sterile water, and the stained nodules were observed under a microscope.

RESULTS

All three soils contained rhizobial bacteria, but there were more pink nodules in Garden 2's roots. Furthermore, Garden 2's agar plate contained the most bacterial growth. On average, the control plants grew the tallest, and the Garden 1 plants were the shortest. All of the stained nodules were a very dark purple, but the exact color is unclear due to how dark it is.

Table 1: Phenotyping of Plants

Location	Control	Garden 1	Garden 2
2.5 weeks post planting 8/1/21	**Shoot color**: green **Height**: 20.9 cm **Roots**: the thin white roots had several pink nodules	**Shoot color**: green **Height**: 15.1 cm **Roots**: the thin white roots had one light pink nodule	**Shoot color**: white **Height**: 20.1 cm **Roots**: the thicker white roots had 3 large pink nodules
3 weeks post planting 8/4/21	**Shoot color**: brown to green **Height**: 19.4 cm **Roots**: the thin white roots had several pink nodules	**Shoot color**: light green **Height**: 16.3 cm **Roots**: the thin white roots had no nodules	**Shoot color**: green **Height**: 11.0 cm (bent) **Roots**: the thin white roots had many smaller pink nodules
3.5 weeks post planting 8/8/21	**Shoot color**: brown to green **Height**: 22.6 cm **Roots**: the thin white roots had several pink nodules	**Shoot color**: light green **Height**: 16.9 cm **Roots**: the thin white roots had several pink nodules (though notably less than the other 2 pots)	**Shoot color**: brown to dark green **Height**: 22.4 cm **Roots**: the thin white roots had several pink nodules
4 weeks post planting 8/12/21	**Shoot color**: brown to dark green **Height**: 28.0 cm **Roots**: the thin white roots had several pink nodules	**Shoot color**: light green to green **Height**: 19.4 cm **Roots**: the thin white roots had several pink nodules (though notably less than the other 2 pots)	**Shoot color**: brown to dark green **Height**: 24.7 cm **Roots**: the thin white roots had several pink nodules

DISCUSSION

In general, the soil in Cypress, TX contains a sufficient amount of rhizobial bacteria as indicated in figure 4. All three pots were found to have pink nodules as shown in Table 1, indicating that nitrogen fixation was taking place in these nodules, and the agar plates showed significant bacterial growth (Figure 4). The soil from Garden 2 has the most rhizobial bacteria, for it had the most pink nodules. These results make sense because Cypress is known for its lush forests and gardens. It's not very difficult to grow plants in Cypress, and this can now be explained by the surplus of rhizobial bacteria in the soil.

In terms of plant growth, the plants growing in the positive control had the highest shoot growth as indicated in figure 2. Further, both Gardens 1 and 2 showed healthy plants with an average shoot height close to that of the shoot height of the positive control.

The results could possibly be skewed because soil collected came from two different gardens. It can be inferred that the soil from most gardens contains rhizobial bacteria, so that could be another explanation for the several pink nodules. If this experiment were to be done again, choosing soil from a location with less plant growth would improve the results.

In conclusion, the soil in Cypress, TX contains rhizobial bacteria, which facilitates plant growth.

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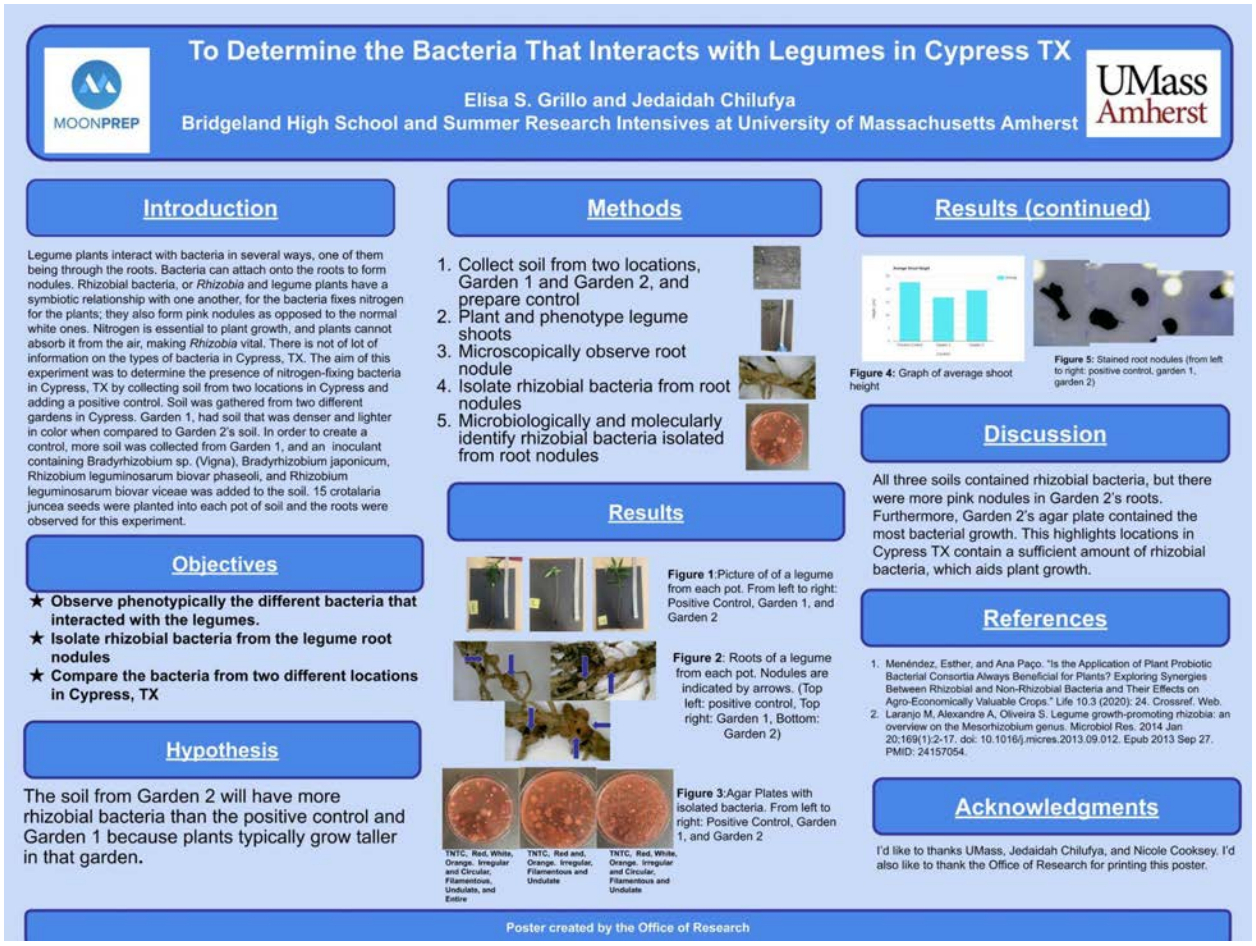


Evana Moses

Rhizobial Bacteria And Its Interactions With Legume Plants

Introduction

A symbiotic host-microbe relationship is one where both organisms in the relationship benefit from the interaction. It is important for organisms to know the difference between a mutualistic and pathogenic relationship when one is introduced to them. In the case of the relationship between bacteria in soil and legume plants, the plants must be able to differentiate between beneficial bacteria, like rhizobacteria and bacteria that could potentially be detrimental to the plant's well-being. Rhizobacteria is a type of bacteria that can be found in different types of soil and in the roots of legume plants. The purpose of this "nitrogen-fixing" bacteria is to take nitrogen from the air and make it into usable compounds for the plants to make use of. This is vital to the prosperity of the plants because they are not able to utilize nitrogen in its elemental state. Because the types of bacteria in every location of soil is so unique, not much is known about the soil in the town of Montgomery, New Jersey. Not much research has even been done on this soil, which makes these results even more important. By collecting soil from two different locations in this town and experimenting with it, we can determine whether rhizobial bacteria is present in the soil, and how effective it is for the survival of the plants. This research will be accomplished through planting and observing the legume plants that were planted in this soil and microbiologically identifying the bacteria in their roots.

Hypothesis

Before starting this research, my hypothesis was that the rhizobial bacteria from the soil in the backyard will be more nitrogen-fixing and beneficial to the legume plants than that of the front yard. All rhizobial bacteria in different soil has unique properties, so the goal of this research was to determine the effects of these different properties on the plants.

Methodology

A total of three pots were filled with soil from two different locations. Two pots were filled with soil from the front yard of my house in Montgomery, New Jersey, and one pot was filled with soil from the backyard of the house. One of the pots from the front yard also had a commercial inoculant added to the soil. This creates a positive control for the experiment, as it is already known what types of bacteria would be present. Around 20 crotalaria juncea seeds were planted in each pot of soil, and germination was observed over the next few days. One plant from each pot was then phenotyped at 2.5 weeks post planting. They were measured and the roots were observed under a compound light microscope. This process was repeated at 3, 3.5, and 4 weeks post planting and all data was recorded. After around 4 weeks post planting, one plant from each pot was extracted from each of the pots. From this plant, a small piece of the root, or a root nodule in the case of the positive control, was cut off. It was then crushed with a pestle and the root was put into a plate of agar media. The petri dish was sealed and the bacteria from all three plants was left to cultivate. Another piece of the root from the plants was also taken at the same time. With these pieces of root, microscope slides were made. This process started with slicing the roots with a scalpel to create a cross section. After the root was cut, it was placed onto a microscope slide. One drop of methylene blue stain was placed on each of the three slides. After one minute the excess blue stain was removed, and the cover slip was placed onto each of the slides. These slides were observed

under a microscope to further our knowledge about the plants in each of the pots.

Results

After all of these experiments, the results were observed carefully. In Chart 1, you can see the results from the phenotypes from each pot. Each bar in the graph represents the average height of the plants from each pot of soil. As you can see, the positive control had the tallest plants, which was expected since we already know about the bacteria that was put into this soil. Between both the front yard and the backyard soil, the chart shows that the plants from the backyard were slightly taller. The tallest plant that I recorded came from the pot of soil from the backyard. I recorded it at 14 centimeters tall, which was 1 centimeter taller than the tallest plant from both the positive control and front yard.

The agar media plates also formulated some results that were observed. All the bacteria that contains nitrogen fixing properties that were put in the agar would turn red. The bacteria that was not nitrogen fixing would still cultivate, but the colonies would remain white. After the agar plates were left for a few days for the bacteria to grow, the growth was observed with the naked eye. After observing the three plates, we could see that there was the most growth on the backyard plate. Image 1 of this dish can be seen to the left. Most of the colonies on this petri dish were red, meaning that they were most likely rhizobacteria. The bacteria on the front yard dish had significantly less colonies. It had one red colony, indicating that there was some nitrogen fixing bacteria in the roots, but not nearly as much as the backyard petri dish.

Discussion

As we already know, there is not much research on this particular topic in the town of Montgomery, so there are no results to compare and contrast to. When looking at the results of our experiments, it is clear that the soil in the backyard is much more beneficial to the prosperity of the legume plants than the front yard soil. The plants grown in the backyard soil were not only taller, with greener stems, but also contained more nitrogen-fixing bacteria in the roots. Because of the greater amount of this type of bacteria, the plants will grow at a faster rate and stay healthy for a longer time. This was seen at 3.5 weeks post-planting as the plant from the front yard was only 7.5 centimeters tall, while the one from the backyard was 9 centimeters tall. In order to further the research on the soil in this area, it might be beneficial to repeat this same research with different types of plants. By performing this variation of these experiments, we can continue to discover more about the bacteria in this particular soil. This will help us to understand why some plants prosper and others wither away.

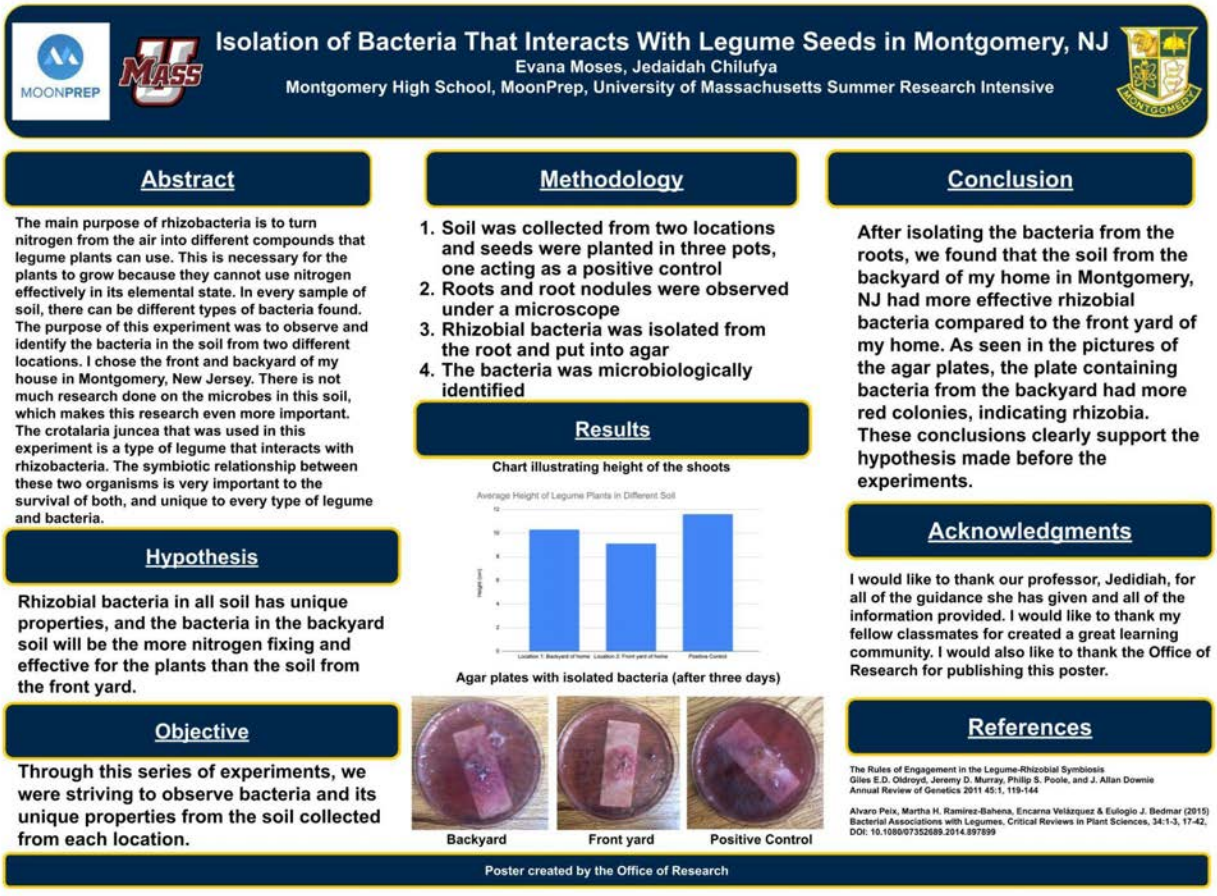
Conclusion

When looking at all the results from these few weeks of experiments, the hypothesis that was made in the beginning of the experiment was proven correct throughout the course of the research. All of the results from isolating the root bacteria from all three of the pots shows that the soil from my

backyard contains the more effective bacteria compared to the soil from my front yard and is conducive for plant growth.

Acknowledgements

I would like to thank our professor Jedaidah Chilufya for guiding all of us through this research and providing us with all the materials we needed to succeed. I would like to thank the University of Massachusetts for creating this course. And finally, I would like to thank all my fellow classmates for creating a great learning community and support system throughout the four weeks of research.



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Jenica Benneaser

Observing The Roots Of Legumes Grown In Soil From Two Different Rivers In Bretton Woods, New Hampshire, And North Woodstock, New Hampshire

Abstract

The purpose of this experiment is to determine the types of microbes present in two different rivers in New Hampshire: The Lost River and Ammonoosuc River. The experiment was started by collecting soil from each location. Two pots of soil were collected from the Ammonoosuc River, one of these pots was inoculated with a nitrogen-fixing inoculant. Once surveyed, legumes were planted in these pots and monitored each week for four weeks. The samples were watered with (how much water) every other day. 2.5 weeks after planting, the plants were phenotyped. 3 weeks after planting, the plants were phenotyped again. Additionally, rhizobacteria were isolated from the plant roots and root segments were observed through microscopy. The essential idea of this experiment is to find out whether the soil has rhizobacteria in it.

Introduction

Nitrogen is an element that is essential to all life. For plants, it is fundamental for plant growth. However, plants cannot use it in its elemental form. One type of plant, legumes, uses a bacterial symbiont called rhizobia. Rhizobia use a process called nitrogen fixation, in which they metabolize atmospheric nitrogen and convert it into compounds that a legume can use. This can be seen in the experiment conducted as legumes, in this case, crotalaria juncea, were also used. There is little information about the presence of bacteria that interacts with Crotalaria juncea in North Woodstock, New Hampshire. Therefore, in this research, our goal was to compare different soils in different locations for the presence of rhizobacterial that would interact with Crotalaria juncea.

Materials

Phenotyping Plants:

- Digging tools
- Paper towels

- Cup/pot or beaker of water
- Flat surface with a dark background
- Ruler or measuring tape or print out of 15 cm ruler (from google search)
- Label

Isolation of Bacteria from Roots:

- Plants (one pot at a time)
- PPE
- Digging tools
- Paper towels
- Cup/pot or beaker of water
- Flat surface with a dark background
- Ruler or measuring tape
- Labels and a pen or sharpie
- Microcentrifuge tubes
- Plastic pestles (blue)
- Dropper
- Microscope slide
- Scalpel
- Sterile water
- Household bleach
- Agar media plates (1 agar media plate per pot)
-

Root Segment Microscopy:

- Microscope
- Microscope slides and coverslips
- Scalpel
- Dropper or pipette
- Toluidine blue stain
- Water
- Plants (one pot at a time)
- PPE
- Digging tools
- Paper towels
- Cup/pot or beaker of water
- Flat surface with a dark background
- Ruler or measuring tape Labels

Methods

- First, the soil was collected from two different locations to be placed in three different pots.
- Afterwards, legumes were planted in each pot.
- The shoot and root nodules of these legumes were observed.
- After this, rhizobial bacteria were isolated from the legumes.
- Finally, these rhizobial bacteria were microbiologically and molecularly identified.

Results:

The plant roots from the legumes grown in the soil from the Ammonoosuc River had healthier roots, with evidence of some root nodules, more rhizobacteria in the roots, and longer root segments than those of the positive control legumes and the legumes grown in the soil from the Lost River.

Discussion:

As stated before, the plants from the Ammonoosuc River soil showed the most improvement. These results were expected as the hypothesis was that the soil from that river would also have the most rhizobacteria, which it did. One reason for this could be that since the land was on a property, it could have been treated with more fertilizer. These findings prove that the addition of fertilizer does help the nitrogen-fixing process.

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Kanksha Koti

To Determine The Bacteria That Interacts With Legume Seeds In Livermore, California

Abstract

In nitrogen fixing plants, legume plants in this particular case, the symbiosis between the rhizobia bacteria and the legume plants is essential to the conversion of ammonia, which is needed for plant roots. The symbiosis between the microbes/bacteria and the plants allow the conversion of nitrogen into more bioavailable forms (Taylor 2020). By observing the interactions between the bacteria and the plants in Livermore, California, the end results were able to demonstrate the beneficial impacts of the symbiosis between them as well as how the development of root nodules came into play. A root nodule is a formation on the root of a plant, which plays a large and crucial role in the process of nitrogen fixation. The goal of this research is to further understand not only the symbiosis between the plants and the rhizobia, but also understand how the growth of the root nodules play a role in the long run in the health and development of the plant. In general, a pink root nodule is indicative of nitrogen fixation, whereas a white one is indicative of lesser to no fixation. To expand research and obtain the most diverse results possible, there were three pots of soil with twenty seeds each that were given around two weeks to germinate before starting the process of root nodule identification. Following the initial germination, there was enough information to be able to create a hypothesis: Based on the type of growth see from the pot with the dirt from the backyard, the microbes are most likely inhibiting the growth of the legume seeds due to their previous location having not been as nutrient rich in comparison to the soil from the front yard. Throughout the course of this research, the obtaining of more results were able to not only confirm this hypothesis, but also demonstrate the importance of rhizobia and legume symbiosis for both their survival, as it is mutualism.

Methodology & Materials

In order to prepare for the research itself, there were twenty legume seeds planted into three pots of soil two weeks in advance to have germination occur before the process of microscopy and phenotyping. In pot number one, it was the soil from the front yard of my house, gathered from digging 6-8 inches deep to obtain the most enriched soil. In pot number two, the same process was repeated, however the soil was from a particular patch in the backyard. In pot number 3, or the positive control, the soil was also from the front yard, however in the soil was mixed healthy and beneficial bacteria meant to enhance plant and root nodule growth and development. After they germinated, at around week two and half, is when the phenotyping and microscopy process was able to commence. Every week, a plant was carefully dug out from each of the pots and respectively measured for height, observed for shoot growth and health, and studied under LCD microscope to identify potential root nodules. While there were none visible the first week of germination, week three was a shifting point that allowed for even more data gathering as the development of the root nodule was able to indicate the symbiosis between the bacteria in each of the pots and the plants. Around week three and three and a half, the height of root nodule development, the nodules -about one to four of them from each plant- were cut off and prepared in

centrifuge tubes and sterilized water before being crushed by pestles and being distributed equally across an agar gel plate specific to to each pot to observe the growth of colonies that would indicate the level of nitrogen fixation occurring in each pot. At the same time, additional nodules were cut off and into half before being cleansed and given a Toluidine stain to be able to highlight the formation of them under the LCD microscope as a microscope slide. The constant repetition of these methods allowed for the best results that ensued and amplified the question of the kind of symbiotic relationship legume plants and rhizobia have with each other.

