FALL 2022

RISING RESEARCHERS

# Journal of Scientific Research Writing

UMassAmherst

HIGH SCHOOL RESEARCH JOURNAL

# 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

Adrien Dai

**The Impact of Microbes and Bacteria on Legume Growth in Great Neck, NY**

**ABSTRACT**
This experiment was conducted to find the impact of microbes and bacteria on legume growth in Great Neck, New York. Methods including soil collection, phenotyping plant roots, and bioinformatics tools to explain symbiosis were used to conduct this experiment.

**INTRODUCTION**
Nitrogen is an essential element for plant growth (Zahran, 1999). There is an abundance of nitrogen in the atmosphere, but plants cannot obtain nitrogen in this form. Bacteria, including Rhizobia, aid plants in nitrogen fixation, and allow plants to obtain the resources required in order to survive (Wang et al., 2012).

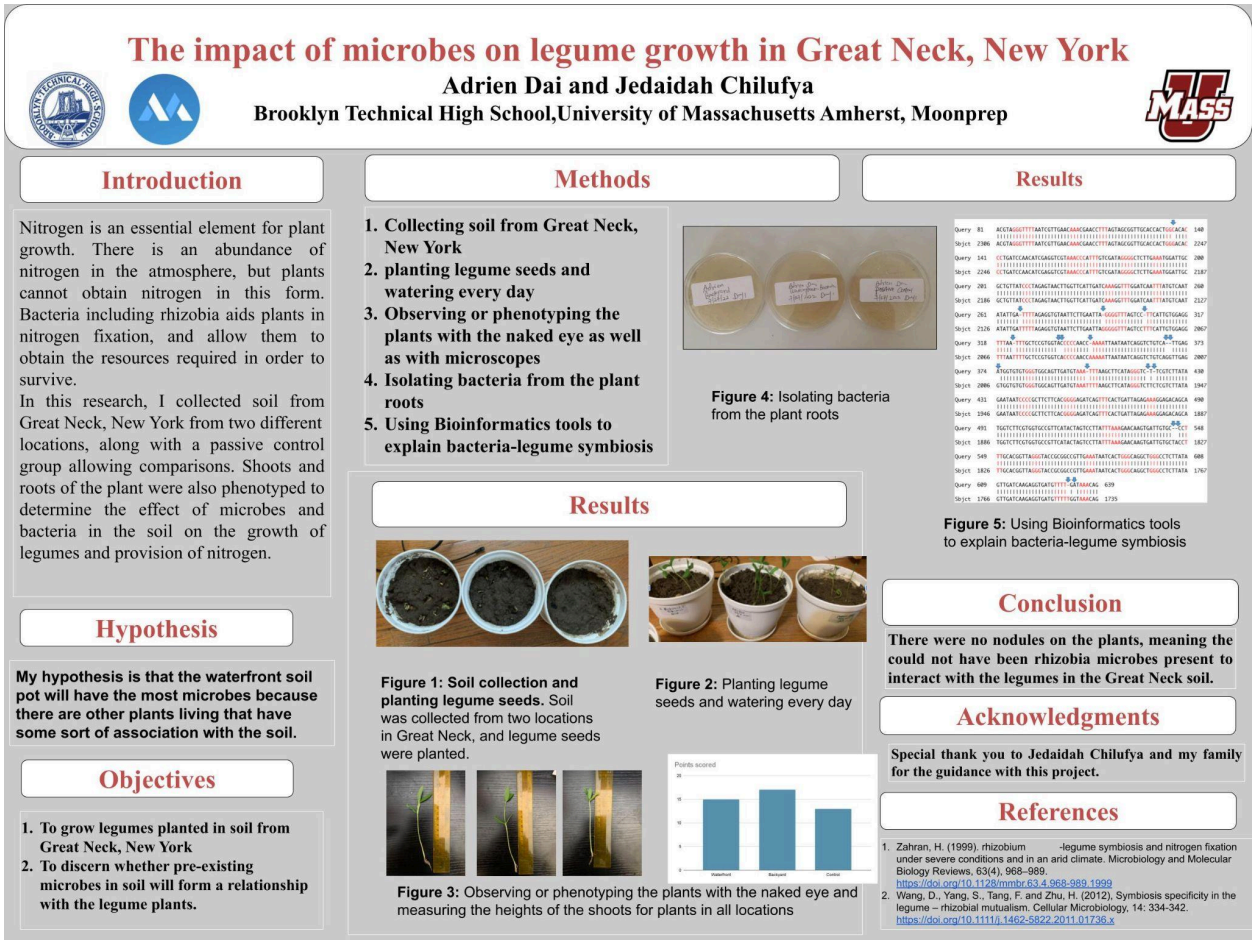
This paper explores the impact of pre-existing bacteria in soil and its effect on legume plant growth. I hypothesize that the soil collected in the Waterfront pot will have the most nodular growth because other plants were observed to have grown in this soil and may have attracted bacteria.

**METHODS**
Soil samples were taken from Great Neck, New York in two different locations: Waterfront and Backyard. The soil was distributed into three different pots labeled Positive Control, Waterfront soil, and Backyard soil. Positive Control consisted of soil from the backyard with added commercial inoculant. Each pot was watered daily and observed. The number of plants and the length of each plant was recorded. Growth was measured in centimeters using a ruler. During week 4, the tallest plant from each pot was removed and phenotyped. Parts of the roots were cut and put in a solution of 75% Clorox and 25% water, and then in a solution of 25% Clorox and 75% of water for comparison. Microscopy was then used to examine the status of nodular growth on the plant roots. Bioinformatics was used to evaluate the status of bacteria and legume symbiosis.

**RESULTS**
There was more plant growth (7 plants total) in the Waterfront pot than those in the Backyard pot (3 plants total). There were only two plants in the Positive Control pot. There were nodules present on plant roots grown in the Positive Control and Waterfront soil, meaning there was interaction between bacteria and legume seeds.

The growth between plants were quite different, having an average of around ~21cm. There were nodules on Positive Control and Waterfront soil. The Waterfront soil plant was 23cm, Backyard soil was 19cm, and Positive Control was 20 cm. The Waterfront soil plant had a 21% increase in root length compared to those grown in the Backyard soil, meaning there could have been more nutrition or different types of bacteria affecting them.

**DISCUSSION**
My results indicate that microbes and bacteria in the soil may not have as large of an impact on plant growth as hypothesized. This may have been a product of a short timeline for experimentation or possible human error. In the future, this data could be used to support further experimentation regarding the interaction between bacteria and plant growth.

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Wang, D., Yang, S., Tang, F. and Zhu, H. (2012). Symbiosis specificity in the legume – rhizobial mutualism. Cellular Microbiology, 14: 334-342. https://doi.org/10.1111/j.1462-5822.2011.01736.x

Akhil Gaddamadugu

# Investigating the Impact of Different Types of Rhizobia on Sunn Hemp Growing in Westborough, Massachusetts

**ABSTRACT**
Crotalaria juncea or Sunn Hemp is a significant sustainable food and feed source in our changing environment, where temperatures are rising, water is becoming limited, and food insecurity is a problem. Crotalaria juncea's capacity to be sustained depends on their interaction with soil rhizobia, which leads to symbiotic nitrogen-fixation to fuel plant development. Crotalaria juncea is a tropical legume that is native to Southeast Asia and is grown in the South of the United States and could also undergo the success that it is having in the south right now in Massachusetts. Due to it being growing in Massachusetts now, the plants are now in a drastically colder environment than compared to Asia where Sunn Hemp is often produced. So hence, the production would be overall lower as it's not a local plant and the conditions in Massachusetts are completely different. To solve this I investigated which rhizobia would result in efficient legume symbiosis in sunn hemp in order to assist achieving high production with this crop.
Introduction

Legume productivity often depends on the symbiotic relationship between the roots of legumes and the nitrogen-fixing bacterium known as rhizobia (Concha & Doerner, 2020). As a result, legumes still thrive in nitrogen-poor soils. Farmers may take advantage of this symbiotic connection to reduce their need for synthetic nitrogen fertilizers produced (Lindström & Mousavi, 2020). Many farmers frequently utilize inefficient inoculants because they are uncertain of which rhizobia strains to use while inoculating their plants. This creates a dilemma whether the Legumes should be left alone with the rhizobia in the soil or artificially add rhizobia to increase production by adding inoculant so farmers can increase their yield? This study aims to address the following questions: What is the effect of inoculation powder on Sunn Hemp? Does it increase the production of Sunn Hemp Legumes?

**RESEARCH OBJECTIVES**
In order to answer these questions, we have to measure the plant to see if inoculation powder increases or decreases the production of Sunn Hemp and to see how the rhizobia changes production.

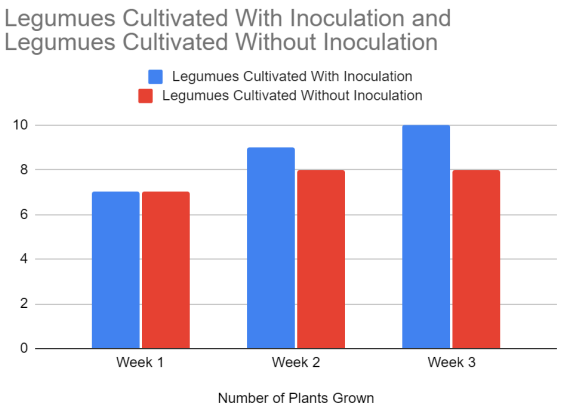
**HYPOTHESIS**
We hypothesized that the Sunn Hemp Legumes cultivated with the commercial inoculant would have higher shoot height and production compared to those planted in native Massachusetts soil because of the rhizobia in the commercial inoculant.