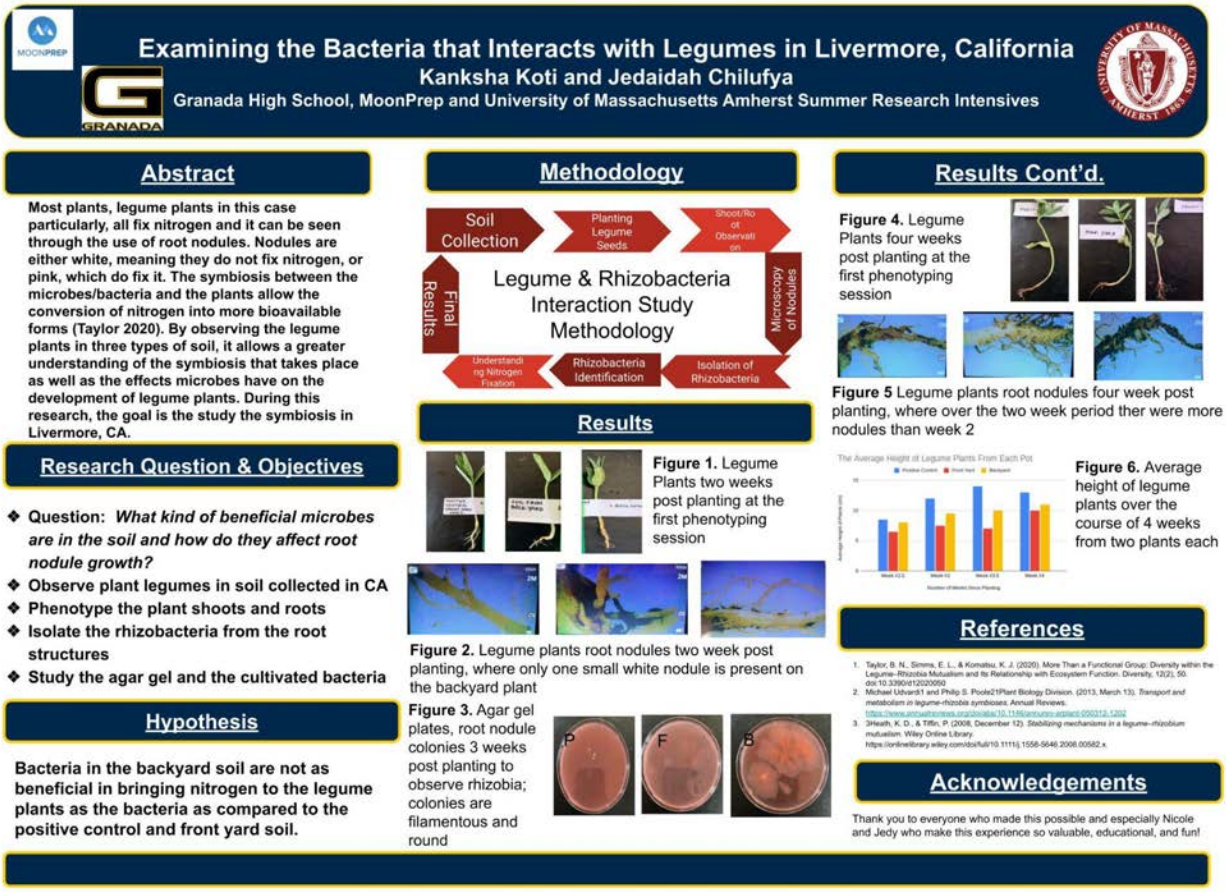
Results

After the first time the plants were phenotyped, the difference between the heights between all three plants were distinct, with the front yard plant throughout the entire process being the shortest in shoot height. However, during the microscopy portion, the plants from the front yard pot had anywhere from two to three nodules at a time. The backyard plants, despite having a positive growth trend, averaging the second most growth amongst the three, had the most limited amount of root nodule growth in general, not just in comparison to the other pots, as there was always zero to one every week during the phenotyping sessions. The positive control had the most success with not only shoot growth and height, but also with the number of root nodules that grew and were presented under the LCD microscope. Not only were they present in quantity, they also had quality, as they were more often than not visible to the naked eye and grew significantly. While at first, specifically around weeks two and two and a half, the growth of the plant roots was stunted due to their presence in small numbers and overall stature, with more time to grow and more time dedicated to tending them, they quickly grew not only in health, but also in quantity. The most indicative part of the research that further highlighted the interaction between the rhizobia and the plants was the results obtained from the cultivation of the bacteria on the agar gel plates. After waiting about two to three days after spreading the crushed nodule and water mixture onto each of the three agar gel plates respective to the plant, the development of the colonies showed where nitrogen was being fixed or not, mainly based on the color of the colonies. In addition to this, the backyard and front yard agar gel plates were more filamentous and dispersed wide across the plate while the bacteria in the positive control plate was more round and concentrated into smaller circles in the original area where the mixture was poured. Further analysis of the root cut root nodules from each of the plants from the three pots concluded the level of success of the symbiotic process between the legume plants and the rhizobia, which was also seen through the average height of each of the plants over a four week period. The gathered results aided in the study of the relationship between the rhizobia and the legume plants and how well they acclimated to each other during the four week long period.

Conclusion & Discussion

As per the original hypothesis -bacteria in the backyard soil are not as beneficial in bringing nitrogen to the legume plants as the bacteria as compared to the positive control and front yard soil-, the results obtained were able to confirm its

accuracy. The distinct differences between the heights of the plants within the first week post germination was the first sign of the different types of rhizobia within each of the pots because the symbiosis was not nearly as successful in the backyard pot as it was in the front yard and positive control pots. This was recurring theme when it was time to dissect and observe the root nodules; because of the difference in the soil and microbe health within the backyard pot, the root nodule growth was extremely stunted and limited, which did not allow for the symbiotic relationship within that pot to be studied to the fullest extent. In contrast, however, the front yard and positive control pots had successful root nodule development and were able to present at least three or more nodules every phenotyping session, which is indicative of a healthier and more beneficial relationship taking place. While these results and analyses were based on visuals, the most representative set of data came from the cultivation of the root nodule bacteria of a plant from each of the three pots on the agar gel plates. The filamentous nature of the colonies in the front yard and backyard plates and the round nature of the positive control gel plate were merely the pattern of the spread, but the color of the bacteria was essential to understanding the nitrogen fixation process. The positive control and front yard plates had white or light pink colonies, which indicate nitrogen fixation was occurring, which is important because “In exchange for reduced nitrogen from the bacteria, the plant provides rhizobia with reduced carbon and all the essential nutrients required for bacterial metabolism” (Udvardi & Poole, 2013). The backyard plate, however, had dark pink colonies, which represents no fixation occurring, which again supports the hypothesis that the backyard soil was not as beneficial for providing healthy rhizobia for legume plant growth. This may also be because in “mutually beneficial species interactions, may be evolutionarily unstable because natural selection should favor “cheaters,” which receive greater fitness benefits than they confer to their symbiotic partner” (Udvardi & Poole, 2013, Trivers 1971), which is seen in stark contrast among the three pots. In the future, this data can be used to narrow down portions of the land in which I cultivated the soil to be healthy and beneficial vs lacking in nutrients to promote even more plant growth, particularly for plants that require the ability of the soil to help them fixate nitrogen. From a scientific perspective, the data and results that were obtained from conducting this experiment can be applied to various parts of science, particularly in medicine, as doctors can narrow down locations and particular situations where types of exposure can increase or decrease the chances of infection and sepsis. With modification of this experiment to a human trial and error process, the chances and spread of infection can be severely reduced in postoperative patients in the CCU and ICU. The collection of data and the ability of it to correlate back to the hypothesis in a correct manner was essential to not only the understanding of the symbiotic relationship between the legume plants and the rhizobia, but also the comprehension of the benefits of the ground in which the experiment was conducted.



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Kristin Van Der Merwe

Identification Of Rhizobial Bacteria On Legume Root Nodules In Prince George, British Columbia And Their Effect On Growth

Abstract

Soil contains a diverse microbial community with which plants establish relationships for optimal growth and protection. The soil bacteria, more commonly known as rhizobia, allows for the formation of root nodules within which the microsymbionts convert atmospheric nitrogen into ammonia, a biological form that can be directly consumed by the plant. The soil's condition therefore plays a critical role in agricultural fertility and crop production. These mutualistic relationships are highly specific, which we explore in this project. We will examine the species of rhizobia found in Canadian soils (Prince George, BC) and determine their compatibility and therefore effects on crotalaria juncea legumes (sunn hemp). Results will be observed through phenotyping and bacteria isolation. This project will be of importance to the agricultural community in Prince George, BC, which will be used to create a thriving agricultural output.

Introduction

The legume family, Leguminosae, is composed of 19,300 species, most of which fix atmospheric nitrogen through a symbiotic relationship with bacteria. Many legume species only enter productive symbiosis with a few, or even single rhizobial species or strains, and vice-versa (11). These bacteria, more commonly known as rhizobia, convert atmospheric nitrogen (N2) into ammonia for the plant to utilize in little swellings known as nodules. These nodules house the bacteria during nitrogen (N) fixation, where they receive fixed carbon (C) in return from the plants photosynthetic processes (10) . This can give the legumes an advantage under low soil nitrogen (N) conditions if other factors are favourable for growth (7). Furthermore, N2 fixation by legumes can be a major input of N into natural and agricultural ecosystems.

The formation of the nodules (nodulation), is what ultimately dictates the compatibility between plant and bacteria . In order for nodule development to occur, there has to be extensive chemical cross-communication between both parties. This selectivity is enforced at all stages of symbiosis, with partner choice beginning during the initial communication between the plant and rhizobia (11). However, it can also be influenced even once nitrogen-fixing nodules have developed on the root (11). Plant roots begin by secreting flavonoids and a vast array of compounds, many of which are a source of nutrients for the bacteria, into the soil that is in direct contact with the roots (rhizosphere) (4). These specific plant emissions activate the nod gene expression of rhizobia mediated by the nodD regulatory gene product (2, 4).

Different legumes each secrete specific flavonoid signals, with which only certain rhizobia will respond to by producing rhizobia specific lipo-chitin oligosaccharides, thereby defining the specificity of the

relationship between the legume host and the infecting rhizobia (10). Another checkpoint that further defines the specificity, is the recognition of the Nod factors (LCO's), if the plant has compatible Nod Factor receptors (NFRs) (2, 4). This allows for the further identification of a friendly rhizobial invader (10). The lipo-chitin oligosaccharides cause the legume root to deform and form a hook-like structure (3, 10). The deformed root traps the compatible rhizobia and begins an inverse tip growth, forming a tube called the infection thread (8). This is where the bacteria travel, by dividing at the leading edge, while the infection thread penetrates cells in the underlying layers of the plant (8).

As this process occurs, the root cortex host cells acquire properties of the stem cells, giving rise to newly generated cells, where the nodules will develop (8). When the rhizobia reach these new cells, they enter through a process similar to endocytosis and transform into nitrogen-fixing organelles known as symbiosomes (8). The bacteria gradually grow and develop into structures known as bacteroids. The bacteroids eventually develop the biochemical properties, which allows them to absorb atmospheric N2 and convert it to N in the form of ammonia. These bacteria stimulate cell division, finalizing the formation of root nodules, where they convert atmospheric nitrogen into ammonia, a biological form that can be directly consumed by the plant (9).This intricate interaction between the bacteria and legumes is highly specific, which we explore in this project. Not all rhizobia bacteria successfully interact with all legumes to provide or fix nitrogen for the plant. Therefore in this project, we will examine the species of rhizobia found in Canadian soils (Prince George, BC) and determine their compatibility and therefore effects on the growth of crotalaria juncea legumes (sunn hemp). The research conducted will be of importance to determine the rhizobia present in Prince George, BC soils and therefore improve crop fertility and production.

Materials and Methods

The research project was conducted through observation and by collecting quantitative data. I collected soil from 2 locations and planted 20 crotalaria juncea legume seeds in each pot, including a positive control containing an inoculant. The first location was the front yard of 6910 Cranbrook Hill road, Prince Geroge, BC. The second location was the Fort Fraser Dump site in Prince George, BC. The soil from the positive control was also collected from location 1. I watered the soil everyday with ¾ cup of water and observed the germination, while proposing a hypothesis. The quantitative data included measuring the growth with tape, measuring the amount of water needed, counting the total germinations and counting the legume seeds planted in each pot. If there were less than two germinated plants in a pot, then a replanting was conducted in a fashion where there was no cross-contamination between the soils. The materials used in the first week included digging tools such as a spade, a liquid measuring cup, a ruler, water and personal protective equipment like gloves and glasses.

In the second week, I conducted root observations. The materials I used were the 3 pots of soil that I collected, a spade, a beaker of water, a dark notebook, a ruler, a digital microscope and PPE. I loosened the soil by wetting it and pressing the spade into the soil and gently lifting it, that way the

desired plant will be uprooted without being damaged. The roots were rinsed off and placed on a dark background next to a ruler, to indicate growth and to phenotype. After this, I observed the roots for the presence of nodules underneath a digital microscope. This was performed with 2 plants from each pot, every week for 3 weeks.

In week 3, I isolated rhizobial bacteria onto agar plates. The materials I used were the 3 pots of soil, microcentrifuge tubes, plastic pestles, a dropper, microscope slides, a scalpel, sterile water, clorox wipes, a sharpie, labels, parafilm, tinfoil and 3 agar media plates. I first phenotyped the roots of two plants of each pot using the same methods and materials as previously stated. I then took one of the phenotyped plants of each pot to cut off a segment of the root with a sterilized scalpel. After that, I placed the root on a clorox wipe and gently patted it down. The root was then placed on a sterilized microscope slide and using the pipette, I submerged the root with the sterile water to wash off the clorox. After that, I sterilized the scalpel and pipette, and placed the root in a microcentrifuge tube, labelled according to the pot of soil I used the plant from. Then I added 0.25 ml of sterile water using the pipette. The sterilized pestel was used to grind the root until the water became cloudy. Next I took an agar plate, sterilized my work area and poured the root suspension onto the agar surface.I swirled the suspension and using another sterile pestel, I spread it across the agar using the streaking method. I turned the agar plate upside down and sealed it with parafilm. I wrapped it in tinfoil and placed it on a windowsill. I repeated this for each pot, using one plant and one agar plate for each.

In the 4th week, I observed a stained root segment under the digital microscope. The Materials i used were a digital microscope, microscope slides, cover slips, a pipette, toluidine blue stain, a scalpel and 3 plants (one from each pot). I first phenotyped the roots of two plants of each pot using the same methods and materials as previously stated. I took a plant after phenotyping it and cut off a segment of the root using a sterilized scalpel and cut that same segment in half as well. The two half segments were placed on a microscope slide and a single drop of toluidine blue stain was placed on top. I left the stain on for 10 seconds and immediately cleaned up the excess stain using a paper towel. I used the pipette to drop sterilized water onto the segments to wash off more stain and used paper towels to absorb the excess water as well. Afterwards, I gently placed the cover slip over the segments and observed them underneath the digital microscope. I repeated this procedure with the other pots of soil ,each time cleaning the digging tool, using new water, a new microscope slide, cover slip and microcentrifuge tube.

Results

The soil from the positive control allowed for the most legume shoot development, however, out of the 20 legume seeds that were planted, only 6 germinated. The roots in the positive control did not have any root nodules, but were the most developed. After the third week, one of the plants died.

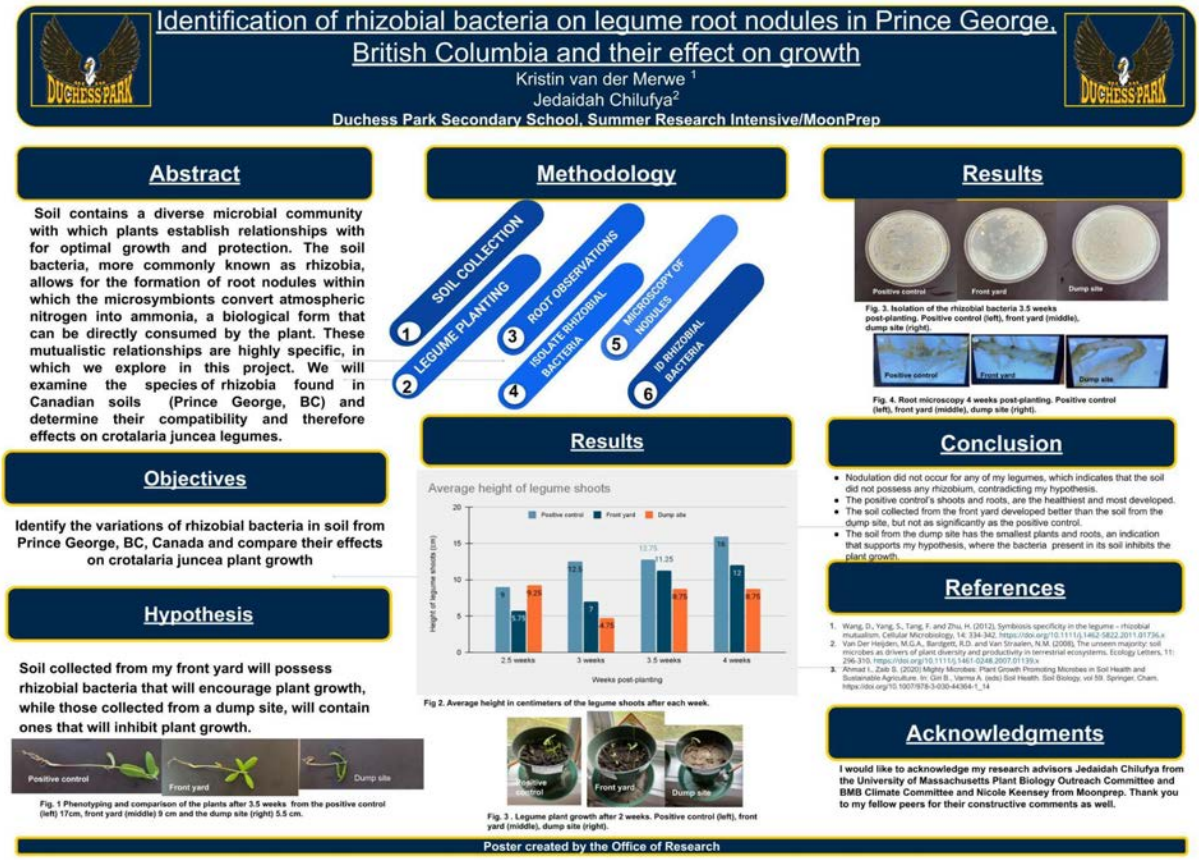
The plants in the soil from location 1 (front yard, 6910 Cranbrook Hill road), had consistent development and growth, but not as successful compared to the positive control. Out of the 20 legume seeds, 6 germinated, but 2 died by the third week. No nodulation occured for these legumes and the roots had a few extensions, but were

still not as developed as the positive control's roots.

The plants from location 2 (Fraser Fort George Dump Site), had the least amount of growth and development out of all the variables used. Out of the 20 legume seeds planted, only 2 germinated and a plant had to be repotted after the first week from the positive control. After the third week, one of the plants died. The roots showed no presence of nodules and the root systems were very small and had little to no branches

Discussion

The positive control had the most suitable environment for crotalaria juncea. The shoots of the legumes, as well as their roots, were the healthiest and most developed. The soil collected from the front yard had fairly favourable soil conditions, but it didn't contribute to the legumes' growth and development as significantly as the positive control. The soil from the dump site had the smallest plants and roots, indicating that soil was not favourable, which was not surprising. Nodulation did not occur for any of my legumes, which could have been due to multiple factors. The first factor being that the soil did not possess any rhizobial bacteria that were compatible with the legumes, the soil conditions hampered the bacteria's ability to enter the roots or the soil already possessed nitrogen fertilizer, so no symbiotic-relationship was needed. I did expect nodulation to occur for the plants in the positive control and the soil from my front yard, seeing that the positive control contained an inoculant and the soil from my front yard contained multiple fully developed plants. This research can be further improved by collecting more soil samples and using different legume species, in order to identify the exact rhizobial species that allows specific legumes to enter symbiosis. The agricultural community in Prince George, BC could use it to their advantage to find the most compatible plants for their soils and increase their agricultural output.



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Madison Cohen

How Does Rhizobial Bacteria Affect The Rate At Which Legumes Grow?

Frequent crop enhancer, Rhizobial Bacteria may have adverse effects on the organism in which it is planted with. The bacterium acts as a primary nitrogen fixator when mixed into a plant pot. Legumes and other plants are unable to utilize nitrogen that is not fixed, making Rhizobial Bacteria a primary factor in this experiment. Rhizobium infects the roots of the plant, infusing them with nitrogen, creating nodules where the nitrogen buildup is held (Thilakarathna, Malinda S.). Little information is known about the presence of Rhizobia in Manhasset, NY that associate with legume plants such as sunn hemp, for their growth. Therefore, we ask, ‘does Rhizobial Bacteria impact the rate at which legumes grow comparatively to those without? The purpose of this microbiological research was to examine the presence and effect of nitrogen fixing bacteria in Manhasset soil on growth of sunn hemp legumes.

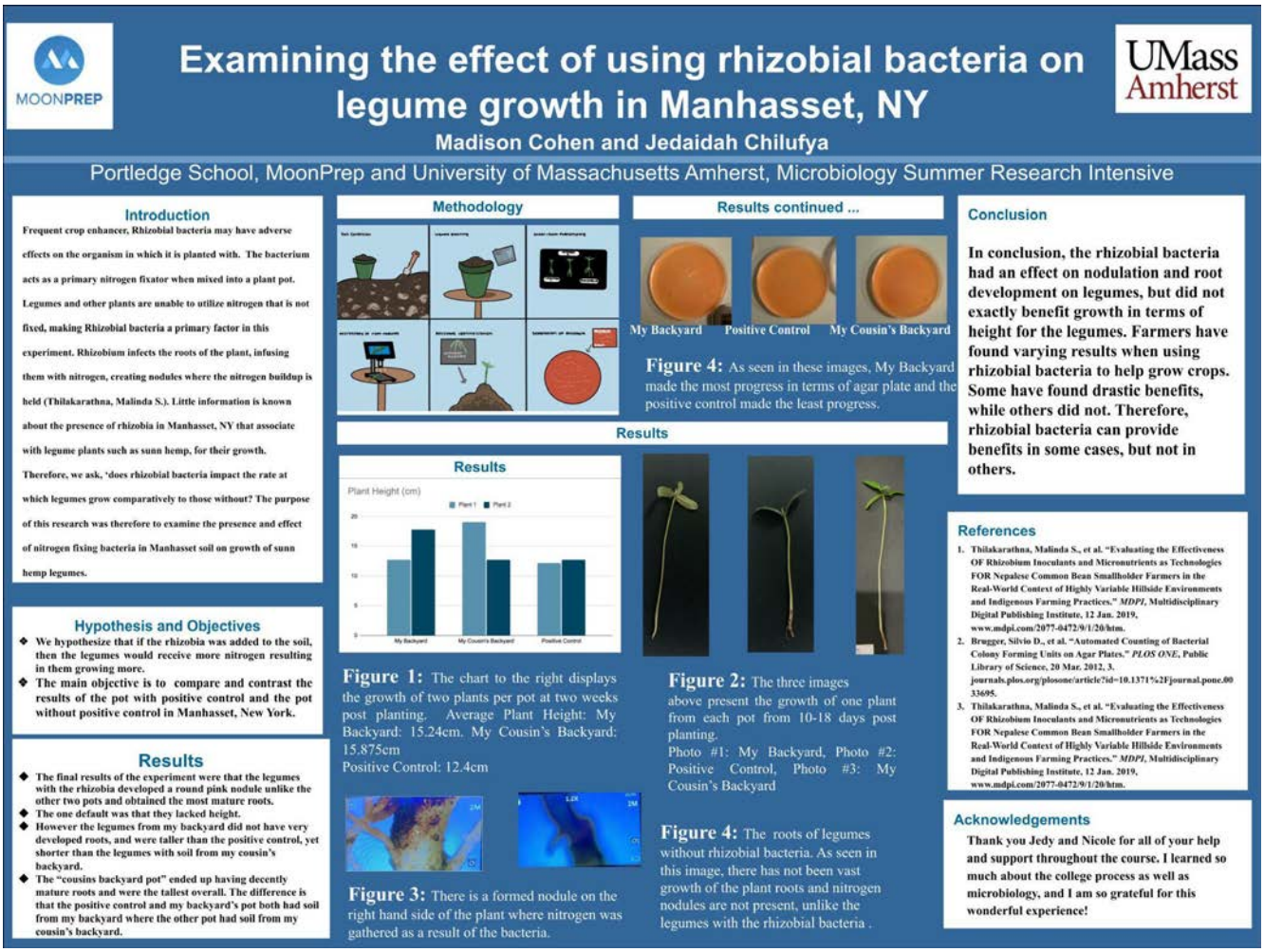
Does Rhizobial Bacteria impact the rate at which legumes grow comparatively to those without? The initial usage for Rhizobial Bacteria is to reduce the effect of adverse conditions that plants are exposed to. Farm crops are a common example of this. Rhizobial Bacteria affect the nitrogen

levels that the plants experience when germinating. This experiment's main objective is to compare and contrast the results of the pot with positive control and the pot without positive control. Originally, we hypothesized that if Rhizobia was added to the soil, then the legumes would receive more nitrogen resulting in exponential growth.