**MATERIALS AND METHODS**
This experiment included comparison of two different planter pots: one Sun Hemp Legume batch with inoculation powder and one Sun Hemp Legumes batch without inoculation powder. Each piece of equipment was sterilized so no other bacteria or microorganisms could interfere with the results. First, we gathered the soil from one common location and planted an equal amount of sterilized seeds in both pots. The next step was to add inoculation powder to one of the pots when planting those seeds. This allowed for comparison of the effects of inoculation powder versus growth without inoculation powder. After this, we watered our plants everyday and kept track of the production by counting the number of sprouts grown. To gather even more data to compare the 2 pots, when the plants were 4 weeks old, we phenotyped the tallest plant out of each pot to compare the lengths of their shoots.

We repeated this process again a week after that to see any potential growth in the crops. After repeating the phenotyping process for a second time, we observed the shoot lengths and found the length grown. Next, when isolating rhizobia in order to place it in our agar dishes, we counted the number of visible colonies of nodules to see how many rhizobia developed. After isolating the nodules in their own respective tray, we waited two days to see the bacterial growth. We compared data collected from each group to better understand which procedure would yield better growth.

RESULTS WITH PICTURES AND TABLES
Table 1 includes a summary of the number of Sun Hemp Legumes plants grown in the two groups (legumes cultivated with inoculation and legumes cultivated without inoculation) over the course of 3 weeks.

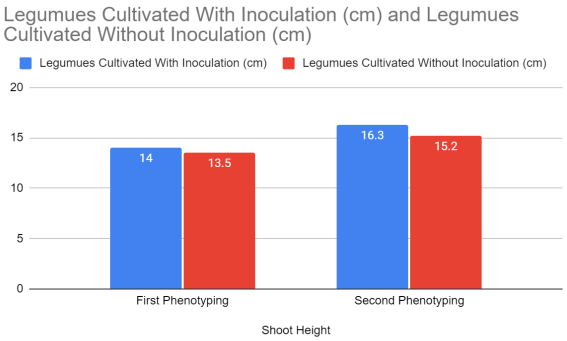
Table. Number of Plants Grown in each Pot



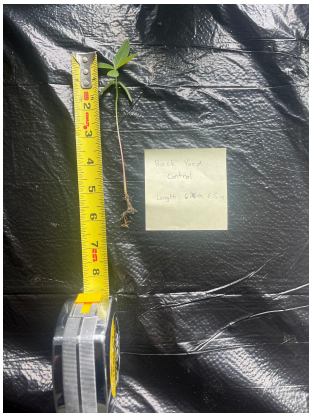

Week 1

Week 2

Week 3

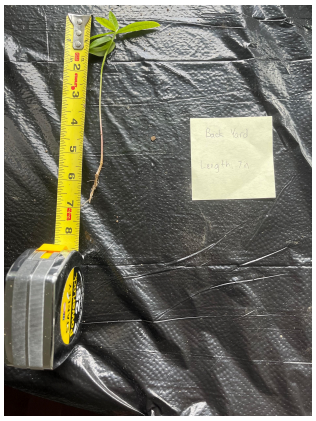



First Phenotyping of Legume Cultivated With Inoculation

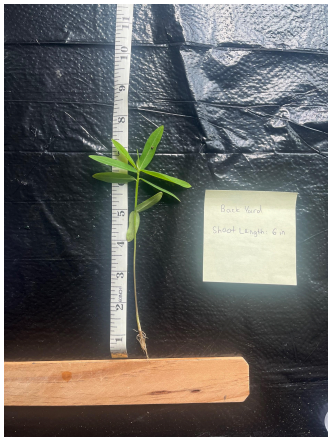


Second Phenotyping of Legume Cultivated With Inoculation

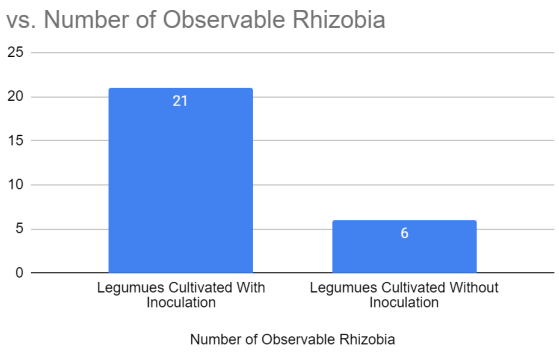
First Phenotyping of Legume Cultivated Without Inoculation