This scientific experiment was conducted in Long Island, New York where various methods were used throughout as captured in **Figure 1**. First, soil was collected from two separate locations, in this case my backyard and my cousin's backyard, which were then distributed into three separate pots. One of three pots contained soil from my cousin's backyard and the other two contained soil from my backyard, except one of two pots also had the Rhizobial Bacteria mixed in. Roughly 20 Legume seeds were then planted into each pot over the course of 4 weeks with the exception of the extra seeds planted to assist in the germination of the plants which is demonstrated via **Figure 2.** (The plants were also closely monitored and attended to throughout the 4 weeks). Approximately 2 weeks post planting, plants from the three pots were phenotyped using a digital microscope, a ruler, and a dark background for vivid photographs. A week later, root nodules were crushed, sterilized, and transferred into media/agar plates where bacteria patterns could be observed. Lastly, the plants were phenotyped for the final time roughly 3.5 weeks post planting for a final inspection of the demonstrated plant growth.

The final results of the experiment were that the legumes with the Rhizobia developed a round pink nodule (as seen in **Figure 3)** unlike the other two pots and obtained the most mature roots. The one default was that they lacked height. However, the Legumes from my backyard did not have very developed roots, and were taller than the positive control, yet shorter than the Legumes with soil from my cousin's backyard. The "cousin's backyard pot" ended up having decently mature roots and were the tallest overall. The clear difference is that the positive control and my backyard's pot both had soil from my backyard where the other pot had soil from my cousin's backyard. Unfortunately, the Agar Plates failed to make progress, so they did not yield or support the results of this experiment.

In conclusion, the Rhizobial Bacteria had an overarching effect on nodulation and root development on legumes, but lacked the benefit of growth in terms of height for the legumes. farmers have found varying results when using Rhizobial Bacteria to help grow crops. In summation, some have found drastic benefits, while others did not. Therefore, Rhizobial Bacteria can provide growth benefits in some cases, but not in others.



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Rohan Singh

Bacteria And Its Impact On Plant Growth

Abstract:

The purpose of this research was to determine the effect of bacteria on legume growth. Soil was found and collected from two different locations for each variable, respectively. The commercial inoculant was used as control. Each soil collection was potted and watered equally and thoroughly. Each pot was provided the same amount of access to direct sunlight for the entirety of the experiment.

Introduction:

The question asked in this experiment was how do microbes found in soil affect the growth of legume plants. Different soil from various areas have several types of microbes, some being harmful while others are beneficial. For Crotalaria Juncea (Sunn Hemp Legume Plants), rhizobia bacteria is beneficial for their growth. This experiment analyzed soil from two different locations and investigated how microbes in the soil benefited the plant. Both of these locations were directly compared to a control group which contains an ample amount of rhizobia bacteria.

Materials and Methods:

This question was answered by monitoring all the experimental variables closely. Each week one plant from each pot was removed and closely examined. The plant height, color, and nodule growth was all measured and noted down. This was repeated twice a week for three weeks after planting the pots. The roots of these plants were closely examined underneath the microscope for key details found on the nodules that indicate presence of rhizobia bacteria. All data and observations were recorded in the E-Lab Notebook. The next part was rhizobia bacteria colony growth. One plant was extracted from each pot once again for the agar media plates. The roots were thoroughly rinsed in order to identify nodules and extract them. Once the nodules had been removed, they were rinsed in bleach and water for cleansing. The nodules were then placed in a tube with water, where it was crushed into a liquid. This liquid was applied to and spread on the agar media plates in order to facilitate bacteria colony growth. The agar media plates were closely monitored over the following 48-72 hours. The last procedure was stained nodule observation. Two to three plants were removed from each pot. The healthiest plant was chosen for the observation. The nodules were removed and cut in half with the inside part facing up. Toluidine blue stain was applied on the nodules, and then water from the pipette rinsed and drained all excess stain. The slide cover was placed

over the nodules and carefully observed under the microscope.

Results:

Positive Control: Highest Average Height; Most Nodules Present; Pink Nodules Present

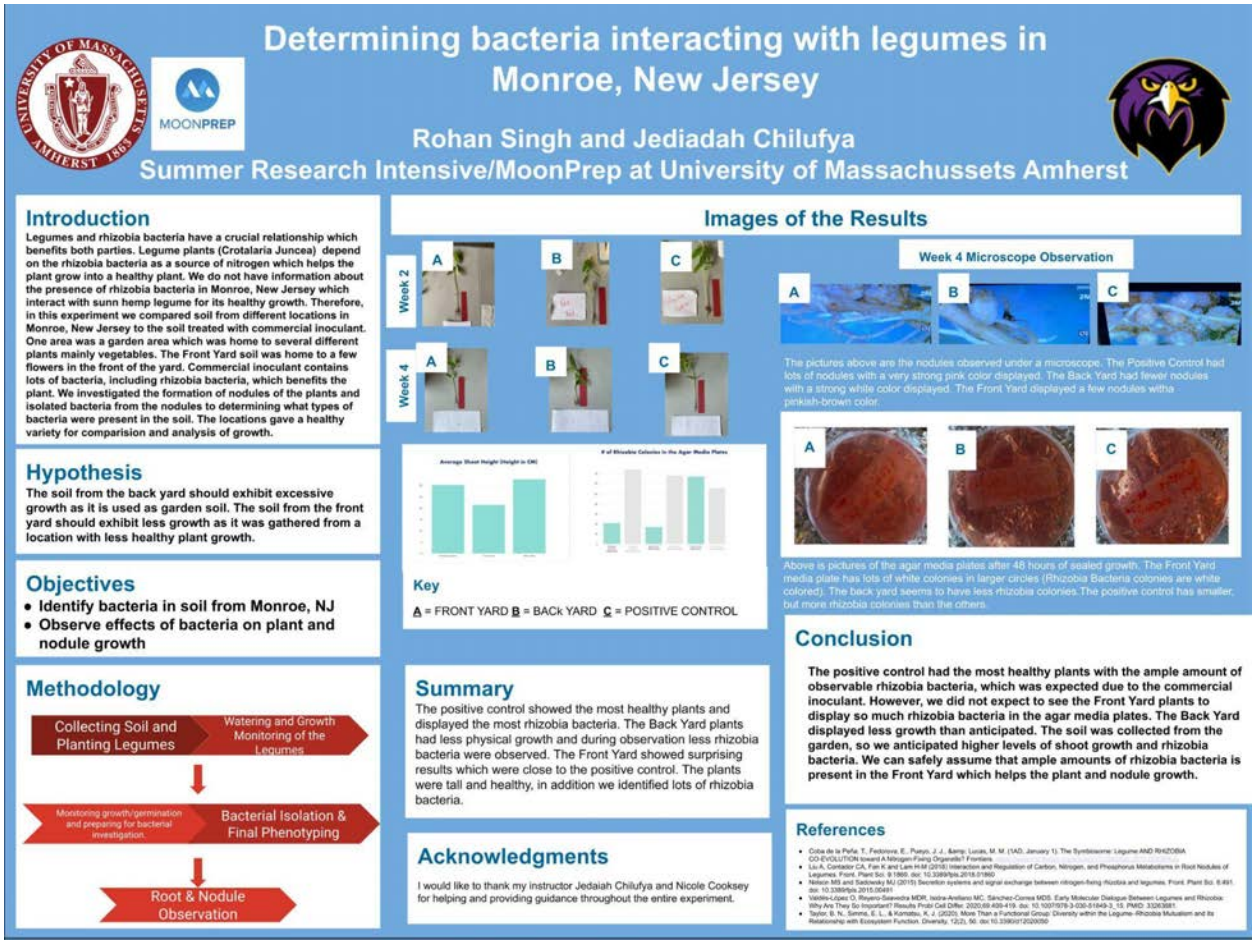
Rear Garden: Avg height 24.75 cm; Not many nodules; Nodules present were pinkish brown

Front Yard: Avg Height 22.4 cm; Nodules Present; Nodules had mainly white color

This graph shows the amount of rhizobia colonies observed from the agar media plates.

Discussion:

Throughout the experiment there were a few surprises. One being the lack of growth and nodules growth in the Rear Garden pot. The Rear Garden was fertile soil that housed several plants commonly found in the garden, however it seems as if the microbes in the soil did not support growth of legumes. Another surprise was agar media plates of the Front Yard. Much more rhizobia bacteria was found growing in this agar media plate compared to the positive control, which was striking to observe. The soil in the Front Yard seems to be on par with the rhizobia levels in the positive control. This data can be used in the future when working with soil from these areas again. Whether it is for planting purposes or for another experiment, we know which soil is best suited to facilitate plant growth.



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Shreya Math

Investigating The Bacterial Populations That Exist In Santa Clara, California That Help Fix Nitrogen For Plants

Abstract

The lack of access to fixed nitrogen limits food production as it limits the production of crop plants. This has led to increases in the use of nitrogenous fertilizer which can be very expensive in order to ensure efficient growth of crops. Due to the fact that there are so many benefits to reduce dependence on nitrogenous fertilizers in crop production, a lot of research is being done on biological nitrogen fixation. Biological nitrogen fixation is the process in which atmospheric nitrogen is converted into a form that is usable by plants. However, this process does not occur in eukaryotes creating a reliance on symbiotic relationships where plants provide fixed carbon to bacteria and in exchange get fixed nitrogen. This is a relationship that is mainly restricted to legumes in agricultural systems. (1) Here we will examine whether the presence of such bacteria exists in Santa Clara, California.

Keywords: nitrogenous fertilizer, biological nitrogen fixation, symbiotic relationship, legumes

Introduction

In order for plants to grow properly they require an abundance of nutrients such as nitrogen. As plants cannot use atmospheric nitrogen they need to form symbiotic relationships with the microbes in the soil. One such plant that does this is the legume plant. (2) The symbiotic relationship that legume plants are able to establish with the microbes, nitrogen-fixing rhizobia bacteria, allows them to gain a big growth advantage as well as providing nitrogen to other crops such as corn. (3) Because it is unknown whether the bacterial populations that fix nitrogen for sunn hemp legumes are located in Santa Clara, California, for this research soil was collected from two areas in Santa Clara, a park and my backyard, and compared to a positive control containing legume benefitting bacteria. The nodules that formed on these plants were then studied to determine which soil had the best bacteria for the plants. If there is a significant difference in the growth of plants in each pot, then it can be inferred that the pot in which there is the best growth has an increased amount of plant-growth-promoting microorganisms as well as the presence of rhizobia and nitrogen because there as differing microorganisms in each pot. I predict that the soil from my backyard will have the best rate of plant growth.

Methods

Week 1: Soil Collection and Legume Seed Planting

This week six pots were labeled with home soil, park soil, and positive control, two of each. The dirt from the park and the dirt from the back yard were placed into two different plastic bags. The two soils were then placed into their designated pots. In order to make the positive control, the two soils were combined and then mixed with a legume benefitting bacteria. Water was put into the pots and left for an hour in order to allow the soil to be completely soaked. Next to plant the seeds, twenty holes were placed in each pot and a seed was put into each hole. The holes were then covered up and placed in front of the back door in order to get light.

Week 2: Watering the Soil and Monitoring Germination/Sprouting of the Legume Seedlings

Starting from Thursday July 15th, 2021 water was put into all six pots every other day. The pots were placed in an area where they had access to sunlight and were checked every day to make sure they were not drying up.

Week 3: Monitoring Plant Growth

Each pot was checked everyday to see whether water was needed and the amount of plants that had germinated was recorded. As there were over three plants in each of the pots no transfer was necessary.

Week 4: Phenotyping Plant Roots and Shoots

Two and a half weeks after planting, one plant was dug out from each of the six pots and cleaned. The shoots and roots were measured and recorded and the root color was recorded.

Week 5: Phenotyping Plants and Isolating Bacteria from Roots Isolating the bacteria - After sterilizing the workspace, take two plants from the same pot and remove the nodules from the roots using a scalpel. Place the nodules in a clorox wipe and gently cleanse and wash with sterilized water. Place the nodules in a labelled microfuge tube and fill with 0.25 mL of water and crush until minimum plant particles are left. Wrap the microfuge tube in foil and place in the fridge. Repeat with each of the pots. Once all pots' bacteria has been isolated spread the mixtures on to different agar growth plates. Close and flip the plates so the media is on top and label with parafilm.

Microscopy of the nodules - After sterilizing the workspace, take one plants from the same pot and remove the nodules from the roots using a scalpel. Next sterilize a microscope slide and cut the nodule in half. Place blue die on the nodules and remove an excess dye using a paper towel. Put the nodules in a microfuge tube with 0.75 mL sterilized water and gently invert to remove any excess dye. Place the nodules on the slide with the cut side facing up and view using the microscope. Repeat with each of the pots.

Results

Germination/plant growth:

Home Backyard, Santa Clara, California: 23 plants

Park, Santa Clara, California: 15 plants

Positive control, with nitrogen fixing bacteria: 23 plants

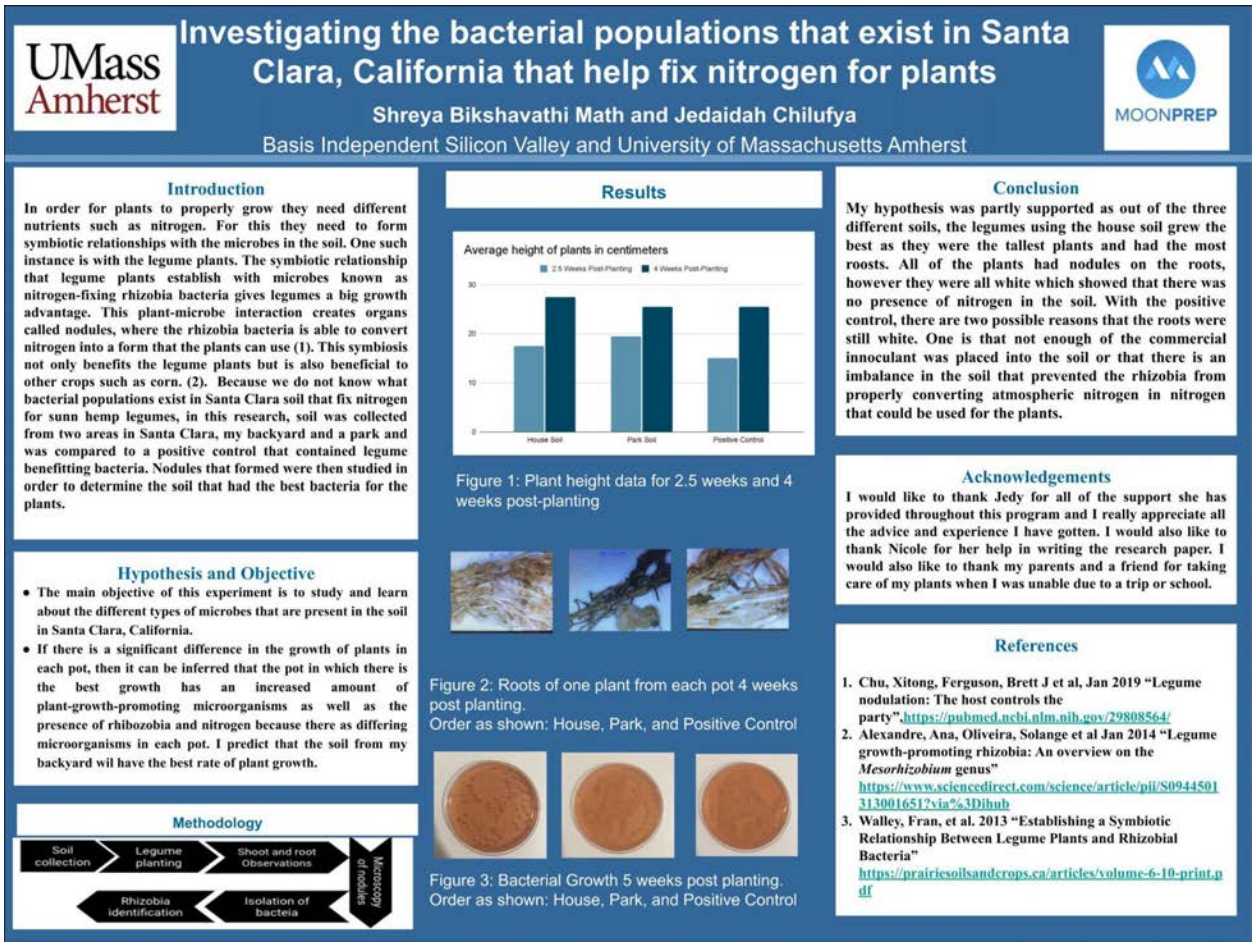
Recording Phenotyping plant shoots and roots

Location	Back yard, Santa Clara, California	Park, Santa Clara, California	Positive control
2.5 weeks post planting 8/3/21	Shoot color: white Height height: 18 cm Roots: 3 cm Shoot color: white. Height height: 17 cm Roots: 8 cm	Shoot color: white Height height: 19 cm Roots: 4.5 cm Shoot color: white Height height: 20 cm Roots: 4 cm	Shoot color: white Height height: 14 cm Roots: 1 cm Shoot color: white Height height: 16 cm Roots: 9.5 cm
4 weeks post planting 8/11/21	Shoot color: white Height height: 25 cm Roots: 7 cm Shoot color: white. Height height: 30 cm Roots: 8 cm *presence of root nodules in both	Shoot color: white Height height: 25 cm Roots: 4 cm Shoot color: white. Height height: 26 cm Roots: 7.5 cm *presence of root nodules in both	Shoot color: white Height height: 27 cm Roots: 6 cm Shoot color: white. Height height: 24 cm Roots: 9 cm *presence of root nodules in both

Discussion

My hypothesis was partially supported as out of the three different soils, the legumes that were growing in the soil from my backyard had the greatest height. I believe this is because there are a lot of animals, mainly cats that roam around in our neighborhood and many excrete their waste in our backyard. All of the pots contained plants which had nodules on them, however they were all white showing the lack of nitrogen-fixation. With the positive control, three possible reasons that the roots were still white are that not enough commercial inoculant was placed in the soil, more time was needed to form the symbiosis, or there was an imbalance in the soil preventing the rhizobia from properly

converting atmospheric nitrogen in to nitrogen that could be used for the plants. This study is significant because it is able to show that the soil in my area of Santa Clara, California is not the most ideal to grow crops in. This study could be furthered by choosing areas further away from each with as well as increasing the amount of time the plants were studied for.



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Sindhu Raghava

Determining The Presence Of Nitrogen-fixing Microbes For Legumes In Soil San Jose, California

ABSTRACT

Rhizobia bacteria form symbiotic relationships with legumes in which the bacteria provide fixed nitrogen to the plants and the plants provide fixed atmospheric carbon dioxide to the bacteria. Some examples of bacteria that form mutualistic relationships with plants are Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, and Azorhizobium. They provide N “fixed” from the atmosphere, while the plant provides C, which is fixed from atmospheric CO2 during photosynthesis. Additionally, legumes that are common crop and forage legumes form symbiotic relationships with rhizobia, and examples are peas, beans, clover, and soy. Specifically, rhizobia bacteria like Sinorhizobium meliloti and cowpea-type rhizobia bacteria have relationships with the plants Medicago truncatula and Crotalaria juncea (also known as sunn hemp), respectively. The products of the mutualistic relationship are nodules, a specialized organ formed by the legume plant that reduces nitrogen to ammonium. Rhizobia tell legume roots to form root nodules, which provides a home for the rhizobia and protects the nitrogenase enzyme. Carbohydrates in the nodule also feed the rhizobia in the nodule. This relationship between rhizobia bacteria and legume plants is very beneficial to both. There is no information about beneficial bacteria that interact with sunn hemp roots in San Jose, California. Therefore, experiments in this research dive deeper into the specific types of bacteria interacting with legume sunn hemp plants grown in soil from different locations in San Jose, California.

The purpose of this experiment was to discover the way bacteria microorganisms present in two selected locations interact with legume plants. The legume seedlings were grown in soil from different locations and watered and given ample sunlight to observe if they were sprouting throughout the weeks. To phenotype the legume plant roots, shoot color was observed, and the roots were examined under a digital microscope to see if there are nodules.

MATERIALS AND METHODS

The materials used in the experiment included a microscope, microscope slides and cover slips, scalpel, dropper or pipette, toluidine blue stain, water, legume plants, digging tools, paper towels, a cup of water, ruler, labels, microcentrifuge tubes, plastic pestles (blue), sterile water in the falcon tube, Clorox wipes, and, agar media plates (1 agar media plate per pot).

The methods for the experiment are soil collection, planting the legume seeds in each of the pots, phenotyping and observing plant roots and nodules under the microscope, isolating the rhizobia bacteria from the root nodules, and identifying the bacteria on each of the agar plates for each pot.

RESULTS

At 4.5 weeks post-planting: Positive Control - plant height is 22 cm; Front Yard - plant height is 21 cm; Backyard - plant height is 34 cm

Plants from all pots developed nodules by 4.5 weeks, but most plants did not have them at 4 weeks.