Second Phenotyping of Legume Cultivated Without Inoculation



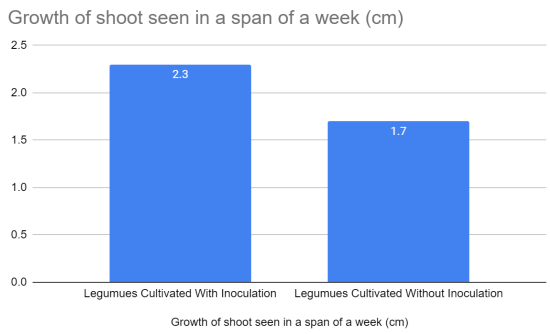
Number of Observable Rhizobia



Legumes Cultivated With Inoculation

Legumes Cultivated With No Inoculant

Growth of shoot seen in a span of a week

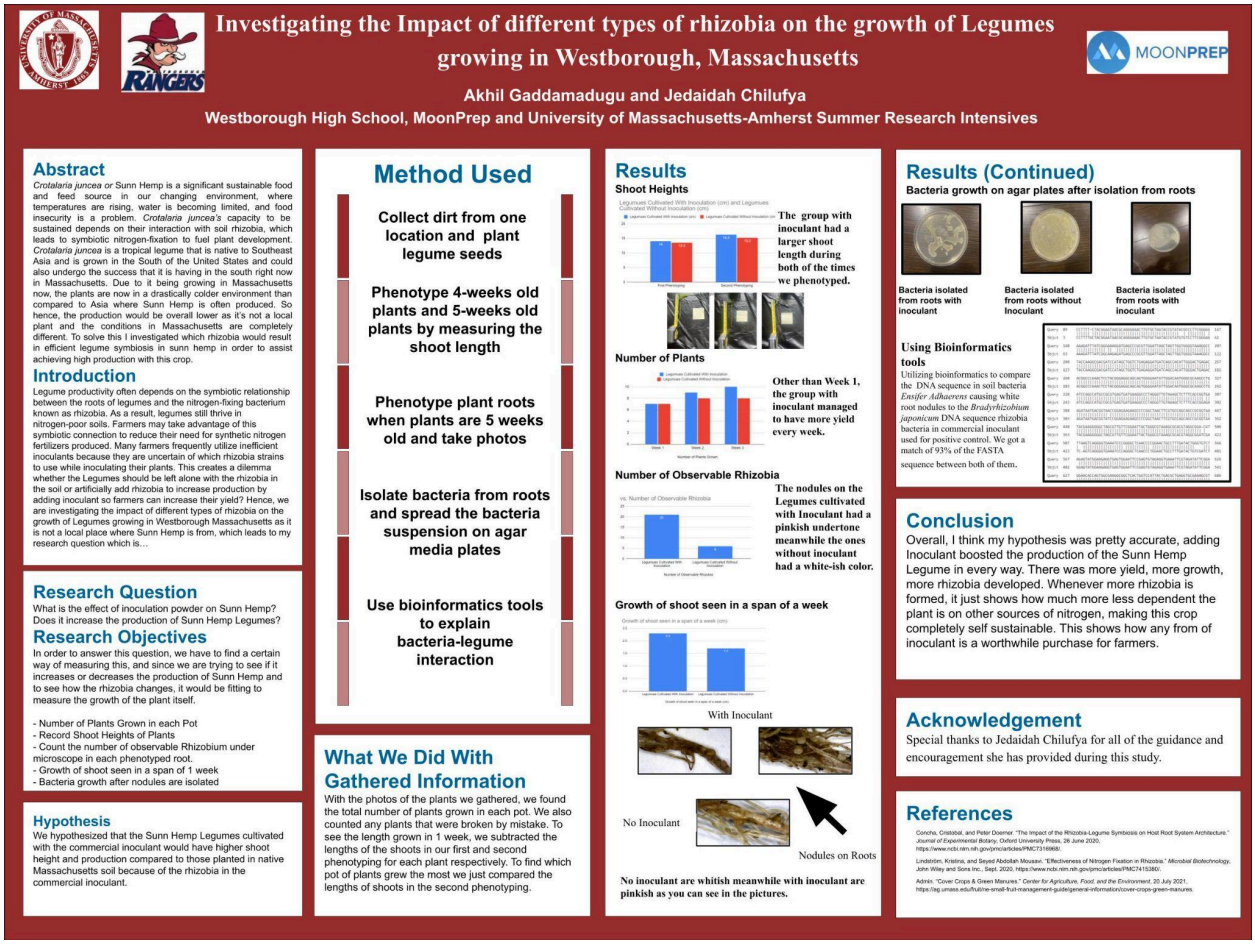



Bacteria growth of Nodules in Agar Plates
Bacteria Nodules With Inoculant

Bacteria of Nodules Without Inoculant

**DISCUSSION**

This experiment supported my hypothesis that Sunn Hemp Legumes cultivated with the commercial inoculant would have higher shoot height and production compared to those planted in native Massachusetts soil. There was more yield, more growth, more rhizobia developed in the pot cultivated with inoculant. The inoculant group was more self sustainable as it produced more rhizobia showing that the plants were less dependent on other sources of nitrogen.