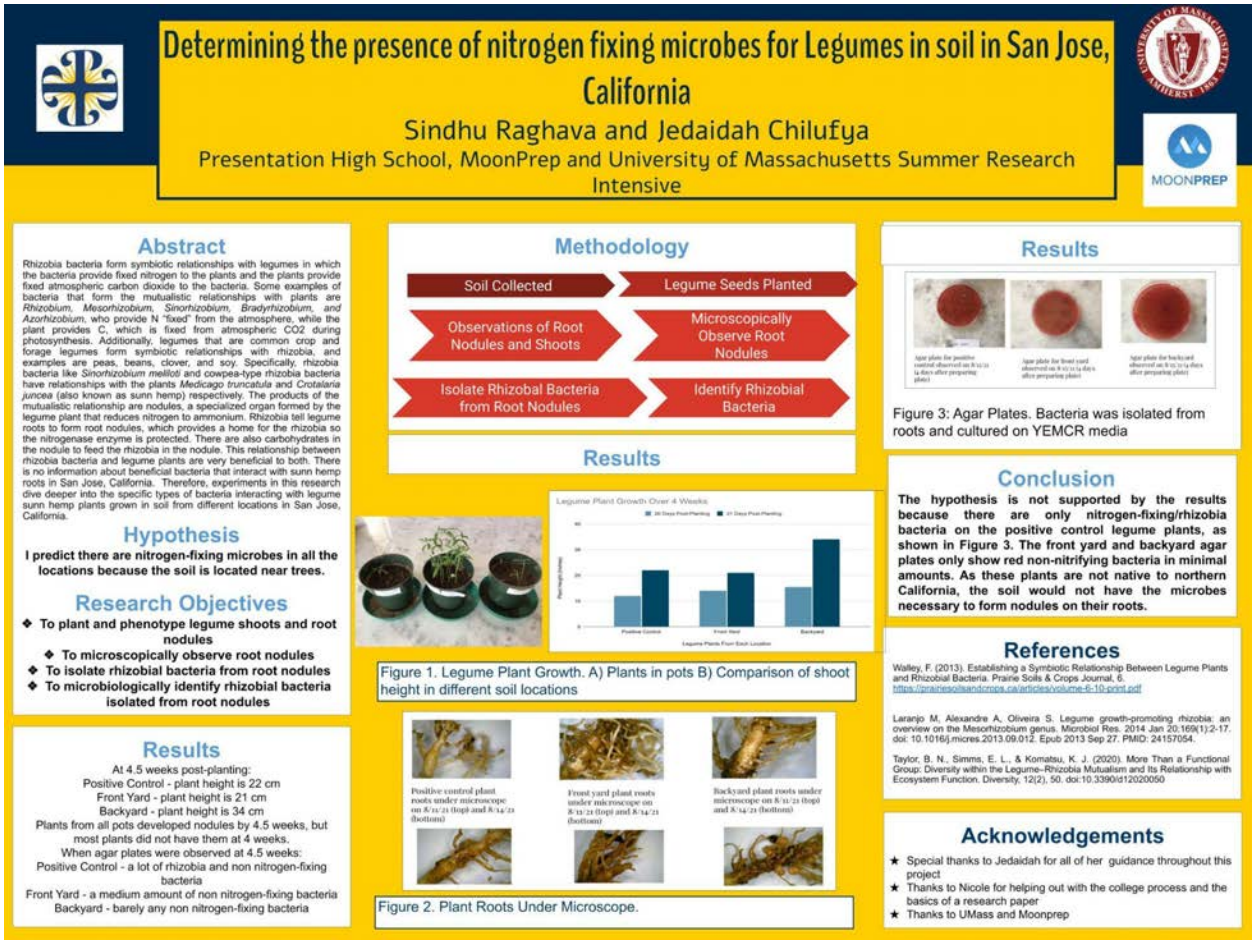
When agar plates were observed at 4.5 weeks: Positive Control - a lot of rhizobia and non-nitrogen-fixing bacteria and bacteria are round, entire, and raised, and there are TMTC; Front Yard - a medium amount of non-nitrogen-fixing bacteria; Backyard - barely any non-nitrogen-fixing bacteria

DISCUSSION

The hypothesis is not supported by the results because there are only nitrogen-fixing/rhizobia bacteria on the positive control legume plants, as shown in Figure 3. The front yard and backyard agar plates only show red non-nitrifying bacteria in minimal amounts.

According to Walley (2013), the nitrogen-fixing capacity of rhizobia can be lost because the genetic material that provides the codes necessary for nodulation to occur exists in plasmids, which can be lost or transferred from rhizobial cells. Furthermore, as the legume plants planted are not native to northern California, the soil would not have the microbes necessary to form nodules on their roots. Combined with the likely loss of plasmids that contain the genetic information of rhizobia, this provides a probable explanation for the unexpected results.

In the future, this data can be used to drive experiments trying to create nodules using soil from similar locations in San Jose, California. Laranjo M, Alexandre A, and Oliveira S. (2014) described, “For the establishment of an effective symbiosis two main classes of bacterial symbiosis genes are needed: nodulation and nitrogen fixation genes” (pp. 2-17). Using nodulation genes that encode enzymes that secrete Nod factors and nitrogen fixation genes that conduct atmospheric nitrogen fixation, researchers can mix these with the soil and plant the legume plants in it to observe any nodule growth that would otherwise not be on the plant roots. If the experiment succeeds and there are nodules on the roots, researchers can put the mixture of genes into the soil in other parts of San Jose to increase the diversity of the environment. As Taylor, B. N., Simms, E. L., & Komatsu, and K. J. (2020) state, the nitrogen-fixing symbiosis can bring more nitrogen into ecosystems and play a role in growing agriculture. Although the results of the experiment conducted were surprising, they ultimately can inspire more experiments that can contribute to environmental diversity and other plant growth.



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Tvisha Chandupatla

The Impact Of Microbes On Legume Plants In New Jersey

Abstract

Microbes are everywhere in this world, and they all work uniquely. Certain

types of microbes can be helpful to plants (such as the *Crotalaria juncea* which can also be called a legume plant or sunn hemp.) In legume plants, *Rhizobia* (a type of microbe) in the soil converts nitrogen into ammonia since the plants cannot get nitrogen from the air. *Rhizobia* from root nodules on the roots of the plant, and they live in those structures. By observing the growth of legume plants in different soil environments, the different microbes in the soil will have different impacts on the plants' health. To figure out which location is best for this legume plant, soil was collected from two places, and a control group was added, which contained an inoculant filled with positive microbes for the sunn hemp plants. Soil from Old Bridge, New Jersey, has different undiscovered microbes, and each bacteria will affect the plant's health differently.

Introduction

How will different microbes in varying soil locations affect the plant's health? Sunn hemp plants form a symbiotic relationship with *Rhizobia*, and this microbe helps fix nitrogen for the plants. In return, the plants pass nutrients, such as carbon dioxide, to the *Rhizobia.* For *Rhizobia* to form root nodules on the plants, the legume plants must first send signals to the soil to find compatible bacteria. Once it does, the *Rhizobia* forms root nodules; they can either be two different colors: white or pink. If the root nodules are white, they are not fixing nitrogen, but if they are pink, they are fixing nitrogen. It is unknown whether the soil in Old Bridge, New Jersey, contains bacteria that fix nitrogen for the sunn hemp.

Methods & Materials

Soil was gathered from two locations: Location 1 is a cul de sac in Old Bridge, New Jersey, and Location 2 is a backyard in Old Bridge, New Jersey. The cul de sac has a couple of trees, and people walk on it every day. In contrast, the backyard is filled with plain grass, barely any trees, and rarely anyone walks upon it. Water was added to the soil, and Location 1 soil is put into pot one, and Location 2 is put into the second pot. For the third pot, there is a mixture of Location 1 soil and a positive control (which is an inoculant containing different microbes: *Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli,* and *Rhizobium leguminosarum biovar viceae)*. Pot three is the control group out of all the pots.

Wooden tweezers were used to poke twenty holes into the soil for all three pots, and the holes were around one inch deep. Each hole was filled with one legume seed, and then all the holes were covered up. The pots were placed near a window with an adequate amount of sunlight and received around 60 mL of water each day.

After two weeks post-planting, the plants were removed from each pot, and their shoots and roots were phenotyped. The roots were washed with water, and each plant was put underneath a digital microscope. They were observed for root nodules (look like miniature spheres connected to plant roots.) To phenotype the shoots, a ruler was used to check the plants' heights. This process is done around twice a week, starting from 2.5 weeks post-planting to 4 weeks post-planting.

From each pot, one plant was taken, and if there were any root nodules, they were cut off from the roots and cleaned with a bleach mixture (contained 0.25 mL water and 0.75 mL bleach). Then, they are put

into a microcentrifuge with 0.25 mL of water. Using a pestle, the root nodules were smashed as much as possible to release any bacteria into the water solution. This solution is put onto a petri dish, spread out with a pestle, and finally sealed. If there weren't any root nodules, a small piece of the root was cut, followed by the same process. The Petri dishes were wrapped in tin foil and kept near a window for around six days.

Results

The plants all grew at different rates, but the location with the highest average shoot height is the backyard (21 cm average). The average for the positive control is 12 cm, and the average for cul de sac is 7 cm. Out of all the pots and their plants, only one plant had root nodules, which was from the positive control pot. All the plants had green shoots.

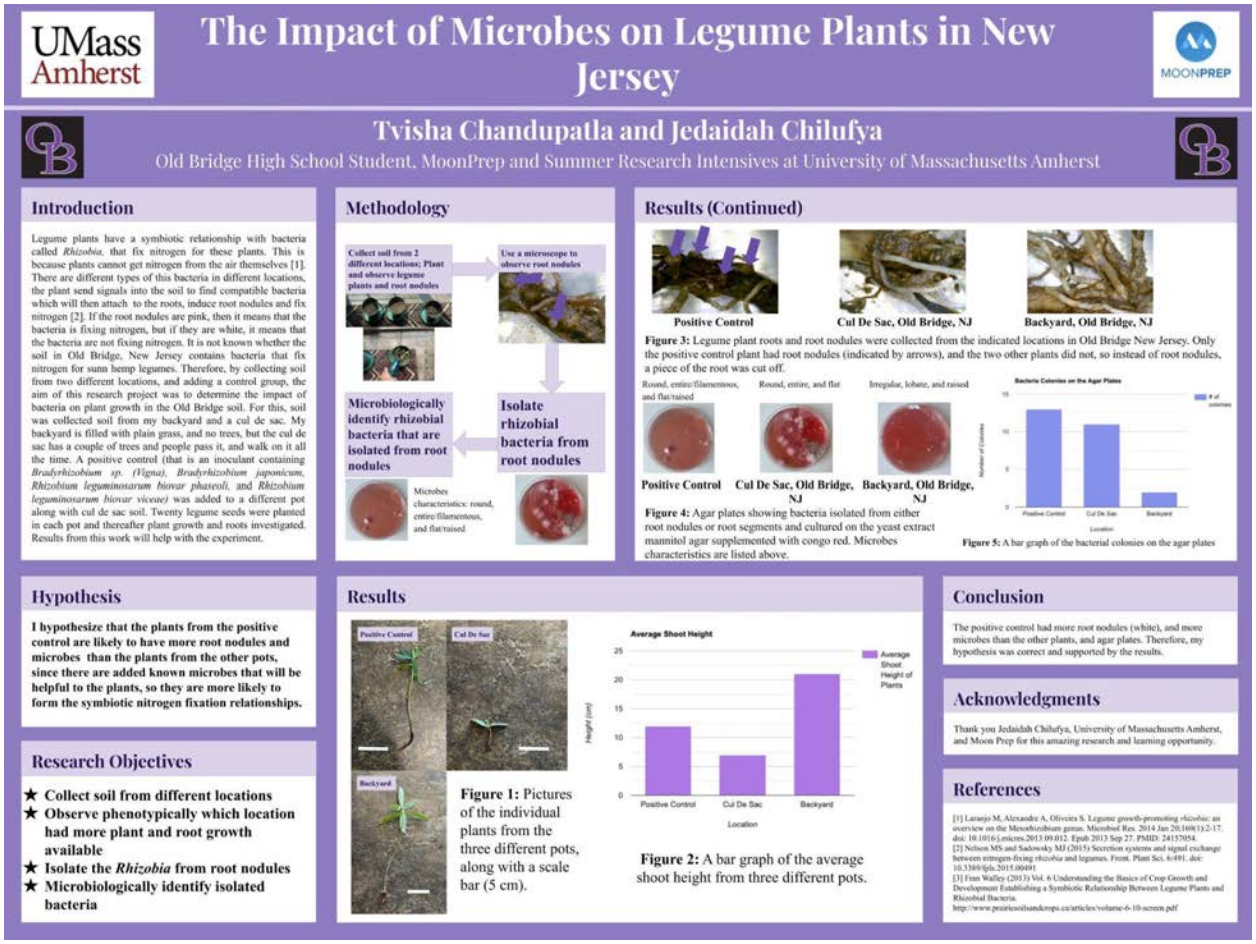
After waiting around six days from the agar plates, the positive control plate had more microbe colonies (13 colonies) than the other plates. The cul de sac plate had 11 colonies and the backyard plate had 2 colonies. Most of the bacteria in the positive control plate were round, entire, and flat. In the cul de sac plate, the colonies were round, entire, and flat. In the backyard, the microbe colonies on the plates were irregular, lobate, and raised. There were many different types of bacteria in each petri dish that varied in colors.

Discussion

From the results, even though the plants from the backyard soil grew taller, the plants from the positive control pot had more root nodules and bacteria. These results were mainly expected since the positive control did have more root nodules and microbes, but it wasn't foreseen that the backyard plants would be taller. This shows that even though there are more microbes on a plant, that doesn't mean that they will become taller than a plant with fewer microbes. It depends on which type of microbes the plant has because some of them could be beneficial to the plant, while others may not.

In the future, if this project is continued, this experiment can be done in different towns and cities in New Jersey and can be compared to the results in Old Bridge. This would be helpful to see how sunn hemp plants grow throughout New Jersey and can be used to see which locations in this state are best for these plants.

Crotalaria Juncea and *Rhizobia* work hand-in-hand, as they both benefit from this mutualistic and symbiotic relationship they have formed with each other.



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Ujwal Linga

To Determine The Bacteria That interacts With Legumes In Edison, NJ

Recent Global demand has led to the use of Legumes as a fertilizer. This is because Legumes have a symbiotic relationship with the Rhizobia bacteria that form special organs called nodules. These nodules can convert atmospheric nitrogen gas into nitrogen that the plant can use. Different legumes interact with different rhizobia. There is no information on whether the soil in Edison, New Jersey contains rhizobia that interact with the legumes to provide nitrogen. Therefore, the goal of this experiment was to determine whether the soil used in the experiment has rhizobia bacteria or not that interacts with the legumes. My hypothesis is that the Legumes from my front yard will have more rhizobia than my backyard as a lot of trees grow in my front yard; while my backyard is extremely dry. To do this I first had to collect soil from two different locations and put it in two different pots. I also had a third control pot that for a fact had rhizobia bacteria. After collecting my soil I planted the legumes in the three pots. Every week or so after the plants started growing I would record the height of the legume plant shoots and observe the roots under a microscope. Once nodules started appearing I would observe them under a microscope every week; then I tried to isolate the rhizobia bacteria from the nodules on the roots from plants in the three pots(the plants from my backyard had no nodules so I used the routes of the plants to try to isolate the rhizobia.) This was the protocol I used for isolating the bacteria:

Materials

- Plants (one pot at a time)
- Digging tools
- Paper towels
- Cup/pot or beaker of water **(get fresh water between pots)**
- Flat surface with a dark background
- Ruler or measuring tape or print out of 15 cm ruler (from google search)
- Labels and a pen or sharpie
- Microcentrifuge tubes
- Plastic pestles (blue)
- Dropper
- Microscope slide
- Scalpel
- Sterile water in the falcon tube (or boil tap water to sterilize it)
- Household bleach (clorox or disinfectant with active ingredient sodium hypochlorite. Clorox wipes are good too.
- Agar media plates (1 agar media plate per pot)

1) Wear your PPE
2) Sterilize your gloves and your work space with 70% ethanol
3) Collect all the materials on the list above
4) Make the diluted bleach sterilizing solution by diluting into the first microcentrifuge tube **(If you have clorox wipes instead, skip this step 4 and move to step 5)** First label the microcentrifuge tube on the top and the side (with B or Bleach)
    a) Then add sterile water in the tube according to the percentage of your active ingredient sodium hypochlorite in your bleach;
        i) If the sodium hypochlorite is **above 5%, add 0.75ml sterile water** in the microcentrifuge tube using the dropper (the line mark **above** the 0.5ml mark on the dropper)
        ii) If the sodium hypochlorite is **below 5%, add 0.25ml sterile water** in the microcentrifuge tube (the line mark **below** the 0.5ml mark on the dropper)

b) Then using the dropper, add bleach in the same microcentrifuge tube accordingly
    i) If you had added **0.75ml sterile water,** then add **0.25 ml of bleach**
    ii) If you had added **0.25ml sterile water,** then add **0.75 ml of bleach**
    iii) Sterilize the dropper by wiping it with a paper towel drenched in 70% ethanol or by using 70% ethanol wipes
    iv) Mix the bleach and water in the microcentrifuge tube by inverting the tube several times.
    v) You are done with making the bleach solution. Close the microcentrifuge tube lid and place the tube aside for your first pot of plants

5) Starting with one pot at a time (either positive control, or location 1 or location 2), carefully dig out all the plants in the pot, wash off soil particles from the roots and check for root nodules by observing under the microscope
6) From all the plants dug out in the pot, **choose one plant to phenotype** and **take a picture of the whole plant like we did last week** (next to a ruler) **(see protocol from last week)** and a picture under the microscope. Add the pictures in your photo uploads Experiment photo uploads
7) Record the plant phenotype and root phenotype in the phenotyping table above under **week 3 post planting** (plant 1 and plant 2) but **also indicate the specific number of days post inoculation** (If you see any root nodules, count and record the total number and their color and record your observations in the phenotyping table above)
8) Once you have a **microscope picture of the roots** on the one plant, choose between **1 to 4 nodules /a small root segment** from one plant **to isolate bacteria from** (for nodules, make sure as much as possible to eventually only obtain the round-ish structures only from the roots)
9) Place the roots of the plant on a **microscope slide** and depending on how many total nodules you have **(save some for microscopy)** cut off **1 to 4 nodules or a small root segment (if there are no nodules)** from the root using a scalpel
10) Clean and sterilize the microscope slide using 70% ethanol
11) Using the scalpel, place the nodules or small root segment in the tube with the bleach solution to sterilize. Close the lid and invert the tube gently several times.
    a) If you have clorox wipes instead, to sterilize, place the nodule or root in the clorox wipe and tap it gently for several seconds within the clorox wipe to sterilize
12) Clean and sterilize the microscope slide using 70% ethanol
13) Using the scalpel, remove the nodule or the root from the bleach tube and place it on the microscope slide
14) Sterilize the scalpel by wiping it with a paper towel drenched in 70% ethanol or by using 70% ethanol wipes
15) Wash off the bleach from the nodule/ small root segment by adding several drops of sterile water (in the falcon tube) on top of the

nodule/small root on the microscope slide using the dropper. Move the nodule/root segment around using the drop and suck/withdraw water several times to completely wash off the bleach. Get rid of the water by squirting it into the beaker/pot/cup of water

16) Sterilize the dropper by wiping it with a paper towel drenched in 70% ethanol or by using 70% ethanol wipes
17) Place the sterilized nodule/root segment into a **new microcentrifuge tube** (using the sterilized scalpel). Label this tube accordingly (Example; P or positive control/Location 1/ Location 2)
18) Using the sterilized dropper, **add 0.25 ml of sterile water into the tube** with the nodule/root (the mark below the 0.5ml mark on the microcentrifuge tube)

<u>If you need a break, you can stop at this stage (step 18)</u> by closing the tube, wrapping it in aluminum foil and placing it in the fridge (not freezer!)

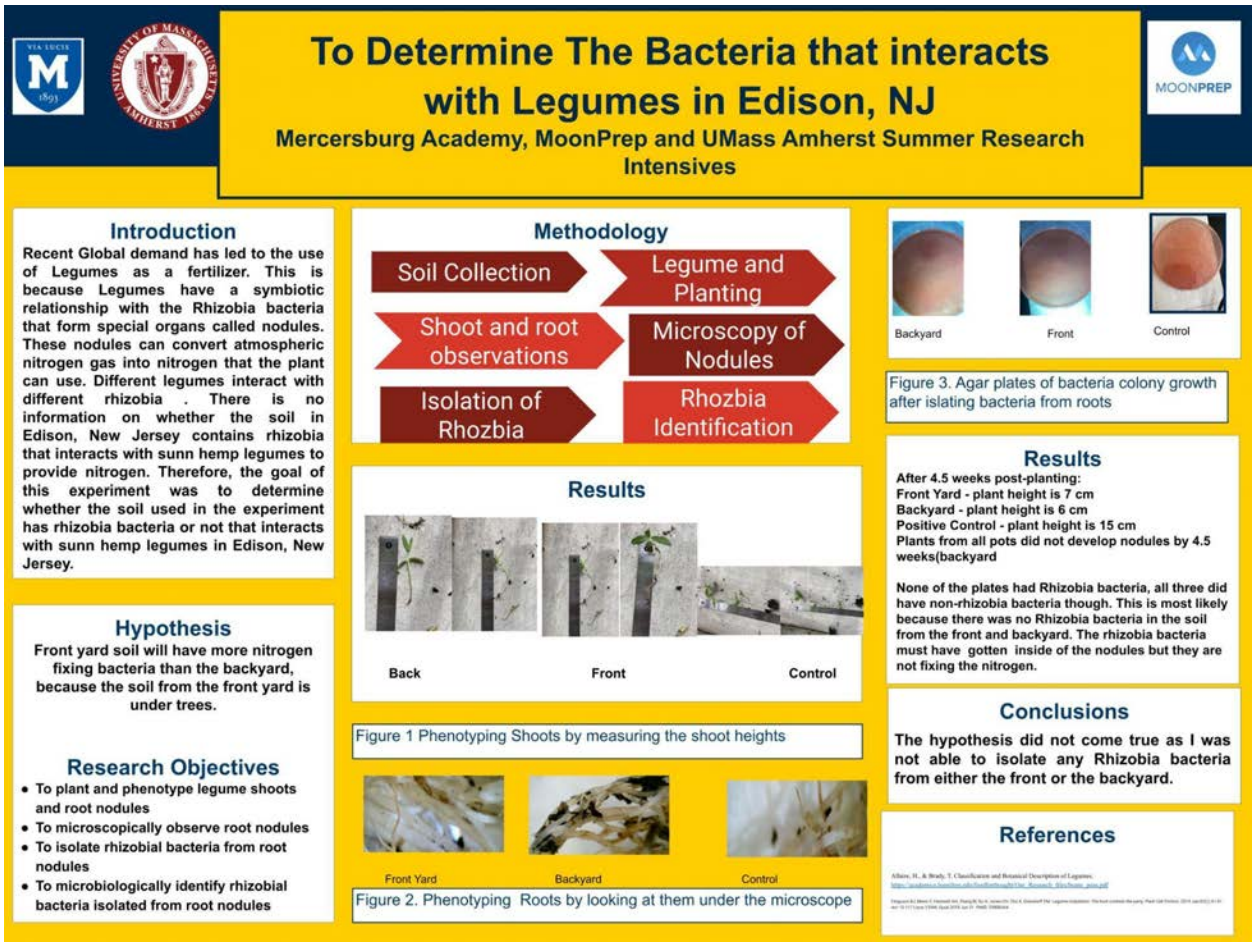
19) The next step is to release the bacteria or isolate bacteria from the nodule/root.
    a) To do this, obtain a plastic pestle (blue) and use it to crush or homogenize the nodule/root (this needs a lot of energy :)).
    b) Keep crushing the nodule/nodule until the water turns a little cloudy and you see only small plant structures in the tube
20) After the crushing is done, you are now ready to place your crushed suspension onto an agar media plate.
    a) To do this, **get one agar media plate** and seal back the rest of the plates
    b) Sterilize your work surface with 70% ethanol and open the agar media plate
    c) Pour all the crushed nodule/root suspension onto the plate and gently swirl the plate around to spread the suspension.
    d) For complete spreading of the suspension on the plate, obtain a new plastic pestle and use it to gently spread the suspension on the entire media surface.
    e) Seal the agar media plate with parafilm, place it upside down (media on top) and wrap it in aluminum foil.
    f) To allow for the bacteria to grow, place the wrapped plate (upside down) near a window (maximum temperature of 30'C) or put a shade by the window if your temperatures go above 30'C.
    g) Check the plate for colony growth after 2 days, then every day after that. If you see anything new growing on your plate, take a picture and post it in your photo uploads Experiment photo uploads for this week. Examples of bacteria growing on agar plates observing-bacteria-in-a-petri-dish.html
    h) **Repeat the procedure from step 4 to step 20 for the remaining 2 pots**. At the end of the experiment, you need to have plant and root phenotypes for all 3 pots and 3 agar media plates (one for each pot).