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Albert Wu

# Observing the Effect of Differing Rhizobia on the Growth of Legume Plants in Great Neck, NY


## ABSTRACT

Nitrogen Fixing bacteria, also known as rhizobia, is often found in legume plants due to sharing a mutually beneficial relationship (Yang et al. 2011). The rhizobia lives in the root nodules of legumes and fixes nitrogen, which is essential for plant growth (Yang et al. 2011). However, the rhizobia in each plant differs due to a variety of factors. (Zahran 1999) This research project was conducted to examine if there is any rhizobia in the soil of Great Neck, New York to interact with legume Crotalaria juncea and cause nodules to form on the roots.


## INTRODUCTION

The purpose of this research is to determine whether preexisting microbes in the soil at Great Neck, New York will affect the growth of the legume Crotalaria juncea. To carry this out, bacteria was isolated from the legumes after growth.

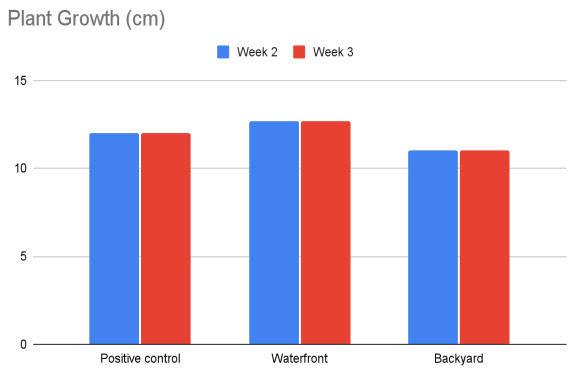
## MATERIALS AND METHODS

Soil was collected from two different locations using gloves and a shovel; one location was from my backyard in Great Neck, New York, and the other was a waterfront in Lake Success. Then, the differing types of soil were each put into their own pots; a backyard soil group, a waterfront soil group, and a positive control group, which was backyard soil with commercial inoculant added. Legume seeds were planted in each pot. The legumes were watered every day for three weeks, and they were phenotyped during the third week using the naked eye and a TOMLOV digital microscope. After week three, bacteria was isolated from the root nodules of the legume plants using two test tubes, one containing 100% water, and another containing a 75% water, 25% bleach solution. The bacteria was cultured then on agar plates for three days.

## RESULTS

By week three, the tallest plant from the waterfront soil group had reached a height of 12.7 cm, and the tallest plant from the backyard soil group was 12 cm tall. In comparison, the tallest plant from the positive control group had a height of 11 cm. Bacteria was isolated from the root nodules of the tallest plant from each group and cultured on agar plates. From day 1 to three of bacteria growth, the waterfront soil group had the most bacteria, with the backyard soil group coming in second, and the positive control group coming in last.

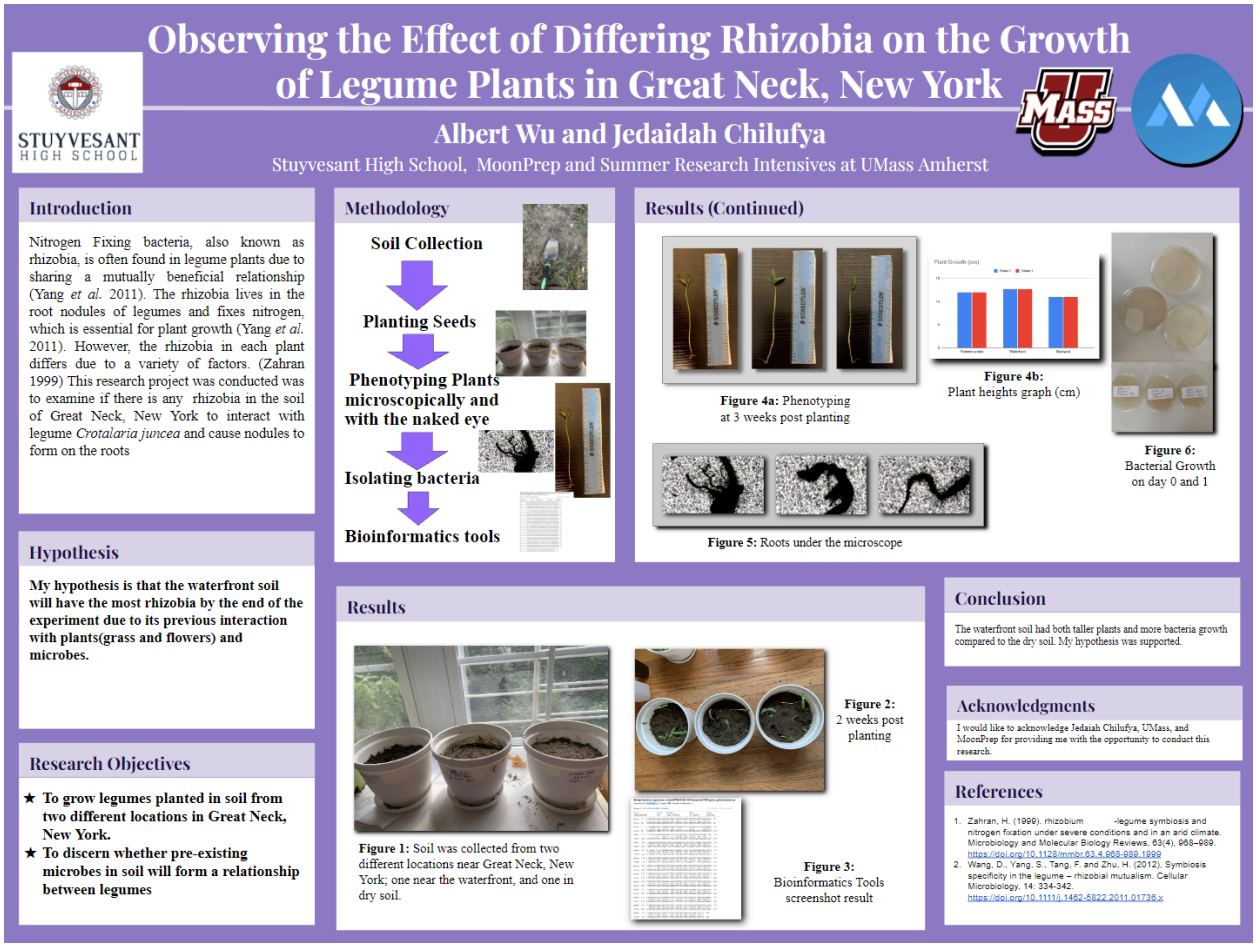
## TABLES AND GRAPHS




## DISCUSSION

The results support the claim that pre-existing microbes in the soil will affect the growth of new plants. The waterfront soil group had the most bacteria growth and plant growth, which supported my original hypothesis, while the backyard soil group came in second and the positive control group came in last. This was because the waterfront soil had the most previous interactions with healthy plants compared to the soil from my backyard,

which was very dry, had minimal plant growth, and did not receive much watering.

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Alicia Cooper

# Growing Legume Plants: Exploring the Microbiology and Bioinformatics in Ashburn, VA


## ABSTRACT

Learning about the relation between bacteria and plants is the key way of knowing the beneficial properties needed for plant growth. Rhizobia is a common nitrogen-fixing bacteria found in soil, typically on legume plants. This particular bacteria forms nodules on the roots of legume plants by converting atmospheric nitrogen to nitrogen the plants are able to use for their nutrients, ammonia. In this experiment, two different locations of soil were used from Ashburn, VA, and tested to observe the presence of Rhizobia and other microbes interacting with the legume plants. The final results of this experiment exhibits whether or not the process of this has occurred through the backyard, frontyard, and positive control, all shown in the phenotyping of roots and isolation of bacteria.


## INTRODUCTION

Rhizobia is a common nitrogen-fixing bacteria found in soil, typically on legume plants. This particular bacteria forms nodules on the roots of legume plants by converting atmospheric nitrogen to nitrogen the plants are able to use for their nutrients (Lindström & Mousavi, 2019). Nitrogen is composed of many molecules, including chlorophyll, a key component for conversion into ammonia and starting photosynthesis (Via, Zanetti & Blanco, 1970). Once the roots are nodulated, the nodules will show a white color then turn into a pinkish tint once the nitrogen has been fixed (Via, Zanetti & Blanco, 1970).The symbiosis of legume-rhizobia starts between the host plant and microbial endophytes, once a bacteria is recognizably suited, the host causes cell division that forms the root nodules. The nodule cells are surrounded by a plant origin membrane which forms a symbiosome, structures similar to organelles, within this nitrogen-fixing bacteria takes place (Wang, Liu & Zhu, 2018). Nod factors are signaling molecules that are essential for symbiosis development whenever there are limiting conditions of nitrogen as it induces nodulation (Wang, Liu & Zhu, 2018). The host has to recognize these factors for nodules to form. This study aims to identify whether or not the process of nitrogen-fixation has occurred through the backyard, frontyard, and positive control. I hypothesize that bacteria, specifically Rhizobia, and fungi will be present in both locations but significantly more in the backyard due to the soil already cultivated by plants.

## MATERIALS AND METHODS

*Germination*

Materials: Three pots and pot saucers, shovel and garden fork for digging, personal protective equipment (PPE), two different locations of soil, legume seeds, commercial inoculant, and a water jug.