Finally, after isolating the bacteria, putting them into 3 agar plates named "front yard, back yard, and control"(depending on where the bacteria was from) I waited a couple of days to see bacteria growth.

My research objectives are:

- To plant and phenotype legume shoots and root nodules
- To microscopically observe root nodules
- To isolate rhizobial bacteria from root nodules
- To microbiologically identify rhizobial bacteria isolated from root nodules

If the bacteria had been rhizobia the agar plates would have had white particles, but the agar plates shown above had no white mass and only had bacteria that were dyed red(non-rhizobia bacteria). This means my hypothesis that the soil from my backyard would contain more rhizobia bacteria is wrong as both my front yard and backyard don't have rhizobia bacteria. One explanation for why the control dish doesn't have any rhizobia showing is that even though the nodules had rhizobia bacteria in them, they weren't fixing nitrogen. This led to them not showing up on the agar plates.



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Varsha Girish

To Determine Which Soil Contains Bacteria That Interacts With Sunn Hemp Legumes In Wexford, PA

Abstract: I planted legume plants in three different pots because their soil was different. I phenotyped them to observe their nodules and isolated bacteria from them to figure out what kind of bacteria microorganisms were to be found. By doing this, I found that location plays a huge role in what to expect in terms of bacteria microorganisms. The growth of the plants, roots, and nodules were all different from one another.

Intro: Bacteria microorganisms are in all kinds of places. There are many factors that can describe the type of bacteria microorganisms to expect. For one, location. The location you plant in is a major factor to finding out the kind of bacteria microorganisms you will see. There is little information about the type of bacteria which are present in the soil in Wexford which interacts with the legume plant, sunn hemp. Therefore, for this research project, I collected soil from different locations and planted my sunn hemp legume seeds in three different pots with three different locations. I did this to observe how the different soil would affect different factors such as the germination and the number of nodules formed. By doing this, we found that Rhizobia bacteria interacts with Crotalaria Juncea to form nodules on the roots of the plants.

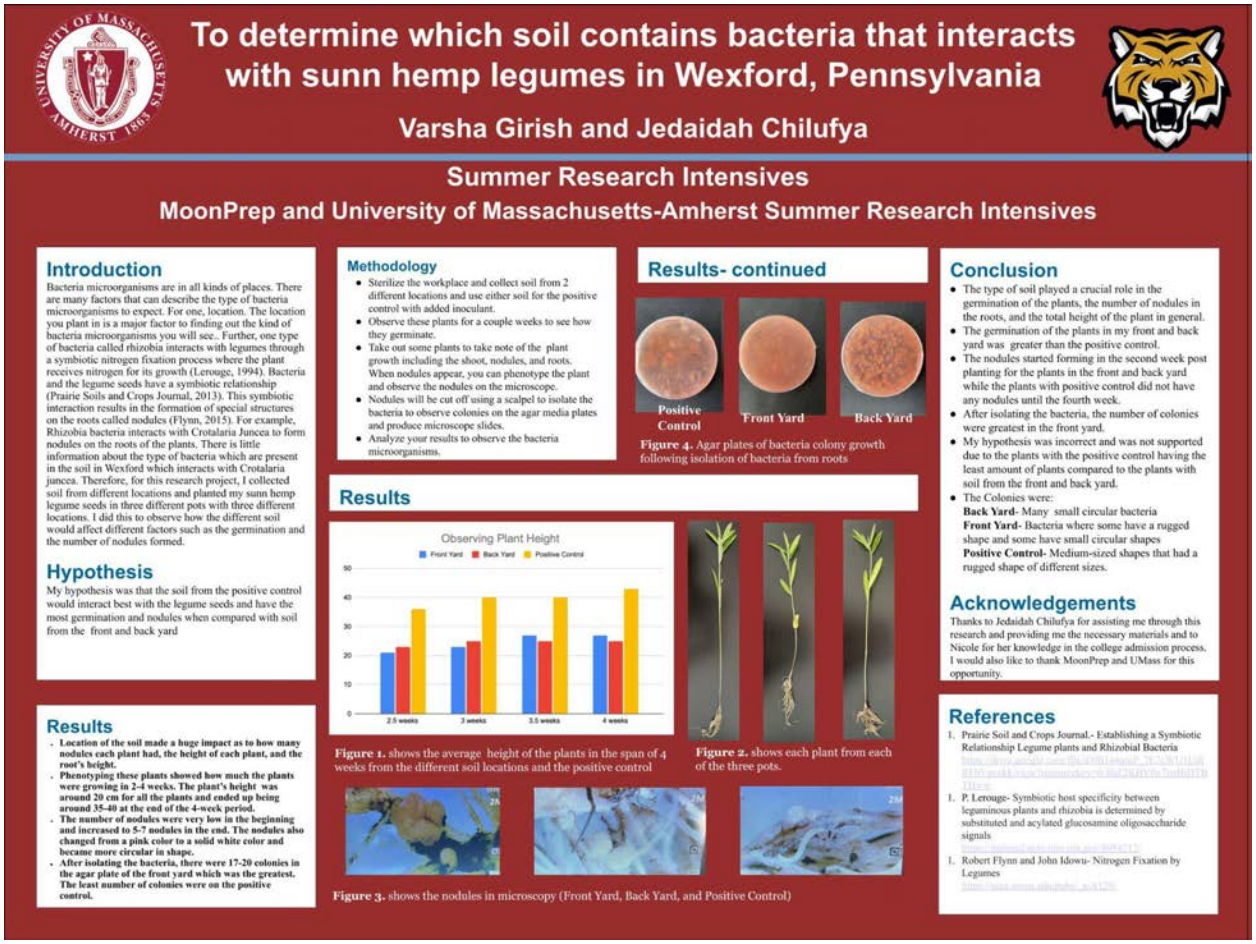
Methods: First, sterilize the workplace and collect soil from 2 different locations and use either soil for the positive control with added inoculant. Second, observe these plants for a couple weeks to see how they germinate. Third, take out some plants to take note of the plant growth including the

shoot, nodules, and roots. When nodules appear, you can phenotype the plant and observe the nodules on the microscope. Fourth, the nodules will be cut off using a scalpel to isolate the bacteria to observe colonies on the agar media plates and produce microscope slides. Lastly, analyze your results to observe the bacteria microorganisms.

Results: Phenotyping these plants showed how much the plants were growing in 2-4 weeks. The plant's height was around 20 cm for all the plants and ended up being around 35-40 at the end of the 4-week period. The number of nodules were very low in the beginning and increased to 5-7 nodules in the end. The nodules also changed from a pink color to a solid white color and became more circular in shape. After isolating the bacteria, there were around 25 colonies in the agar plate of the front yard which was the greatest. The least number of colonies were on the positive control.

Discussion:

My most significant results are the plant germination and the number of nodules formed. These factors helped me phenotype my plant which also helped me figure out whether the plant had enough water and sunlight. The results helped access my soil and the fact that location played a huge role in determining whether or not there was more plant germination. My results did not support my hypothesis. The positive control reacted the poorest with the legume seeds in the soil. I was very surprised as I thought that having the inoculant would increase the germination of the plant. I can use this data in the future to figure out if this is the same for the state of Pennsylvania or even the surrounding areas around me. I only took data from my neighborhood, there may be different factors 2-3 miles away from me. I can take away that small factors have significant impacts. The location, a small factor, had a large impact as to whether or not the roots have nodules and germination of the legumes.



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Independent Student Researchers

The following students did not participate in Rising Researchers. They have conducted research outside of the UMass research intensive. After careful review, we have deemed their research worthy of publication in our scientific research journal.

Aditya Purugulla

The Effect of the Global Pandemic on the Doctor-Patient Relationship

Introduction

With the start of the COVID-19 global pandemic, there was no doubt that life around the world would change. As the situation grew worse, more people started to need medical assistance and this greatly affected the dynamic of hospitals around the world. Medical supplies, doctors, and hospital beds were all in high-demand and this continued to grow with the rising number of COVID-19 cases. With this change in the dynamic of hospitals, doctors and patients both need quality healthcare due to the large number of patients being admitted to hospitals. We need to look at alternative ways to improve the quality of healthcare. One prominently overlooked solution is improving the basis of all doctor's visits: The doctor-patient relationship.

The doctor-patient relationship allows for a meaningful exchange of information between doctors and their patients. This creates a trusting relationship and a medium in which data can be gathered, plans can be made, and healing and support are provided for the patient. (Goold and Lipkin 1). Therefore, by improving this relationship, we can greatly impact the quality and effectiveness of healthcare. This is ultimately the goal of this research study, which is to examine how and in what ways the doctor-patient relationship changed as a result of the global pandemic.

Looking at a deeper analysis of the COVID-19 pandemic, most of the efforts to ease the pandemic were focused on adults and the elderly, as the CDC warned that increasing age was a risk factor for COVID-19. Very little attention was diverted to teens, who have in fact been "removed from normal social, physical, and educational interactions", leading to an overall decline in their mental state (Drillinger 1). This was mainly due to the restrictions of lockdown and social distancing procedures.. Therefore, this study will aim to focus on teenagers by exploring the communication patterns with their doctors. The researcher plans to observe how the doctor-patient relationship has changed over the course of the pandemic. In order to accomplish this, surveys will be used in the form of a needs assessment that measures how patients and doctors are communicating with each other, as an indicator of the effectiveness of the doctor-patient relationship.

Materials and Methods

Participants

The researcher chose a population of high school teenagers across Frisco, Texas. The social barriers and the quarantine regulations as a result of COVID-19 have affected teenagers' mental health to a great extent, limiting their normal social interactions overall and having a negative impact. Therefore, by conducting a needs assessment directed towards

teenagers, the researcher is able to see ways to improve communication with their doctors and take responsibility for their own health. Picking a time period for this research was relatively easy, as during this time of the research study, the pandemic was still ongoing. The research was then conducted over the course of 3 months, from November of 2020 to January of 2021.

Materials and Procedure

The next step in the research method was to create the survey that would be distributed to high school students across Frisco, Texas. Since this was a needs assessment, the researcher needed to focus on the obstacles that were present when patients were communicating with doctors as well as how the communication has changed as a result of the pandemic. In order to measure the level of communication, qualitative and quantitative data-centered questions were asked in order to achieve an accurate conclusion. The goal of making this questionnaire was to identify many variables that could lead to a trend which will reveal how the doctor-patient relationship has changed due to the pandemic. Google Forms was the survey method used, and in order to store the results of the survey for easy access and comparison, Google Sheets was implemented.

In addition to the patient survey results, results from a survey done by a medical research group was used to encompass the doctor's perspective. These results were used due to the fact that doctors were widely unavailable to contact during the time of this research study. However, the questions and results of the survey were still influential and provided data that would ultimately contribute to the researchers findings and conclusion.

Results

Perceived Needs

The first half of the research study was to assess the perceived needs of patients and doctors in the doctor-patient relationship. Through an analysis of past documentations and articles of the doctor-patient Relationship, the perceived needs were found to be:

- **Trust -** Patients who trust and "like" their physician had higher levels of satisfaction. This indicates that trust is needed for patients and doctors to communicate with each other effectively (Bennett et al).
- **Knowledge -** When doctors discovered patient concerns and addressed patient expectations, patient satisfaction increased, which indicates that patients expect doctors to have a formidable amount of knowledge (Korsch BM, Negrete VF).
- **Regard -** Ratings of a physician's friendliness, warmth, emotional support, and caring have often been associated with patient satisfaction, indicating that patients communicate more readily with their doctors if they are able to empathize with them (Korsch BM, Freemon B).

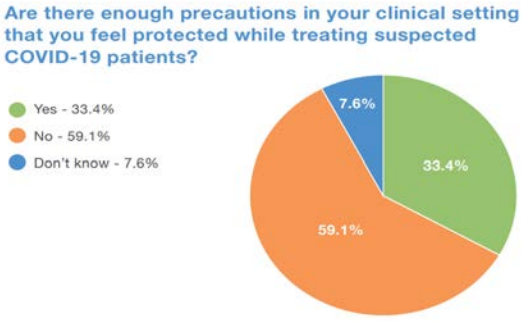
The perceived needs identified served as an indication of the doctor-patient relationship before the onset of the COVID-19 pandemic.

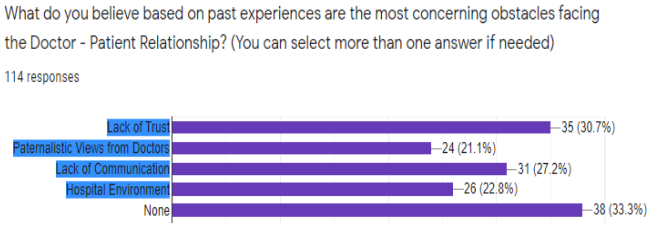
Expressed Needs

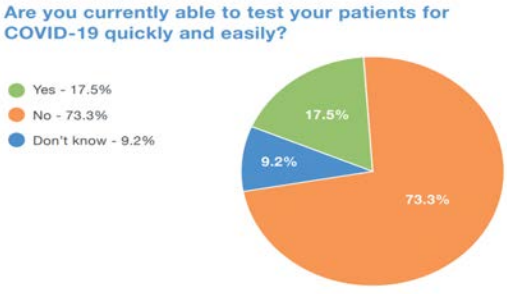
The expressed needs, which composed the second half of the data collection, was an assessment of the doctor-patient relationship after the COVID-19 pandemic. In the patient (teenager) survey, a majority of the respondents said that their ability to communicate with their doctors has stayed the same during the pandemic, with the second most popular response being it has gotten worse, and the least selected answer being that their ability to communicate has gotten better. When asked to rate the level of communication with their doctors on a scale of 1-10, most of the teenagers responded with 10. Next, when asked about

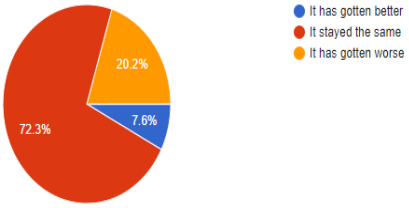
the obstacles facing the doctor-patient relationship 4 major problems were identified by patients: lack of trust, paternalistic views from doctors, lack of communication, and the hospital environment. The results of the survey given to the doctors showed a majority expressed they did not feel safe while treating COVID-19 patients. Lastly, when asked about whether or not they were able to test their patients quickly and easily, once again, a majority of doctors answered negatively

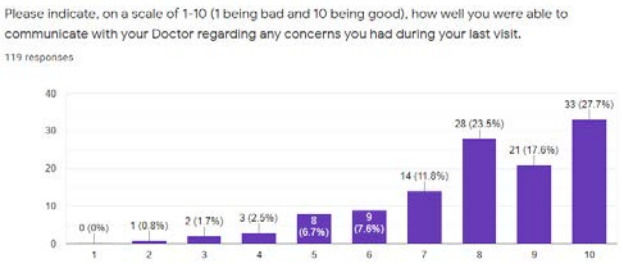
Tables, Graphs and Photos











Discussion

The original hypothesis for this conducted research study was that the COVID-19 pandemic would have a negative impact on the Doctor-Patient Relationship. Looking at the results of the patient survey, we can see that a majority of patients felt their communication with their doctor was sufficient. In addition to this, comparing the perceived needs, to the expressed needs found in the results of the research, we can see the majority of the needs between teenagers and their doctors stayed the same, as trust, loyalty, and empathy relied at the core of all the potential problems identified by the patients. This indicates that this hypothesis is proven to be false, due to the fact that there was no observed negative correlation between the onset of the COVID-19 pandemic and the doctor-patient relationship, as a majority of patients stated that their level of communication with their doctors has stayed the same.

However, with the rapid influx of new COVID-19 cases each day, hospital beds are filling up quickly and doctors are getting more and more patients. This prompts the idea that doctors actually need improved communication with their patients. Perhaps

the pandemic has prompted a new need for increased communication between doctors and their patients, rather than negatively impacting the relationship between these two. This is further reinforced by the results of the doctor survey.They feel that precautions were not implemented well in their hospital environment. Therefore, it can be concluded that the COVID-19 pandemic has caused a need for more communication between doctors and teenagers because of the increased emphasis of safer hospital environments.

Conclusion

Overall, the research study done on the impact of the doctor-patient relationship and how it was impacted as a result of the COVID-19 pandemic provided a new understanding, which is that the pandemic has caused a need for increased communication between patients and doctors. This could include implementing regulations or regular check-ups in order to ensure that the factors such as trust, a healthy environment, loyalty and empathy are all preserved in the hospital environment. Doing a further analysis of the results, the researcher found that one prominent obstacle to communication among a handful of teenagers was their parents. These teens stated that their parents made them feel uncomfortable, which comes as a hindrance to a safe environment. Therefore, it is suggested that future researchers explore this topic in more depth and arrive at an understanding which states if this is another result of the pandemic or an already existing obstacle to the doctor-patient relationship.

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Anokhi Amara

Detection of Leukemia with Cyclin D and B Flow Cytometry

ABSTRACT: Leukemia has been a prominent disease for many generations, yet current tests are ineffective and often detect tumorous growth too late. The purpose of this research is to investigate the relationship between cyclin concentrations (D and B) and the different types of leukemia (ALL, CLL, AML, CML) and use the relationship to detect the presence of cancer. Concentrations of the various cyclins activate the signaling pathways of Cyclin-Dependent-Kinase (CDK). CDKs are kinases, enzymes that phosphorylate specific target proteins, and they play an important role in the regulation of the cell cycle by controlling cellular division. The activity of CDKs rises and falls with the changes in the concentration of its cyclin partner, and this concentration trend regulates the normal division rate of cells. In non-cancerous cells, cyclin concentrations follow a certain pattern that is uniform among subgroups of cells (Figure 1). However, in cancerous cells, this pattern is altered because the cell cycle is unregulated as cells divide and metastasize in an uncontrolled way. This research proves that the alteration of cyclin D and B concentrations has been an indicator of different types of leukemia. The proposed medical test will utilize Fluorescent-Activated Flow Cytometry to quantitatively determine levels of cyclins B and D. By comparing the levels of cyclins in cancerous cells to those of normal cells, standards can be established and abnormal levels will point to the risk or presence of leukemia. This cyclin tracking test will revolutionize the diagnosis of cancer by detecting abnormal cyclin concentrations before the proliferation of white blood cells, which would allow scientists and doctors to have a greater time period to advise patients on the reduction of controllable risk factors and to give them treatments at earlier stages.

MATERIALS AND METHODS: As most labs were shut down due to the pandemic, I used different resources to gain data. I compiled and analyzed over 50 medical research papers regarding CDK and proposed my research to various professionals every week. Few of the people I talked to included an Internal Medicine specialist, Hematology and Oncology specialist, AP Biology Teacher, Ph.D. student for Biomedical Engineering research, and a Biomedical Sciences Major. They pointed out flaws in my previous designs and offered ideas for how the testing of levels would be most accurate and inexpensive. I analyzed types of testing like IHC, PCR, Western blotting, and flow cytometry with the help of professionals.

RESULTS: CDKs are kinases, which are enzymes that phosphorylate (to attach phosphate groups) specific target proteins. When a cyclin attaches to a CDK, it activates the CDK. As displayed in Figure 2, cyclin D activates CDK4/6, cyclin A activates CDK1/2, and cyclin B activates CDK 1. The activated CDK is directed to a specific set of target proteins that control the checkpoints of the cell cycle [1]. Each phase of the cell cycle is correlated with specific cyclin-dependent kinases (CDK)/cyclin complexes. During the G1 phase, the synthesis of Cyclin D increases. Cyclin D binds with CDK4/6 to promote the entry into S phase. During the S phase, the activation of CDK2 by cyclin A inhibits the phosphorylation of areas involved with the replication of DNA. Cyclin A is highly synthesized in the S phase and throughout the last stages of G2. During the G2 phase, CDK1 is the primary regulator of the cell cycle. This cycle is shown in Figure 2 below [3].

According to Nature Reviews Drug Discovery, a scientific journal, “The CDK4/6–RB axis is critical to cell cycle entry; therefore, it is unsurprising that the vast majority of cancers subvert this axis to promote proliferation” [4]. This clearly states that CDK4/6 is key in regulating the cell cycle, and in many cancers, cells manipulate the levels of the conjugate

cyclin to allow for rapid, uncontrollable division. The same study states, “Deregulated cyclin D protein expression, gene translocation, and gene amplification are observed in many tumor types, and a plethora of functional data support the specific oncogenic activity of cyclin D1” [4]. In many cancers, cyclin D levels deviate from normal levels as shown in Figure 3.

This overamplification of cyclin D has been associated with both CLL and ALL. In the study of “Cyclin D expression in chronic lymphocytic leukemia” led by James T Paul, three cyclin D isoforms in 43 leukemia patients were examined and correlated with the findings of clinical features. Two significant conclusions reached from this were that “the mean cyclin D1 and D3 levels were 4 to 6-fold higher in CLL cells than in normal blast and peripheral blood cells” and that “the relative overexpression of cyclins D1 and D3 in CLL were unrelated to gene amplification, as assessed by Southern blotting” [5]. There is a clear overamplification of all 3 subgroups of Cyclin D. With cyclin D3 concentrations up to 6-fold higher than normal, the inflated concentration acts as a significant indicator of leukemia. The second conclusion stated that the overexpression of cyclins did not correlate with gene amplification or a mutation of the gene that produces this cyclin. Even if the genes that transcribe for cyclin D (NCBI 595, HGNC 1583, and NCBI 896) are located and assessed for changes, DNA sequencing or genetic testing will not provide an accurate representation of whether a patient is prone to getting cancer as not all types of leukemia cause an amplification or mutation to the genes. This is why the actual products of the genes (cyclins) should be measured to ensure accurate leukemia detection. In a similar way to CLL, ALL also causes an increase in the concentrations of cyclin D. In a study led by Salah Aref, the expression of cyclin D1 was analyzed at protein level in 10 patients with acute lymphoblastic leukemia (ALL) and 11 normal controls. The results were similar to those of CLL. It concludes, “overexpression of cyclin D1 was evident in ALL groups as compared to that in healthy control. The ALL cases with cyclin D1 overexpression were significantly correlated to high blast cell counts in the peripheral blood” [6]. Again, there is an overexpression of cyclin D1 present. This study is significant because it shows the correlation of overexpression and the abundance of blast cells in peripheral or flowing blood. Having at least 20 percent blasts is a key factor for a diagnosis of ALL, so because cyclin D1 levels act in a way that reflects the abundance of blast cells, it is clearly a good indication that high cyclin D levels play an important role in the proliferation of leukemia. Another study conducted by A Sauerbrey also analyzed cyclin levels in ALL patients with similar conclusions to the Aref study. “The blast cells of the relapsed patients contained significantly higher levels of cyclin D1” and “The cyclin D1 expression ... inversely correlated to the expression of the retinoblastoma tumor suppressor gene ($r = -0.27$, $p = 0.03$)” [7]. Along with the amplification of cyclin D, there was an inverse correlation of high cyclin D and expression of the tumor suppressor gene. Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, or dictate cell apoptosis. As Cyclin D1 levels increased, the expression of these tumor suppressor genes decreased which resulted in the further deregulation of the cell cycle as DNA mistakes are not getting stopped for repair. The last type of cancer that has a positive correlation with cyclin D is CML. The study conducted for CML discussed the amplification of cyclin D again, and stated, “differences in cyclin D1 expression between CLL and CML patients were also confirmed on protein levels by western blotting” [8]. The differences could play a significant factor in determining the type of leukemia a patient could have just based on the level of cyclin.