To begin the experiment, soil was collected and placed into two pots from the backyard and front yard in Ashburn, VA using a garden fork and shovel. Positive control soil was collected from the front yard and placed in the third pot. After labeling the correct locations on the pots, 15 holes were poked into each pot and sprinkled with commercial inoculant. 2-3 legume seeds were added into each hole then covered with the surrounding soil with another 20 ml of water added for hydration. If excess water was found on the bottom of the pots, it was removed to prevent clogging of the roots.

*Phenotyping Plants*

Materials: LCD Digital Microscope, 75% disinfecting wipes, PPE, ruler, black

background, water jug and paper towels.

Growth of plants is observed over the course of three weeks. 2.5 weeks post planting, a plant from each pot was carefully removed and measured in centimeters against a black background by the shoots with a ruler. After a week, a second phenotype of the shoots on a different set of plants were done to compare the improvement in growth. These same plants were observed under a microscope for root nodules.

*Isolation of Bacteria*
Materials: PPE, paper towels, plastic pestles, scalpel, swabs, microcentrifuge tubes, agar media plates, 75% disinfecting wipes, clorox wipes, and a water jug.

The same plants used for the second part of phenotyping were cut by the root/root nodule, crushed by a pestele, and placed inside a tube with a 0.5 water mark. A swab was used to spread the isolated bacteria onto the media plates. Growth of bacteria was checked everyday for four days. The surface and tools were sterilized with wipes between each plant.

**RESULTS**
Phenotyping of each of the three trials were compared at 2.5 weeks and 3.5 weeks. Table 1 shows the results of shoot phenotyping at 2.5 weeks indicating the largest number of plants grown and tallest height seen in the backyard trial. Table 2 shows the results of shoot phenotyping at week 3.5 showing a similar pattern to Table 1.

Table 1. Shoot Phenotyping at 2.5 Weeks

| | # of Plants Germinated | Average Plant Height (cm) |
|---|---|---|
| Backyard | 17 | 17 |
| Frontyard | 9 | 13.5 |
| Positive Control | 12 | 12.5 |

Table 2. Shoot Phenotyping at 3.5 Weeks

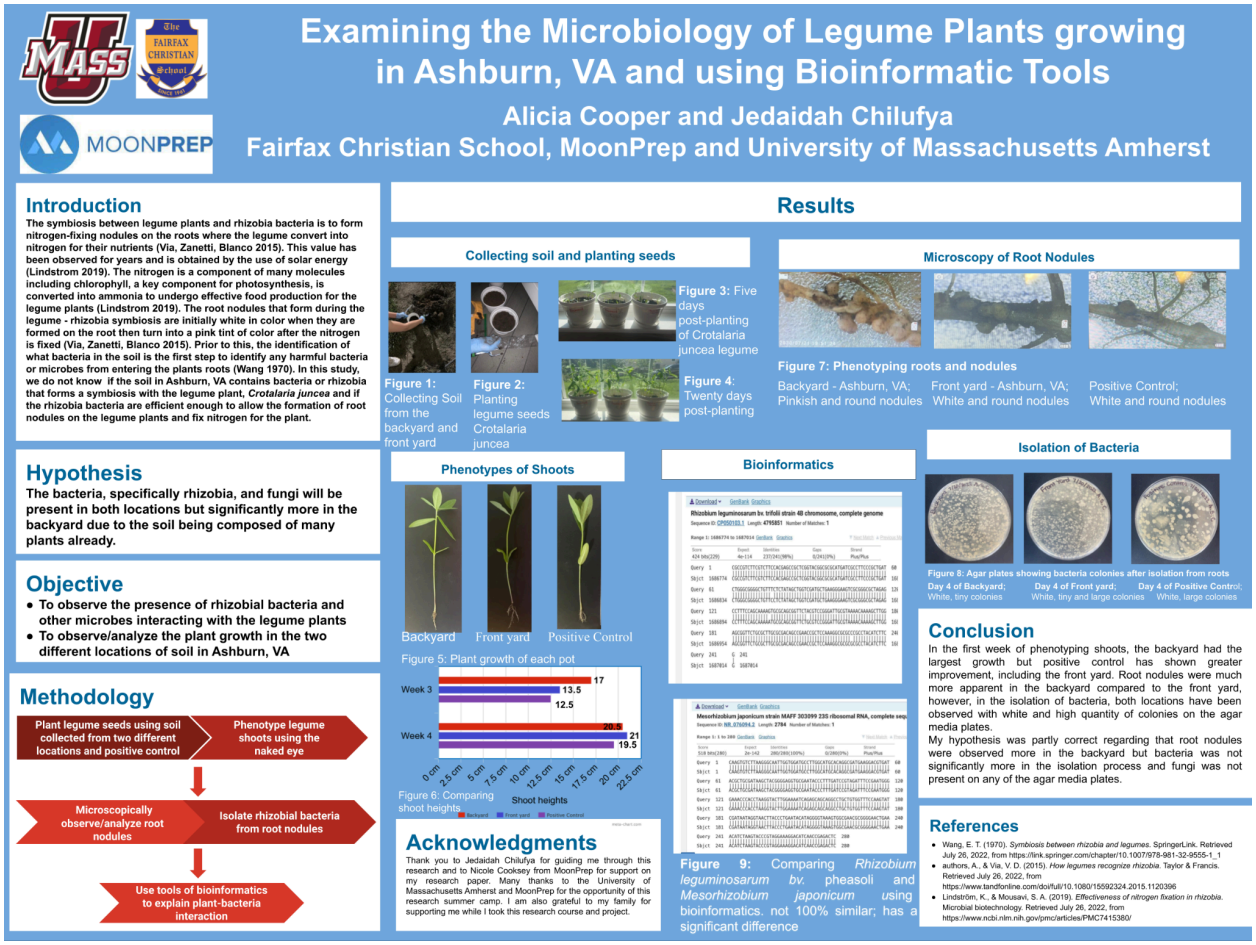
| | # of Plants Germinated | Average Plant Height (cm) |
|---|---|---|
| Backyard | 20 | 20.5 |
| Frontyard | 11 | 21 |
| Positive Control | 16 | 19.5 |