There is also an overamplification of cyclin in AML; however, the expression of cyclin B is increased in these types of leukemia. In a study led by Elisabeth Ersvaer, the concentrations of Cyclin B1 were analyzed in the cytoplasm of human acute myelogenous leukemia cells. Results proved that “primary human AML cells show aberrant cytoplasmic expression of cyclin B1 for a majority of patients” and “Confocal microscopy demonstrated that 32/42 (76%) patient samples

showed abnormal cytoplasmic expression" [9]. This study proves the abnormalities in the overall cyclin B concentration in AML cells. These aberrant levels of cyclin B can act as an indicator of AML as the majority of patients presented with that. Another research paper proves this conclusion as well. This study was led by Haixia Li, and tested the effect of MiRNA on the regulation of the cell cycle by targeting cyclin B2. In the control groups of cells affected by AML and regular cells the idea of high cyclin B2 levels were proven. "Consistently, the protein levels of cyclin B2 were all higher in the three leukemia cell lines (THP-1, Molt-4 and K562), than in the normal HS-5 cells" [10]. This general observation is significant as it proves that cyclin B levels can be used as an indicator of AML.

DISCUSSION: The proposed test for the detection of leukemia will effectively detect the cyclin concentrations of cyclin B and D through a blood sample utilizing fluorescence activated flow cytometry. This test is noninvasive as it only requires a blood sample, and results would be produced quickly which would give the doctors quick direction to advise or treat the patient.

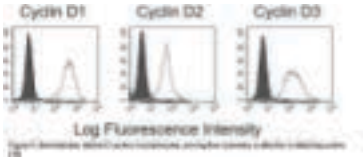
Flow cytometry is used to count and analyze the shape or intracellular contents of a sample of cells. These values are determined by software programs to produce quantitative values. Flow cytometers have 6 main parts: the sample, fluid that moves the sample, lasers, optics that gather the light, detectors that sense light, and a computer system that outputs the data.

In this procedure, a blood sample would be extracted from a patient. This sample would be fixed so that the cells can be preserved and stabilized so antibodies can have a good surface to bind to. Next, the blood cells will be permeabilized with a chemical called Triton X-100. This will lyse the cell and allow it's intracellular components to be exposed. Blocking antibodies will be released into the sample, which will stop the binding of other antibodies to the targeted antigen, cyclin. Then, 2 different antibodies that are specific for cyclin B and D will be placed in the sample to attach to their respective proteins. The 2 antibodies will be developed so that they are specific for all subtypes of cyclin D (1,2, and 3) or all subgroups of cyclin B (1,2, and 3). This has been proven possible by David Kaplan, who successfully found the levels of D cyclins in lymphocytes using flow cytometry [12]. In this proposal, both primary antibodies for cyclins B and D will be conjugated to a fluorophore, which will be useful in detecting the concentration of cyclins through light. After the initial sample is prepared, it is inserted into the cytometer. When the solution is inserted, a sheath fluid is flowing at a higher rate around the sample, which ensures that all of the particles are travelling along the same axis at approximately the same velocity. As the cells pass through the laser in a single file line at the "interrogation point," the laser beam scatters in different directions: forward and side scatter. This side scatter is important because it will detect the amount of fluorescence present [13]. The computer processor uses this scattering of light to create a graph with axes for the fluorescence intensity and the number of cells with that intensity. The quantitative value for the concentration of cyclins can be determined from the graphs. These numbers can then be compared with one another based on the Chi-Square test. The Chi-Square test will compare the observed and expected values for cyclin levels and determine if the difference is statistically significant/ if levels are really abnormal.
This will link with a computer program that will assess the levels into no risk, risk, and high risk (leukemia) based on a certain range of concentrations. If considered risk or high risk, the doctor can accurately advise and treat the patient. In addition, both antibodies for cyclin B and D will be labelled with different colored fluorescent dye (i.e blue and green), so the type of leukemia can also be determined based on the fluorescence color chosen. As done in the previous Kaplan study, different cyclins were attached to by multiple fluorescence activated antibodies and run in the same test , demonstrating that results are still accurate when different types of proteins undergo fluorescent-activation [12].

The difference between Klan's experiment and this proposed innovation is that Kaplan solely used normal leukocytes and tested cyclin

levels on those. This proposed innovation connects both parts of the research presented about cyclin concentrations being deregulated and Kaplan's proof that flow cytometry correctly detects cyclin levels.

TABLES, GRAPHS and PHOTOS:



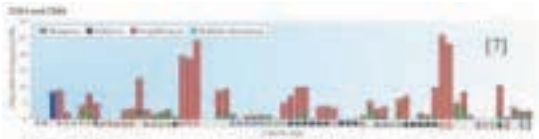
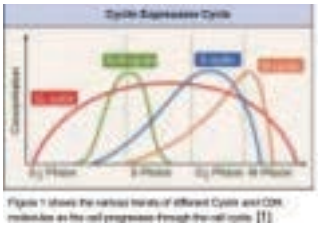


Figure 3. This demonstrates how CDK4/6 levels fluctuate in different cancer types



BENEFIT TO SOCIETY: This innovation will have a great impact on the diagnosis of leukemia as it is versatile and can act as both a confirmatory and early detection test. For early detection, the cyclin level of the blood will be measured along with the annual blood test to assess the patient's risk of developing leukemia. The computer software will generate an answer of no risk, risk, and high risk. These classifications will allow the doctor to treat the patient
accordingly and suggest ways to decrease potential risk. This will make a big impact for children because out of all children who develop cancer, 30% will develop some form of leukemia [2]. The test can also be used as a confirmatory test. Currently flow cytometry is used by oncologists for CLL to determine whether or not there are structural abnormalities. This current test used by doctors, however, can be inaccurate at times because the blood cells may not be in a far enough stage to develop abnormalities. My proposed test would be useful in those cases because
abnormal cyclin levels will be detectable in this test sample, even though there are no visible abnormalities. This proposed test could also be used in place or together with biopsies. The most common way to perform a biopsy for leukemia is to extract marrow with the use of a needle from the hip. This procedure is quite invasive and takes up to 3 weeks to get results because the percentage of abnormal cells in the bone marrow is determined. The proposed solution of flow cytometry will be useful to ensure that a bone marrow biopsy is only performed when absolutely necessary and when all other signs point to leukemia including WBC count and high cyclin
levels. In addition, this innovation can produce results about cyclin concentration fast, which will improve the system when a patient comes into the ER with high WBC count. Normally, doctors rush to treat this patient in a general approach because this count can indicate many diseases like pneumonia, kidney disease, or leukemia. This proposed innovation will speed up that process by testing cyclin levels, which would allow doctors to understand if the problem is in the mitotic division of the cell cycle or something else. This is much faster than waiting up to 3 weeks for a bone marrow biopsy, so the innovation will improve ER care.
Overall, there are many uses for this proposed innovation that will revolutionize the field of oncology. It will beneficially impact society and the diagnosis of leukemia when implemented into common use by medical professionals.

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Anvitha Nadendla

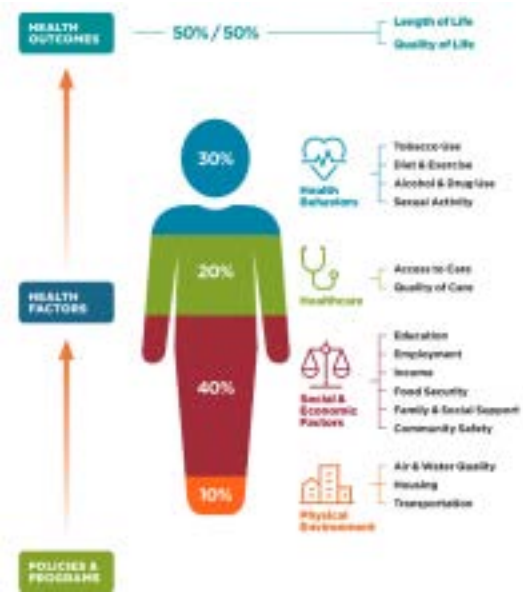
Leveraging Social Determinants of Health to Improve Patient Outcomes and Reduce Healthcare Costs

Abstract

National health spending is projected to grow at an average rate of 5.5 percent per year and to reach nearly $6.0 trillion by 2027 [(1)]. While the national health spending is increasing, there is a clear lack of progress in improving health disparities during the past 25 years [(2)]. The efforts to improve health in the U.S. have traditionally looked to the health care system as the key driver of health and health outcomes. However, there has been increased recognition that improving health and achieving health equity requires broader approaches that address social, economic, and environmental factors that influence health [(3)]. As shown in Figure 1[(4)], clinical factors account for only about 20 percent of health care needs, while the other 80 percent depend on social & economic factors, health behaviors, and physical environment.

Link:
https://drive.google.com/file/d/1lrMwS_SaWmzFHaBtgnjijyIgyFZIiMrD/view?usp=sharing

The World Health Organization (WHO) defines Social Determinants of Health (SDoH) as the non-medical factors that influence health outcomes. They are conditions in which people are born, grow, live, and age, and the wider set of forces shaping the conditions of daily life and include variables such as socioeconomic status, education, neighborhood and physical environment, employment, and social support networks, as well as access to healthcare. Per a



recent JAMA Network™ publication, SDoHs direct contribution accounted for 5.8 percent of the variation in price-adjusted Medicare per beneficiary spending across countries after controlling

for patient demographic characteristics, clinical risk, and supply of health care resources [5], which equates to about $46 billion based on 2019 Medicare spend [6]. Overall, significant improvement opportunities exist in today's healthcare to go beyond clinical factors to enhance health outcomes, reduce geographic spending variation, and control increasing health care costs.

INTRODUCTION:

Integrating SDoH data into the care plan provides valuable insights and opportunities to offer comprehensive care that goes beyond treating the medical condition but also potentially addresses the underlying root causes (e.g., lack of housing, food deserts, limited transportation, etc.), and timely & critical interventions.

A recent Kaiser Family Foundation (KFF) research shows that health disparities are driven by social and economic inequalities[7](Figure 2). These disparities are estimated to increase healthcare spending by $93 billion and cause lost productivity of $42 billion annually [8].

Figure 2

Link:
https://drive.google.com/file/d/1v7L_lub_lUtFNAT0YGqgzNSB3xGOlnlV/view?usp=sharing

This paper reviews the results of leveraging SDoH and applying advanced data models on about 40,000 Behavioral Health (BH) population data with about $600 million medical and pharmacy annual spend. Traditionally, population health management primarily focused on analyzing medical and pharmacy utilization data to understand the population that an organization is serving and offer them care management and control the medical costs. Could this approach be improved by i) enriching Medical and Pharmacy data with SDoH ii) applying advanced modeling techniques for early detection of certain medical conditions to offer timely care management and interventions for overall improved health outcomes, reduce health disparities, and reduce healthcare costs in the long run?

MATERIALS AND METHODS (Based on my SDoH project during Internship with AArete, 13 July, 2021- 30 August, 2021):

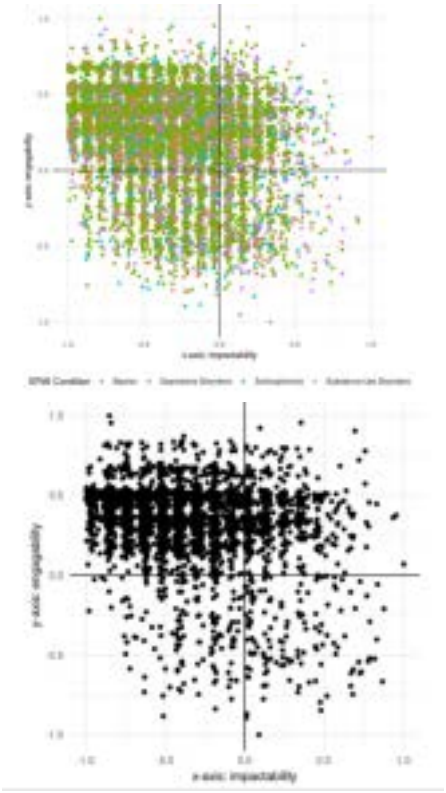
For the purposes of this analysis, I defined impactability as potential for future medical cost reduction and engageability as patient's responsiveness to care management. Our analysis focused on creating cohorts of populations with high potential across impactability and engageability. Initially, I stratified the BH population into Serious and Persistent Mental Illness (SPMI) that consists of medical conditions like Major Depression, Bipolar Disorders, Schizophrenia and Borderline Personality Disorder and Non-SPMI categories to normalize for utilization and care management levels. In the baseline data, the SPMI category has approximately 24,000 patients with $385 million spend, and the Non-SPMI category has 21,000 patients with $255 million annual spend.

Approach: I created a comprehensive dataset using traditionally available Medical and Pharmacy claims data and integrating it with SDoH data elements like race, urban/rural, etc. I developed a methodology, which considers various factors and assigns engageability and impactability scores in the range -1 to +1 to each patient in the baseline data, with +1 indicating the highest potential for impactability and engageability. Engageability defines patients' attention and focus on their personal health, whereas impactability is associated with existing chronic conditions and medical behaviors. In addition, in the NonSPMI category of patients, I applied a predictive model with >70% accuracy level to identify "rising risk members" who are likely to become SPMI patients for proactive treatment and timely interventions prior to a patient being diagnosed with a particular medical condition. SDoH points help enrich the scoring process and also normalize the bias associated with communities with limited access to care.

RESULTS:

As shown in Figure 3, impactability and engageability scores are mapped for both SPMI and non-SPMI populations to identify the patients with highest opportunity

Figure 3 (patients mapped on impactability and engageability scores)



I identified a vulnerable population of 1,109 and a rising risk population of 235 patients from SPMI and non-SPMI baseline populations of approximately 24,000 and 21,000 patients. These members represent 2 percent of the baseline data and account for 7 percent of the total spend. It's estimated that based on a 35 percent engageability, conservatively $2 million annual recurring cost avoidance can be realized on the sample population of data after adjusting for the care manager's expenses and other costs.

DISCUSSION:

The results from 40,000 BH patients' data are encouraging. Designing and developing an

optimal predictive model may take a few iterations before a reliable model with high confidence levels is applied. The application of predictive models and other advanced data techniques has huge potential to proactively identify rising risk members and offer them care management.

SDoH such as poverty, unequal access to health care, lack of education, stigma, and racism are underlying contributing factors of health inequities. SDoH data are not always available consistently across counties in the U.S., but the ability to proactively identify and address the SDoH barriers for populations of patients is moving in the right direction. The key is to identify the right population cohorts so the health organizations can initiate customized and patient-centric care management and adherence through active patient engagement.

Another application with great potential is to improve non-optimized medication therapy (e.g., medication nonadherence); the estimated annual cost of prescription drug-related morbidity and mortality resulting from this is in the range of $495.3 billion to $672.7 billion [(11)]. SDoH challenges (e.g., Lack of transportation) drives a considerable portion of medication nonadherence. Studies have consistently shown that 20 percent to 30 percent of medication prescriptions are never filled and that approximately 50 percent of medications for chronic disease are not taken as prescribed. This lack of adherence has dramatic effects on health and hospitalizations.

Another related example is that select state Medicaid programs (e.g., CA, NY, TX) are supporting providers focused on SDoH through Delivery System Reform Incentive Payment (DSRIP) initiatives. DSRIP initiatives link Medicaid funding for eligible providers to process and performance metrics, which may involve addressing social needs and factors. For example, in New York, DSRIP initiatives had a goal of improving health outcomes and reducing avoidable hospital use by 25 percent and made considerable progress in 4 years since they launched the initiative in April 2014 [(9), (10)]

Overall, addressing SDoH will have a significant impact on health outcomes at the individual level and a positive impact on controlling rising healthcare costs in the long run. Additionally, it can also have a positive impact on reducing health disparities and help us achieve health equity.

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Divya Ramaswamy

Rapid Prompting Method: Why it Needs More Research

ABSTRACT:

To confirm whether Rapid Prompting Method (RPM) is a suitable learning device for people with autism, researchers need to conduct ample research. Currently, there is limited research on the long-term effects of RPM, and there is only demonstrated knowledge of the initial stages, not the entire program. Moreover, before research can be accomplished, scientists need to consult individuals with nonverbal autism, as well as practitioners of RPM to get a full understanding of what the learning method truly is. None of this is possible if RPM is discredited through comparisons to other different communication methods like Facilitated Communication before it is given a chance at scientific validity.

INTRODUCTION:

Rapid Prompting Method (RPM) is a form of communication developed by Soma Mukhopadhyay for her autistic son, Tito (https://www.halo-soma.org/, 2012). Since its creation, thousands of autistic children and their families have used RPM to reach communication levels never thought possible before. RPM involves a communicator asking questions and the autistic user pointing at boards with “yes” and “no”, only choosing between them, before gradually making their way to a letterboard. It relies on “presumed competence,” or the argument that we simply have to accept that autistic individuals understand what they point to on the letterboard. Through meticulous training, users eventually train themselves to use the letterboard while it is

flat down on a table, then incrementally move towards eliminating all influences of the communicator. This usually happens in small steps, as it may begin with the communicator simply being a few feet away, then moves towards the corner of the room, and eventually out of sight. There are alternative methods, as a fundamental of RPM is for it to be designed for the user, and some people choose to hand write, type or even verbally pronounce the letters and words they are communicating. While the process is slow, many older individuals have shown remarkable progress and have even progressed beyond the need for RPM.

MATERIALS AND METHODS:

The question for this experiment was why scientists do not advocate for the Rapid Prompting Method, and to discover flaws in their methodology for discrediting it as well as provide insight into the most accurate way to research RPM.

RESULTS:

The result of the research was the finding of multiple deficiencies. One common comparison is to Facilitated Communication (FC), a discredited form of communication for autistic individuals. Mukhopadhyay clearly laid out that the foundations of RPM may begin with a letterboard and communicator, like FC, but these two methods diverge once autistic people develop the basic skills.

TABLES, GRAPHS and PHOTOS:

If you present your data in a table or graph if appropriate. Be sure to include a title describing what the table is displaying. If you can summarize the information in one sentence, then a table or graph is not necessary.

The general consensus is that RPM must be thoroughly studied to be accepted by the scientific community, though the method of the study is unclear.

DISCUSSION:

Highlight the most significant results and explain how these results related back to your original question. Were these results expected or were you surprised? Why or why not? How can you use this data in the future? What can you take away from this experiment and apply to other areas?

RPM has been compared to a scientifically discredited communication method known as Facilitated Communication, and is now mistrusted by the scientific community due to the similarities between these two methods. Facilitated Communication also often uses letter boards or keyboards, sometimes requiring a “communicator” to guide the autistic person’s hands while they type. While the beginning of both methods may be similar, they lead to very different scenarios and cannot be compared. Facilitated Communication also concerned scientists as several families were accused of abuse through the facilitator before research proved it inaccurate (Travers et al., 2014).

The scientific community’s disregard for RPM is based on several misunderstandings and, to some level, blatant ableism. One of the largest misunderstandings is that the ultimate goal of RPM is to rely completely on a “facilitator,” or a communicator, and a letterboard. This insinuates that autistic people cannot communicate without certain hand signals, and the board has even been compared to “ouija boards,” indicating that any form of communication from an autistic individual has no more substance than fantasy.

Moreover, even with the unsubstantiated connection with FC, the article, Facilitated Communication Denies People With Disabilities Their Voice. Research & Practice for Persons with Severe Disabilities, fails to mention the various flaws in FC research. One of the most notable parts of Autism spectrum disorder is how every individual is entirely unique. The studies that use multiple subjects are automatically flawed as the method that works for one individual has no guarantee to work for everyone else. To complete the study, the subjects must have been subject to conditions slightly different from their usual settings. There is ample evidence that autistic brains do not observe the same way as neurotypical ones, and even minor changes could result in huge setbacks. Body language, breathing, details on the walls, and even the knowledge that they

were being studied all could have created flaws in studies.

"Voices from the past: Comparing the rapid prompting method and facilitated communication" is one of such flawed stance (Tostanoski et al., 2014). The authors clearly state that they are aware that the long-term goal of RPM is for students to be able to write and type independently, yet still repeatedly compare RPM to Facilitated Communication (Tostanoski et al., 2014). However, many autistic people who achieve mastery with the letter board and communicator do not stop there. They continue on to learn how to answer questions that the communicator is not told, slowly moving away from prompting and any forms of nonverbal communication or encouragement. Furthermore, some autistic people are trained in how to answer questions with their communicator a safe distance away, or even in a different room altogether so as not to unconsciously sway any answer. Some of the most inspirational former users of RPM are now able to type entire presentations, novels, and social media posts with no communicator present. It stands then, that if there is no communicator present and the person is given free rein with the keyboard, that every word they say must be entirely of their own, else the keyboard would have to start typing itself. If these such people are able to communicate completely independently, there must be some indication that the steps leading up to it, namely RPM, hold some value.

Several people with non-verbal autism, such as Hari Srinivasan, a student at the University of California, Berkeley, are able to communicate independently, having once used a letterboard. While the communicator may have some degree of influence over the person using RPM while holding the letterboard, the goal is to progress to a state with little to no dependency, something most research on RPM fails to acknowledge. The most clear example of this is in the article, "Facilitated Communication Denies People With Disabilities Their Voice," in which the authors state, "Facilitated Communication (FC) has been rebranded as "supported typing" and repackaged as Rapid Prompting Method, but remains a disproven intervention for people with disabilities" (Travers et al., 2014). This point of view demonstrates all the issues with current research on RPM—that it simply has not been done. The very accusation that it is just a repackaged version of a completely different form of communication bears no more weight than comparing RPM to American Sign Language, and does little more than discourage people from actively researching and attempting to perfect systems of communication for autistic individuals.

The very same studies discrediting Facilitated Communication are being used to discredit RPM, ignoring the words of several members of the autistic community, achieving nothing other than silencing thousands of people who only recently gained a voice. This shows not only a lack of understanding of the fundamentals of RPM, but also blatant ignorance and the clear eagerness of the scientific community to remove all forms of communication from nonverbal autistic people unless somehow backed by testable, undeniable evidence. The strong stigma against letter boards and any form of communication with letter boards, as seen in the comparisons with FC, have also prevented individuals who succeeded with RPM from coming forward with their observations. Now, many individuals in the autistic community avoid referring to RPM, which prevents proper research and prevents a large collection of anecdotal evidence.

There are also some preliminary studies that are not fully developed. Researchers at St. Angela's College have found a decrease in "stimming," or engaging in repetitive behaviors, after using RPM as a communication method (Deacy et al., 2016). Such research admits that more studies have to be done, but also refrains from discrediting RPM before doing any said research on it.

By the negative feedback RPM has received, it could be assumed that such research has been thoroughly done, with the aid of nonverbal autistic people who can type independently (to ensure their help is theirs and theirs only). Yet little to no research has been

done, and such a vital part of so many people's lives has been completely disparaged on the basis that there is not sufficient evidence, else citing yet another research article on FC. RPM is misjudged as a communication method rather than a learning method, and there is no funding being collected nor research being done to in any way prove whether RPM works. Therefore, the only current way to credit or discredit RPM would be a longitudinal study on RPM users, to catalog how the education device has aided them in their journey to communicating independently. Such witnesses would have opinions untainted by a "translator" prompting or signaling, and would be able to demonstrate not how RPM can create a short-term, immediate result, but rather how it can aid with growth and independence of autistic individuals over time. In addition, those that do research should have nonverbal autistics that communicate independently as advisors. It is commonly noted that the loudest voices to speak about the issues of disabled people should be disabled people themselves, and a collection of evidence from undeniably credible sources would be a staunch pillar for or against RPM.

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Ethan Chiu

Investigating Uveal Melanoma Using a Seurat scRNA-seq Analysis of SEAM Organoid Gene Clusters.

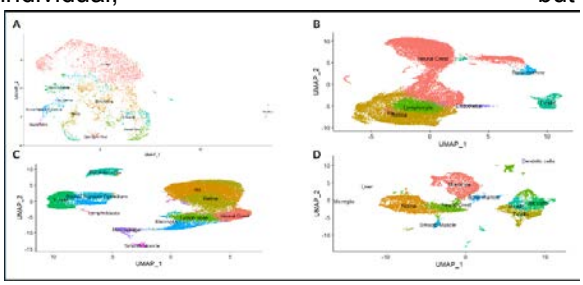
Abstract – Uveal Melanoma (UM) is the most common eye cancer with a mortality rate of 80%. However, only 1% of patients have detectable UM at metastasis, and UM exhibits punctuated early growth. The origin and proliferation of UM were investigated using single-cell RNA sequencing analysis as it is currently the best method to define cell states and phenotypes. The Seurat R toolkit was used to separate the cells of a self-formed ectodermal autonomous multi-zone (SEAM) organoid and UM cells into gene clusters. These gene clusters were then identified using canonical gene markers and compared in samples with and without UM. In this way, the origin of UM was attributed to certain genetic clusters and targeted for future treatments. Distinct cell clusters with unique gene expression patterns were found in the UM datasets, which provided insight into the mechanisms underlying UM

progression and facilitated comparisons between the control SEAM organoid and the experimental UM gene datasets. Moreover, the potential of the SEAM organoid model in modeling UM metastasis was verified. In the future, both in vitro and in vivo experiments will be performed to confirm the results of the scRNA-seq analysis. Additionally, RNA interference with lentivirus therapy will be tested using the SEAM organoid model to determine whether it effectively minimizes the UM phenotype.

I. Introduction

Uveal melanoma (UM) is the most common cancer of the eye and the second most common form of melanoma. The four-year mortality rate of patients with metastatic UM is approximately 80% [11], with a median survival period of fewer than six months [14]. Only 1-3% of patients have detectable metastases at diagnosis [11], and UM exhibits punctuated early growth [3], so rapid and early treatment is critical to long-term survival. Unfortunately, within 2.4 years after primary tumor treatment, 50% of UM patients develop metastasis, often to the liver [8], so the minority of patients who have successfully treated their initial occurrence of UM are still at long-term risk [5]. Given the poor prognosis of those diagnosed with metastatic UM and the lack of a documented cure, a deeper understanding of the origin and mechanism behind UM cell proliferation is essential to developing future treatments. In fact, BAP1, SF3B1, or EIF1AX mutations are critical to UM proliferation [3], and BAP1 loss leads to tumor cells devolving to have the stem cell-like properties associated with the neural crest [7].

Currently, researchers use animal and human cell lines to test UM treatments. Unfortunately, animals have different genetic makeups than humans, leading to potential inaccuracies. However, human tissue samples cannot be controlled for and are not only highly individual, but



also prone to contamination and mutation, preventing universal reproducible results. Created by Dr. Blenkinsop of the Icahn School of Medicine, the SEAM organoid model is a cluster of selectively differentiated stem cells produced in vitro to overcome the limitations of animal and human cell lines. By analyzing if the SEAM model accurately reflects the human eye, the possibility of effectively testing UM treatments on the model can be determined.

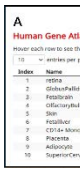
Single-cell transcriptional analysis (scRNA-seq) shows the gene expression profiles of individual cells within a gene population and is currently the best method to define cell states and phenotypes [16]. Currently, Seurat is the most accurate and efficient scRNA-seq analysis toolkit [18]. Since gene expression patterns can be identified using gene clustering analysis, rare cell types and developmental processes can be mapped, facilitating the location of diagnostic and therapeutic targets for various eye-related diseases. The plot created by the scRNA-sequencing analysis visually represents the data compared to an online database of genes like Enrichr and GeneCards. The plot can be used to identify whether the SEAM organoid model is truly effective in replicating every region of the human eye for future UM treatment purposes. Moreover, the data can then be compared to UM scRNA-seq datasets to identify the mutated genes that need to be targeted for potential therapies.

The gene expression and number of cells will be evaluated to determine whether the SEAM organoid model accurately represents each eye region. Additionally, the gene clusters containing genes specific to UM can be identified by creating a map of SEAM organoid scRNA-seq gene clusters and comparing it to UM's gene clusters, which will indicate genes involved in uncontrolled cell proliferation and allow for targeted therapies to be developed.

II. Materials and Methods

Seurat scRNA-seq analysis

Load the dplyr, Seurat, and patchwork libraries into RStudio. Load the dataset containing the pre-sequenced SEAM ocular cell model using Read10X. Initialize the resulting Seurat object with the raw (non-normalized) data. Select and filter the cells based on Quality Control (QC) metrics by visualizing the QC metrics using a feature scatter plot to exclude empty droplets with very few genes, cell multiplets with an abnormally high gene count, and dying cells with burst mitochondria. Remove the unwanted cells by inputting the new dataset parameters. Normalize the feature expression measurements using the total expression and



log-transform the result. Apply a linear transformation scaling to shift gene expression so the mean-variance is zero across all cells and the cell variance is one, so highly expressed genes will not dominate. Perform principal component analysis (PCA) linear dimensional reduction to increase interpretability and minimize information loss through creating new uncorrelated variables to maximize variance. Identify significant PCs with low p-value features by finding the true dimensionality of the dataset by observing the ElbowPlot function. Run the FindNeighbors function to cluster the genes by finding the distance between neighboring genes. Run the FindCluster function to group genes together using the Louvain algorithm. Run non-linear dimensionality reduction using the RunUMAP function and create a dimensional plot using the UMAP reduction. Find the positive markers for every cluster with a minimum percentage of 0.25 and a log fold change threshold of 0.25, and group the resulting clusters together. Visualize the clusters by creating a UMAP plot.

Cluster identification

Export the seam.markers dataset to a .csv file and copy all the .csv file genes from each cluster into a spreadsheet, separating each cluster into a different tab. Copy the genes from each cluster and input them into Enrichr to identify the probable cluster identities. Insert screenshots of the Mouse Gene Atlas Table and Human Gene Atlas Table into respective cluster tabs. Conduct a literature review and consult the GeneCards human genome database to find specific canonical gene markers identifying each region of the eye. Gather the gene markers into a separate spreadsheet tab. Use identified gene markers to classify clusters as specific regions of the eye.

Seurat scRNA-seq cluster labeling

Assign the cell-type identity to gene clusters using the new.clusters.ids function and create an annotated UMAP plot. Determine the accuracy of the SEAM model in reflecting the human eye based on cluster identities. Identify the potential of using the SEAM model to investigate UM based on the presence of neural crest cluster identities. Investigate annotated clusters and note the location and number of UM genes including GNAQ, GNA11, PLCB4, CYSLTR2, EIF1AX, SF3B1, BAP1, HTR2B, and PLCB4 in both the UM tumor cell and SEAM eye organoid clusters. Pinpoint differing clusters and note them as potential locations from which metastasizing gene mutations may have arisen.

III. Results

The SEAM and UM clusters were identified based on their region of origin in the eye using gene markers from literature reviews and the Enrichr and GeneCards databases (Fig. 1), and subsequently labeled using the scRNA-seq analysis program onto UMAP plots (Fig. 2). Furthermore, differing expressions of UM genes like BAP1 were found among corresponding SEAM and UM clusters, and the BAP1 genes involved in UM metastasis were mapped and identified within the UM clusters.

Figure 1. Cluster identity predictions were made using Enrichr, canonical gene marker identification, and GeneCards analysis. (A) The Enrichr tool estimates possible cluster identities based on their statistical significance (created by student researcher). (B) Clusters were identified using canonical gene markers (created by student researcher).

Figure 2. UMAP plots of the SEAM organoid model and UM samples with their scRNA-seq analysis clusters labeled. (A) UMAP plot of the SEAM organoid model (created by student researcher). (B) UMAP plot of the UM class 1 primary sample (created by student researcher). (C) UMAP plot of the UM class 1 metastatic sample (created by student researcher). (D) UMAP plot of the UM class 2 metastatic sample (created by student researcher).

IV. Discussion

The SEAM and UM eye region clusters were identified through Enrichr, literature reviews, and GeneCards analysis, so the genes corresponding to each cluster could be labeled according to their best-fit eye region. Since differing expressions of UM genes like BAP1 were found among corresponding SEAM and UM clusters, and BAP1 genes involved in UM metastasis were mapped within the UM clusters, therapies can be developed that identify and target BAP1 mutations. Additionally, based on the presence of a neural crest cluster, which eventually develops into the regions from which UM metastasizes, the SEAM organoid was verified as a promising model for analyzing the genetic origin and potential treatments for UM. As a new model free from the limitations of animal and human cell lines, the SEAM human eye organoid can be used to test treatments for

not only UM, but also other ophthalmic pathologies. Additionally, since the UM genes were found in the SEAM and UM datasets, targeted therapies can be developed for the specific cluster locations indicated.

V. Conclusions

Consistent with past results that SF3B1 and EIF1AX mutations cause UM class 1 while BAP1 mutations are critical to proliferation in UM class 2 (Durante, 2020), the results indicated specific clusters where the gene mutations appeared, allowing for a targeted therapy to be developed for the specific location that the clusters expressing the UM genes represent. The targeted therapy can subsequently be evaluated using a SEAM organoid model, which lacks the limitations of animal and human cell lines and was proven to be effective with the scRNA-seq analysis.

Identified UM mutations will be induced in a maturing SEAM organoid model, which will be treated with potential treatments such as RNA interference with lentiviral vector gene therapy to knock down those UM mutations and evaluated using scRNA-seq analysis. Efficacy will be verified via an ANOVA test.

Alongside the gene therapy, tetracycline antibiotic treatment will be evaluated for its effectiveness in targeting the specific neural crest mesenchymal stem cells that often give rise to UM. To prevent oxidative phosphorylation, the mitochondrial biogenesis inhibition effect of tetracycline antibiotics will be used. The effects on the endoplasmic reticulum stress response and its resulting impact on the mitochondria will be evaluated. Additionally, tetracycline antibiotics' downregulation of DNA-PK to prevent tumor metastasis and make tumor cells more radiosensitive will be utilized. Tetracycline antibiotics will be introduced into both normal SEAM-organoid and UM cells with small interfering RNA (siRNA) transfection to evaluate the genetic differences between the two types of cells. Immunohistochemistry will be integrated to visually locate, track, and confirm the path of the tetracycline antigen and identify specific regions of the UM cell tissues affected by the tetracycline antibiotics. An ANOVA test will be performed to verify the significance of the results statistically. Additionally, the effectiveness of ipilimumab, pembrolizumab, and nivolumab on treating deidentified UM tumor cells and SEAM-organoid cell models will be evaluated. The locations affected by the combined treatment will be determined using scRNA-seq analysis.

Ultimately, as the SEAM organoid model was verified as accurately depicting the human eye, treatments for other ophthalmic pathologies that afflict the eye, such as age-related macular degeneration, will also be evaluated and treatments tested using the SEAM model.

Acknowledgment

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Sophia Lerebours

Risk Compensation Among Young Adults During the COVID-19 Pandemic

ABSTRACT

Young adults have been attributed to an increased spread of the SARS-CoV-2 virus during the coronavirus disease 2019 (COVID-19) pandemic. Face masks and social distancing protocols (including physical distancing) have been found to limit the spread of the virus. Reducing the spread of the virus is key to returning to normalcy. A potential cause of young adults spreading the virus is risk compensating behavior. According to risk compensation theory (also known as risk homeostasis theory), young adults may respond to a reduced risk of contracting COVID-19 by taking more risks, such as compromising physical distance. A survey was used to assess whether young adults are engaging in risk compensation in regards to social distancing and mask wearing in Nassau and Suffolk counties in New York (n = 97). I found evidence that self mask wearing and stranger mask wearing have a statistically

significant effect on physical distancing ($p < 0.001$) while the linear relationships between risk perception and mask wearing and risk perception and social distancing had very weak correlations. Therefore, there is some evidence to suggest that this target demographic is indeed engaging in risk compensating behaviors. To combat risk compensation, further research should examine more untraditional avenues of communicating public health information to young adults.

Keywords: risk compensation theory, risk homeostasis theory, COVID-19 pandemic, SARS-CoV-2, social distancing

Introduction

Throughout the coronavirus disease 2019 (COVID-19) pandemic, social distancing and mask wearing have played an integral role in slowing the spread of SARS-CoV-2 virus, the virus responsible for the disease. Social distancing, commonly associated with the idea of remaining six feet apart from others, is defined in public health as non pharmaceutical interventions intended to limit contact between individuals to reduce the spread of the SARS-CoV-2 virus including physical distancing, quarantine, and isolation policies. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) strongly encourage social distancing measures. General mask wearing has repeatedly been demonstrated to reduce the spread of the virus (Pryor, 2020; Chu et al., 2020). There has been discussion about the efficacy of different types of masks on stopping the spread, with researchers examining the effects of the materials used in cloth masks on filtration (Konda et al., 2020; O'Kelly et al., 2020). The results of these studies have been confounding with some results almost seeming contradictory, but overall, mask wearing itself has been proven to be essential in reducing the spread of SARS-CoV-2 virus. Social distancing and mask wearing have been the key components of public health policies during the COVID-19 pandemic.

Social distancing protocols have been changing as more and more has been learned about the virus. Originally, the CDC recommended a 14-day quarantine after coming into contact with someone who has COVID 19, but the latest research by the CDC shows that quarantine can end after 10 or even 7 days as long as certain criteria are met (Pryor, 2020). Although plans for combatting the virus have been developed with care, implementation and enforcement has varied around the world. While some countries have issued strict mask mandates, in the United States, mask wearing policies vary by state, contributing to the higher rates seen in the country.

After over one year since the virus emerged in Wuhan, China, there have been many medical developments. Currently, the CDC and FDA have authorized and recommended three different COVID-19 vaccines created by Moderna, Pfizer-BioNTech, and Johnson & Johnson/Janssen (Centers for Disease Control and Prevention, 2021a). The vaccines created by the first two companies are mRNA vaccines that cause the cell to produce a surface antigen from the SARS-CoV-2 virus so the immune system can create antibodies. The latter company uses a viral vector to accomplish the same task. Though there have been some cases severe side effects of the various vaccines (such as thrombosis with thrombocytopenia syndrome in patients who took the Johnson and Johnson/Janssen vaccine), the United States has seen the mass distribution of vaccines with 277,290,173 doses administered as of 9:00 am ET on May 19th, 2021 (MacNeil et al., 2020; Centers for Disease Control and Prevention, 2021b). At the same time, four different variants of the virus have been discovered. The United Kingdom (UK) identified a variant called B.1.1.7 in the fall of 2020, and around the same time, a variant now referred to as B.1.351 emerged in South Africa. Later in January 2021, a third variant was found in Brazilians known as the P.1 (World Health Organization, 2021).

Despite knowing more about the virus, it has continued to spread in the United States. This is in part due to younger people socializing and facilitating transmission. In a press conference regarding COVID-19 in the Western Pacific, the WHO expressed concerns about younger people driving the spread of the virus (Kasai, 2020). Asian countries that initially brought the spread of the virus to low rates were seeing a resurgence in cases primarily driven by younger cohorts. Clusters of cases have also been linked to higher education institutions in

the United States: institutions with a generally young population (Wilson et al., 2020). Younger people are generally less susceptible to contracting the virus because their immune systems are more versatile and can better respond to infections. If they contract the SARS-CoV-2 virus, they are also less likely to develop a severe case that could require hospitalization. This, however, does not justify socialization because they are spreading the virus to more susceptible populations such as the elderly. Dr. Jeffrey Harris, a physician and economist on the faculty of the Economics Department of the Massachusetts Institute of Technology, explored this phenomenon using publicly available COVID-19 data from sixteen counties in Florida over three months to conclude that young adults are contracting the virus and spreading it to older, less mobile people (Harris, 2020). This is supported by the findings of Dr. Alexandra Oster and her team who noted early increases in percent positivity of persons under the age of 25 years old were followed by weeks of increased positivity in those 25 years old and older (Oster et al., 2020). Unsafe socialization also places a burden on the healthcare system with more people asking for tests and demanding care.

Literature Review

Risk Compensation Theory

One possible explanation for this behavior is risk compensation. Risk compensation theory, also known as risk homeostasis theory, begins with the idea that people will adjust their risk taking behavior based on their evaluation of the risks. For example, when driving in the rain, drivers tend to go at slower speeds because slippery roads could result in a higher chance of an accident. This is known as behavioral compensation; the drivers are compensating for the increased risk of a collision. The theory itself explains that when a safety measure is introduced, people will alter their behavior as a result of a perceived decrease in risk. In the case of the COVID-19 pandemic, the introduction of mask wearing and social distancing could result in risk compensation in the United States in young adults.

Risk compensation has been studied over the past half century in regards to safety interventions. Two of the most pivotal researchers were Sam Peltzman and G. J. S. Wilde. Peltzman looked at risk compensation in an economic sense when he published his paper titled “The effects of automobile safety regulation,” (Peltzman, 1975). Peltzman took a hard stance, arguing that the safety regulations were ineffective due to the results of his economic analysis of the Federal Motor Vehicle Safety Standards that had recently been passed. Although his work was debated for decades after it was published, Peltzman introduced the concept of risk compensation as a major issue in road safety, sparking a discussion. Wilde is responsible for popularizing risk compensation theory through his works, even coining the term. He took a more psychological approach to the idea, proposing that there is a target level of risk that people are looking to attain. This means that if the risk decreases due to a safety intervention, people will be willing to engage in riskier behavior to maintain that target level of risk. This was explained in his book, *Target Risk* (Wilde, 2014). Wilde used Sweden’s change from driving on the left to driving on the right as an example of risk compensation. The change in driving regulations caused a decrease in fatality in making right turns at first, but after eighteen months, the fatality rate was comparable to that before the change. From the research of Peltzman and Wilde, it is clear that risk compensation is commonly studied using already existing data to draw behavioral conclusions.

Hedlund, who worked for the National Highway Traffic Safety Administration for 22 years in research and management positions, outlined the four criteria for risk compensation theory in his paper titled “Risky business: safety regulations, risk compensation, and individual behavior" (Hedlund, 2000). The four conditions are as follows:

1. Visibility. The safety measure needs to cause an obvious change. In the case of COVID-19, the safety measures are mask wearing and physical distancing, both of which have drastically altered daily life. 2. Effect. The safety measure needs to affect physical performance, attitude, and risk perception. Mask wearing has a noticeable physical effect; many people feel discomfort. There is also a sentiment of annoyance associated with mask wearing. People generally feel less at risk of contracting a case of COVID-19 when sporting a mask. The

same follows for physical distancing.

3. Motivation. There needs to be motivation for compensating behavior. In the case of the COVID-19 pandemic, a desire for more “usual” forms of socialization serve as motivation. Virtual meetings and parties are becoming increasingly ubiquitous; there is a yearning for normalcy.
4. Control. The behavior of individuals cannot be tightly controlled in order for risk compensation to occur. In the United States, while mask wearing may be required in certain public places, there are still some opportunities for people to choose when or when not to wear a mask. While social distancing is encouraged, it is more difficult to enforce than mask wearing.

All of these conditions have been met during the COVID-19 pandemic, meaning that it is appropriate to study risk compensation.

Because risk compensation theory is relatively new, it has been met with resistance and criticism. Some examples of risk compensation such as the relationship between bicycle helmets and cycling speed have papers with contradicting evidence. Ross Owen Phillips, a Senior Research Psychologist at the Norwegian Centre for Transport Research, and his team concluded that risk compensation was occurring in bicycle users, specifically that bicycle users were likely to ride faster when wearing a helmet (Phillips et al., 2011). Igor Radun, a researcher at the University of Helsinki, and his team actually concluded that there is a lack of evidence that risk compensation is occurring in this case, explaining that a particular study of risk compensating behavior in regards to helmets biking was incorrect and produced misleading conclusions (Radun et al., 2018). The contradictions to risk compensation theory are in no way stating that the theory cannot occur; the studies are simply disputing whether it is occurring in a specific instance. Therefore, it is assumed that the theory itself holds true. Not only is there contradicting evidence, but also the weaponization of risk compensation theory to fight against safety measures and protocols, an extremely harmful practice. Phillips et al. went on to urge caution in the implementation of helmet-wearing laws. Researchers utilizing the theory to advocate against safety legislation results is a cause of opposition to risk compensation theory. However, the reason I am studying risk compensation is so public policies and information tactics can be adjusted to try to reduce the prevalence of the phenomenon. In no way am I attempting to argue that social distancing protocols should be removed because of the presence of risk compensating behaviors. If risk compensation is occurring, educational tactics should be used to try to dissuade compensating behaviors.

Risk Compensation During the COVID-19 Pandemic

Risk compensation has been studied, though not thoroughly, in regards to COVID-19 pandemic. In Denmark, Frederik Jørgensen, a postdoctoral researcher and assistant professor in the department of Political Science at Aarhus University, and his team used daily nationally representative surveys to examine risk compensation (Jørgensen et al., 2020). A mask mandate was issued mid-August 2020 so the researchers used survey responses from before the mandate, during announcement, and after the enactment of the policy to determine whether risk compensation occurred. They concluded that there was a small level of risk compensation, specifically people were less likely to avoid settings with many people. Risk compensation was also studied in England, though the researchers used a different approach. Ashley Luckman, a research fellow at the Warwick School of Business, and his team used online experiments to determine whether masks affect the amount of distance between two people (Luckman et al., 2020). The participants were presented with scenarios involving them with a stranger. They had to indicate how close they would stand near the stranger if the stranger, the participant, both people, or neither were wearing masks. This is an extremely effective way to study risk compensation because the researchers get to observe the decision making process of their participants. Luckman et al. did not solely rely on the behavioral reports of participants like Jørgensen et al. did. The researchers found that risk compensation was occurring and it was higher in those who believed that masks were effective and those who were younger (18-40 years old). Both of these studies show that risk compensation can also be studied using surveys.

Gap

Although there has been research, none of these studies were conducted in the United States. The management of the COVID-19 pandemic has been extremely different in the United States as compared to England and Denmark, therefore the results from these studies are not representative of the United States, specifically Nassau and Suffolk counties in New York. Both Jørgensen et al. and Luckman et al. conducted their research during the summer of 2020, earlier in the pandemic when disputes about the effectiveness of certain protocols were more widely disputed. The researchers also did not focus on a specific demographic, though Luckman et al. had data separated by age. It is essential to look at young adults, defined in this paper as people ages 18-24, because of their involvement in spreading the virus. Risk compensation could be responsible for behaviors endangering not only individual health, but also the public health of society.

This ultimately leads to the research question: *To what extent are young adults in Nassau County and Suffolk County in New York exhibiting risk compensating behaviors in regards to social distancing and mask wearing?* I hypothesize that young adults in Nassau and Suffolk counties are engaging in risk compensating behavior in terms of social distancing and mask wearing to a great extent. By examining the thought process behind the behaviors responsible for spreading the SARS-CoV-2 virus, we can gain a better understanding of how to correct these behaviors for the betterment of society.

Methodology

Originally, a mixed methods study consisting of a survey and analysis of data from local health departments was proposed. Data on COVID-19 transmission, case, and hospitalization for both young adults and the general population would have been examined to demonstrate the extent to which young adults are transmitting the virus at a faster rate than the general population in Nassau and Suffolk counties. The analysis of COVID-19 data from local health departments was intended to support the reasoning for examining young adults. It would have revealed the extent to which young adults are spreading the disease, highlighting the significance of this research. The request to the health departments was for data over a two month time period so trends could be analyzed over time. However, I was unable to obtain the necessary information from the health departments. Nassau and Suffolk counties were hit extremely hard during the pandemic given their proximity to New York City (which was devastated by the virus due to high population density), resulting in an extreme workload with much of the data being stored physically. After months of trying to contact representatives of the department who would be capable of fulfilling my request, I learned that it would take many additional months before the data could be delivered to me. Because this would fall outside of the limited timeframe of the AP Research course, I redesigned my method to be centered around a survey. A survey was administered to young adults ages 18-24 via Qualtrics to determine whether that demographic is engaging in risk compensating behaviors. The mixed method study would have allowed for the collection of the data needed to answer the research question.

Risk compensation has primarily been studied in two ways: through the use of surveys and data analysis. Jørgensen et al. used surveys to examine risk perception over time during the COVID-19 pandemic. Other studies have used surveys to study at risk compensation in regards to the helmet-wearing habits of bicycle riders (Schleinitz et al., 2018; Phillips et al., 2011). Because surveys and questionnaires have been used to study risk compensation, it is logical that a survey is used to study this topic. In the case of data analysis, researchers have previously used datasets to draw conclusions about risk compensation (Peltzman, 1975; Wilde, 2014). This was not possible in this study because there were no publicly-available datasets about the mask wearing and social distancing habits for my target demographic. Therefore, a survey was the best method of collecting data to study risk compensation in the case of my research.

Survey questions fell into three main categories: risk perception, mask wearing and social distancing behaviors, and scenario-based questions. All questions were multiple choice and most of the data was translated into numbers for statistical analysis. After answering general information about their gender and age, participants answered

questions that would gauge their perception of the risk of COVID-19 to themselves and to society. The risk perception was measured by the answers to two questions from the survey: “To what degree do you feel you are exposed to COVID-19?” and “To what degree do you feel COVID-19 threatens the public health of American society?.” Jørgensen et al. used this technique to establish what they termed a “threat appraisal covariate,” serving the same purpose. Mask wearing and social distancing questions were inspired by the surveys of Luckman et al. and Jørgensen et al. Answers to these questions were compared to risk perception to study whether risk compensation was occurring. If there was a strong, positive correlation between risk perception and either mask wearing or social distancing, this would be suggestive of risk compensation because a higher perceived risk would translate to higher adherence to the respective policies. Eight scenario-based questions allowed for the examination of whether an individual’s social distancing choices vary based on mask wearing, specifically if mask wearing makes them feel as though they can stand closer than the recommended distance. This set of questions was modelled after the research of Luckman et al. who studied the effects of mask wearing, location (indoors versus outdoors), and activity (standing versus sitting versus walking) on physical distancing while my research only looked at the first two factors. Testing for activity would have resulted in twenty-four scenario based questions, bringing my survey to a total of forty-four questions. This would have made the survey extremely long, most likely decreasing the survey completion rate. Also, location is a factor that can be selected for by event officials and school administrators while activity cannot be controlled. Therefore, even if activity had a significant effect on physical distancing, this conclusion would not really have practical implications.

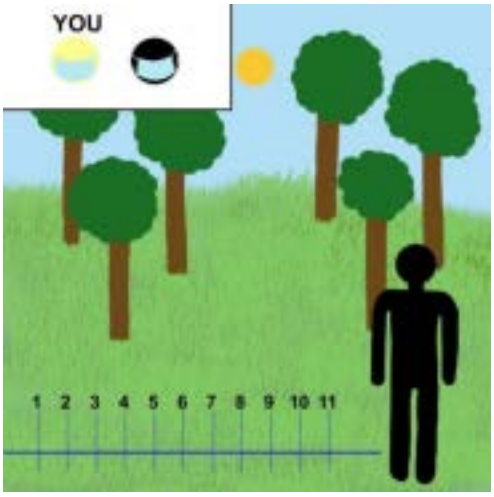

Figure 1. Sample scenario-based question from survey. The key in the top left as well as the background indicated the conditions of the scenario (in this case, the participant and the stranger were both wearing masks in an outdoor setting).

The survey was distributed across both counties. Young adults were primarily targeted in two ways: at educational facilities through professors and in public areas commonly frequented by that demographic such as local coffee shops. Flyers were physically posted advertising the lottery for a $20 Amazon gift card. To access the survey, participants could simply scan the QR code or they could manually type in the URL into a search browser. Those who received the flyer electronically could click the hyperlink to start the survey. The data was kept in a password protected location. Email addresses were submitted as an optional entry into the gift card lottery and were deleted after the winner of the lottery was announced. After passing a second IRB, the survey was also made directly available to students at a local university. Participants that took the survey through that local university were eligible for class credit. All participants consented to participate in the survey after reading a summary of the purpose, procedures, risks and benefits, and confidentiality of the survey. A complete list of the replicable survey questions and their sources and purpose can be found in Appendix A.

This study is intended to test whether risk compensation theory explains the behavior of young adults during the COVID-19 pandemic. Risk compensation

theory describes a causal relationship, however, the data collected through this method can only indicate whether there is a correlation. Causation can only be indicated through an experimental design. Survey data was also collected on general mask wearing behavior and social distancing to examine any other potential trends that could explain why young adults are spreading the virus. Results from this data are not the focus of my paper due to the lack of connection to my research question but some noteworthy findings are in Appendix E. The risk perception, mask wearing and social distancing behaviors, and scenario-based questions allowed me to determine whether individuals are engaging in risk compensation.

Results

Participants and Exclusions

The participants were 97 Suffolk and Nassau county residents who completed the survey through Qualtrics. 73 subjects were female, ages 18 to 24 with a mean age of 19.4536. Any person outside of the designated age range who tried to participate in the survey had their survey terminated after inputting their age. Although studying participants ages 14 to 24 would have allowed for a comprehensive analysis of adolescents (an age group recognized and researched internationally while young adults are less empirically defined), logistically, it would have been difficult to obtain the proper consent from the parents and legal guardians of the children while preserving the anonymity of the survey. It was also relatively challenging to sample people ages 18 to 24 years old as a researcher who is a minor as I do not regularly come into contact with my target demographic. I had to travel to various locations to garner enough participation. Participants from a local university received class credit for participation.
All other participants were eligible to participate in a lottery for a $20 gift card; they had the option to submit an email address for a chance to win the prize.

Risk Perception

A threat perception score was created to analyze the risk perception of young adults, similar to the threat appraisal score used in the research of Jørgensen et al. The threat perception score was the sum of the answers to the following two questions, each measured on a scale of one to ten with one being the lowest answer: “To what degree do you feel you are exposed to COVID-19?” and “To what degree do you feel COVID-19 threatens the public health of American society?.” Mask wearing was studied using a mask composite score, the sum of the answers to three questions: “Do you wear a mask when socializing?” (1-5), “To what degree do you feel wearing a mask prevents you from catching COVID-19?” (0-10), and “To what degree do you feel wearing a mask prevents you from spreading COVID-19?” (0-10). Data from the question “How many times have you worn a mask within the past week?“ was not used because in retrospect, it became clear that mask requirements at schools and in public places could have affected responses. Similarly, social distancing was studied with the answer to the question: “In the past week, how many people have you come into close contact with (besides the people you live with)? Close contact is defined as interaction with a person within six feet for at least ten minutes.” Scatter plots were used to examine threat perception versus mask composite and threat perception versus close contact. A scatter plot is typically used to visually determine if there is a trend in data points. Participants who did not answer all of the questions for a specific score were not included in any analysis of that score (ex. participant did not answer both threat perception questions therefore not included in either scatter plot). A linear regression model of threat perception versus the mask composite score showed a weak linear correlation between the two variables with r = 0.3412 and r squared = 0.1164 (*Figure 1)*. For the model to have been suggestive of risk compensation, there should have been a strong, positive correlation. The scatter plot of threat perception versus close contact showed little to no relationship between the two variables, indicated by the almost completely horizontal line (*Figure 2)*.

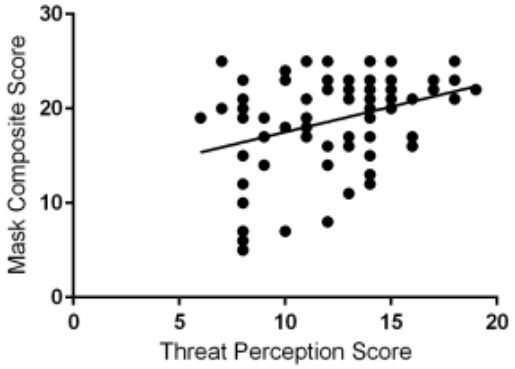

Figure 2. Risk perception vs. mask wearing. Scatter plot showing the weak correlation between risk perception and mask wearing. Risk perception is measured by the threat perception score and mask wearing is measured by the mask composite score.

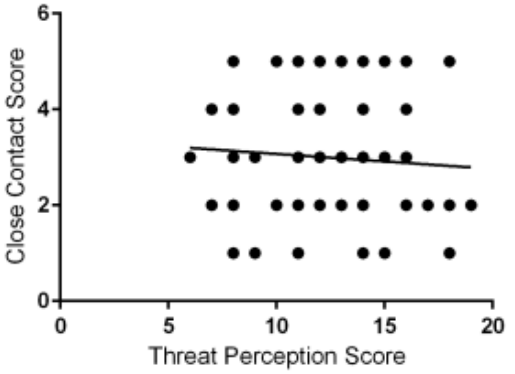

Figure 3. Risk perception vs. social distancing. Scatter plot showing the weak correlation between risk perception and social distancing. Risk perception is measured by the threat perception score and social distancing is measured by the close contact score.

Physical Distancing Scenarios

The scenario-based questions were analyzed separately to more closely follow the work of Luckman et al. A two - way analysis of variance (ANOVA) test was conducted to analyze the potential effects of location and mask wearing on physical distancing because Luckman et al. ran an ANOVA test as well (see *Table 1)*. I was primarily concerned with the effects of mask wearing as a statistically significant effect of mask wearing on physical distancing would have been indicative of risk compensation.

Table 1. Displays the results from the two-way ANOVA test. Location did not have a significant effect while mask wearing did as indicated by $p < 0.0001$.

	SS	dF	MS	F-Statistic	p
Location	16.67	1	16.67	1.42	0.2338
Mask Wearing	645.42	3	215.14	18.3	< 0.0001
Location x Mask Wearing	1.33	3	0.44	0.04	0.9893
Error	8440.49	718	11.76		
Total	9103.91	725			

With an alpha significance level of $\alpha = 0.05$, the mask wearing produced a significant result ($p < 0.001$). To determine precisely which relationships were significant, I then conducted a Tukey HSD test which produced a critical value of 0.9 ($\alpha = 0.05$). 0.9 was the minimum difference between the means of the four combinations of mask wearing (neither person wearing a mask, both people wearing a mask, “you” wearing a mask, or the stranger wearing a mask) for the relationship to be statistically significant. Therefore, the differences between both people wearing a mask and the other three groups were statistically significant (see *Figure 3)*.

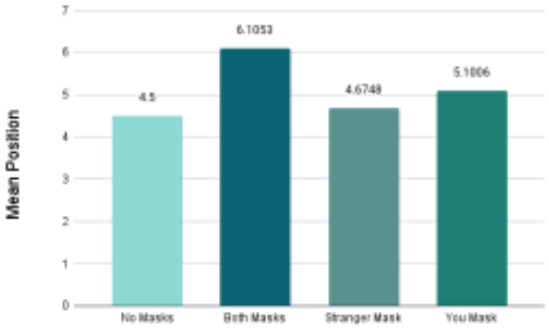

Figure 4. Mean position for each mask combination. The differences between the following groups were greater than 0.9, making them significant: no masks vs. both masks, stranger mask vs. both mask, and you mask vs. both mask.

Discussion

Both scatter plots do not provide evidence of risk compensation occurring among young adults in Suffolk and Nassau counties due to the weak correlations. The analysis of close contact, however, was limited by the survey response options. Because this question was multiple choice, participants could only choose between five answers, limiting the sensitivity of the question. Luckman et a l. actually asked subjects to input the number of people they had come into close contact with, however, I initially thought that this would result in respondents skipping the question simply because it is difficult to produce such a number. My statistical analysis of these variables differs from that of Jørgensen et al. because the researchers examined responses to their survey over the course of many months while time was not a factor studied in my research. Also, Jørgensen et al. could directly compare data from before and after a mask mandate while I was not nor could not have been looking at a specific policy change. Originally, I had intended to use the results of multiple questions to study the relationship between risk perception and social distancing, however, when I started statistical analysis, I realized that most of the participants never had to quarantine or isolate themselves. This meant that data from questions about the adherence to quarantine and isolation protocols was essentially unusable.

The statistically significant differences in means are evidence for risk compensation. The evidence is not strong because only some of the differences were statistically significant. If the difference between every possible combination of the means, then it would be clear that mask wearing has a strong effect on physical distancing. The insignificant effect of location on the physical distancing means signifies that location should not be a major factor when planning events. Although having an event outdoors generally provides more space for people to spread out, my findings show that being outside does not guarantee that people will stand further apart. It is also important to realize that the means of all four groups were all greater than 4. Because the closest position that maintained physical distancing standards was position 4, these means suggest that subjects are not adequately physical distancing at all. Please note that the further the distance from the stranger, the lower the position number is. The scenario-based questions provided some evidence of risk compensation because of the three significant differences between the mean position chosen for the four different combinations of masks.

Conclusion

Evidence of risk compensation among young adults in Suffolk and Nassau counties during the COVID-19 pandemic, specifically in the area of physical distancing, supports my hypothesis. Subjects indicated that they would stand closer to an individual if both people were wearing masks which is consistent with risk compensation, the theory that when a person feels safer after the adoption of a safety measure (in this case masks), they will engage in riskier behaviors. This aligns with the conclusions of Luckman et al. and Jørgensen et al., who find that risk compensation is occurring among residents of England and Denmark respectively. The analysis of the risk perception versus mask wearing and risk perception versus social distancing did not support the hypothesis.

Implications and Further Research

Evidence that risk compensation is occurring among young adults has significant implications for public health policies and strategies for Suffolk and Nassau counties. This risk compensating behavior can contribute to an increased spread of the SARS-CoV-2 virus which is more likely to mutate if it continues to spread frequently. Because this behavior has such serious ramifications, my research supports the call for young adults to get vaccinated. We must prioritize the vaccination of young adults because of risk compensating behavior and because they have been linked to an increased spread of the virus due to their social mobility (Harris, 2020). Risk

compensation often occurs because of misunderstandings and misconceptions about the severity of engaging in certain risky behaviors. Evidence from my research supporting the idea that young adults are not necessarily aware of all of the regulations and guidelines about COVID-19 includes the fact that 38% of subjects did not know the maximum size for a social gathering dictated by NYS Health Department at the time of the survey (10 people). To inform this demographic, health departments can continue to explore untraditional avenues of communication, especially the use of social media platforms such as Twitter, Tiktok, and Instagram.

Future research should be conducted to combat the spread of COVID-19 among young adults and to draw stronger conclusions about risk compensation among the aforementioned demographic (especially in the United States). As previously discussed, young adults are not the most informed on all of the information regarding COVID-19. Surveys should be conducted to explore the knowledge and opinions of young adults on newer developments such as vaccines and variants. Data from these surveys would allow officials to get a better understanding of the specific misconceptions that need to be targeted. Furthermore, risk compensation could be studied experimentally by implementing the scenario-based physical distancing questions in real life. This would allow for the study of additional variables, such as whether the stranger was exhibiting symptoms of COVID-19, as well as drawing a much more definitive conclusion about risk compensation among young adults during the COVID-19 pandemic.

Limitations

Though there is some evidence of risk compensating behavior among young adults during the COVID-19 pandemic, there are limitations that reduce the strength of the findings. The sample size of 97 people is on the smaller side to generalize the findings to the hundreds of thousands of young adults who reside in Nassau and Suffolk counties. However, $n > 30$, meaning that the distribution of the sample means can be assumed to be approximately normal (due to the Central Limit Theorem) and by extension, the significant difference between the sample means could be assumed to be representative of the population. Another limitation is that the survey did not account for location of residence. This means that theoretically, a person visiting from outside of the target geographic area could have taken the survey, though the chances of this are unlikely. Additionally, there were many partial responses which weakened the quality of the data available for analysis. The survey questions were not mandatory to protect the subjects right to nonresponse, however, it seemed as though some questions were accidently skipped (for example, answering seven out of the eight scenario-based questions). Instead of $n = 97$, the number of responses was as low as 71 for a particular question. While I was only able to study correlation, Jørgensen et al. were able to study a causal relationship because they examined trends over the course of three months during the implementation of a mask mandate in Denmark. This allowed them to account for other variables that could have affected behaviors, and as a result, study a causal relationship. Because risk compensation theory describes a causal relationship, the findings of my study are not as strong as those of Luckman et al.

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Trisha Aiyer

Halobacterium and Gene Regulatory Pathways: Light-Dependent and Light-Independent Energy Production via Cell Signaling

Abstract

This paper is intended to highlight research done on a microorganism through trials designed to measure the impact of environmental factors. For this experiment, halobacteria colonies introduced to a sugar solution and limited light access were tested. A well tray was used to divide halobacterium and a growth medium solution into sixteen separate trials. The addition of sugar to the wells resulted in an increase in GGPP production but led to a decrease in net energy production as halobacterium utilized the light-independent pathway.

Background

Halobacterium are unicellular organisms with a single membrane with a rod-shaped exterior and flagella. They reproduce through binary fission and are also extremophiles; this means that they thrive under extreme conditions. These bacteria thrive in salt concentrations of 4.3M and temperatures of 37-42 degrees Celsius. They also occupy pH ranges of 5.5-8.5. To acquire energy, they rely on bacteriorhodopsin which is a light-responsive protein that helps to create chemical gradients via proton pumps resulting in energy products. In addition to this method of energy production, halobacterium can also use fermentation of arginine when there is a lack of light. They can also use the metabolization of sugars through redox reactions with amino acids in the trichloroacetic acid cycle.

Introduction

The primary objective of this experiment was to answer “What is the impact of inducing the light-independent pathway on halobacterium energy production.” The intent was to observe how halobacterium, which thrives in extremely saline solutions, would react to a new source of possible energy. For these reasons, the method of storing the sugar-influenced bacterium in a dark room yielded the best results as it directed the pathway to become light-independent.

Materials and Methods

In order to explore this question, 5 mL of growth solution was put into each of the sixteen

wells in the tray. Next, 1 mL of halobacterium was added to each. After dividing the well tray into two, eight of the wells were introduced to a pinch of sugar (composed of 6 crystals.) The wells with sugar were then covered before placing the entire tray in direct sunlight. The halobacterium were exposed to a week of sunlight before results were recorded.

Results, Tables, Graphs and Photos

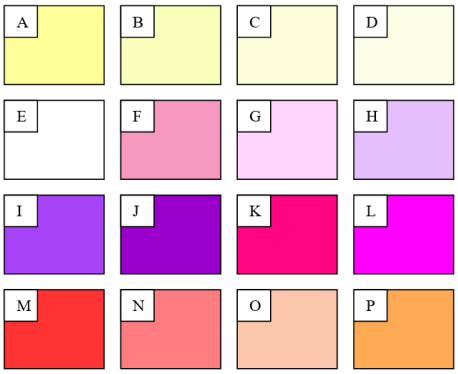
Observations from the wells of Halobacterium:

Row	A				B				C				D			
Well Number	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Growth? Y/N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Sugar? Y/N	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N
Clarity? O/T	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Absorbency?	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.106	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145	0.123 - 0.145
Color Chart?	O	O	O	O	O	O	O	O	F	F	F	F	F	F	F	F

*Clarity is discussed in terms of opaque or transparent.

*Absorbency was measured before adding sugar and after the full week with a spectrophotometer.

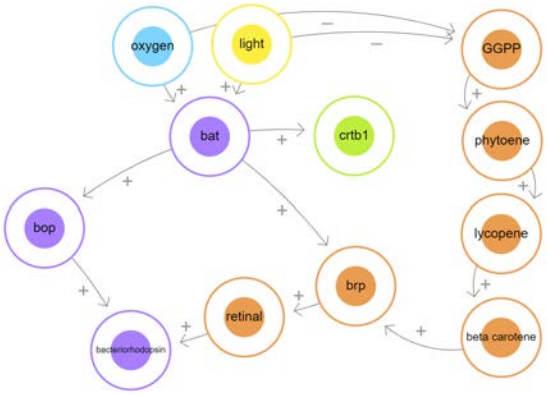
*The Color Chart is seen below.



Discussion

The addition of sugar in a halobacterium solution did result in the production of energy as seen in the absorbency numbers not dropping to zero indicating that there were halobacteriums that were able to adapt and utilize light-independent pathways. However, the halobacterium without sugar but access to light had an increase in absorbency and thus had a higher population of bacteria indicating that light-dependent pathways yielded more energy to grow populations. I expected these results because halobacteria typically utilize the pathway of bat to bop to bacteriorhodopsin which is efficient when oxygen and light are present. When forced to resort to the light-independent pathway of GGPP to phytoene to lycopene to beta carotene to brp to retinal then bacteriorhodopsin, it takes more energy than it provides resulting in the decrease of population.

Addressing the color changes in the bacteria, bacteriorhodopsin is formed along with gas vesicles which help with oxygen and light exposure. The vesicles have a protein layer that creates the unique pink color that signifies a healthy and energy-producing bacterium. Thus, when the bacteria that were introduced to sugar and darkness turned a more orange shade, it indicates the beta carotene pathway is more prominent as the pigment no longer matches the characteristic pink. The control group maintained its pink hue as it continued to follow the light-dependent pathway. This data can be used to observe similar processes in other species because halobacteria work as model organisms since they reproduce quickly and display traits found in many other organisms. For example, the information gathered about gene regulatory networks and cell signaling can lead to progress in microbiology and systems biology-related works. Thus, this data is applicable to many other species.



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