Phenotyping of the roots showed the backyard with the most apparent number of root nodules (pinkish and round), front yard with root nodules on every branch (white and round), and positive control with least amount of root nodules (white and round). Lastly, isolation of bacteria was conducted and the backyard showed white, small and crowded colonies, the front yard showed white, both small and large, crowded colonies, and the

positive control showed  white, very large colonies.

## DISCUSSION

No plants died over the course of germination or phenotyping for both locations but the surprising factor is the advance in growth for the front yard as the soil showed significantly more dryness than the backyard. The backyard started off as the largest growth but the front yard and positive control showed a greater improvement. Root nodules were more apparent in the backyard compared to the front yard, however, in the isolation process of the bacteria, both locations showcased white and crowded colonies throughout the agar media plates. My hypothesis is partly correct regarding the root nodules but the backyard bacteria was not significantly more in the media plates and no fungi was observed for both locations. The importance of this experiment relates to the question asked regarding the type of microbes present in the soil. This process can identify what aids and hinders the growth of plants in the comfort of your soil.

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Anika Sharmila

**Analyzing the Interaction of Nitrogen Fixing Bacteria within Legume Plants in Wichita Falls, TX**

## INTRODUCTION

It has become evident with our current climate and gradual decrease of both water and food supply that legumes have become undoubtedly important as a sustainable source of consumption. The sustainability of legumes is in their association with soil rhizobia which result in a symbiotic process called nitrogen fixation, which will provide nitrogen for the plant to grow efficiently. If the interaction transpires smoothly, it should as a result create root nodules, which are small tumor shaped substances lying along the root of the plant where the rhizobial bacteria lie within. The rhizobia has to be able to fix nitrogen within the nodules so that the legume plant can grow properly, but there are some factors that potentially may prohibit this process. This study will examine the interaction of nitrogen fixing bacteria that will fix the Crotalaria juncea legume from areas within Wichita Falls, Texas in order to determine whether Texas has sufficient planting soils for legumes to thrive in. The study will also take into account the environment in which the legume plants are growing in and utilize that information in order to analyze how the different soils within the city will be able to interact with the legume plants, and whether or not some do so more efficiently by observing for root nodules and overall plant growth.

The question I asked in this study is which area of soil within my city would allow for the most efficient cultivation for the Crotalaria juncea legume, taking into consideration the environment that Texas provides these plants with. My initial hypothesis was that the front yard soil will have more bacteria that will allow for the legume seeds to grow more efficiently due to the variety of plants that watered throughout the week, specifically observing for any sprouts that started to appear due to germination of the legume seeds. Throughout this process, it was also required to take note of any unusual appearances of unwanted material as well as the days in which the seed sprouts emerged within any of the pots.

While continuing the watering of the legume plants, the first phenotype was conducted. This phenotyping was focusing on the shoots of the plants within the different pots, and this was done by carefully removing the desired legume plant out of their pots, which was possible after adding water into the pots and making the soil softer to maneuver. Once the plant was taken out, it was washed gently with water and dried off with a paper towel. The plant was then set onto a black surface beside a measuring device such as a ruler. The measurements were noted in accordance to each location, and then planted carefully into their respective pots. A second phenotyping was conducted subsequent to the first, this time also measuring the roots of the plants. In addition to this process, a microscopy of the roots was done, which used essentially the same steps as the phenotyping while using a microscope.

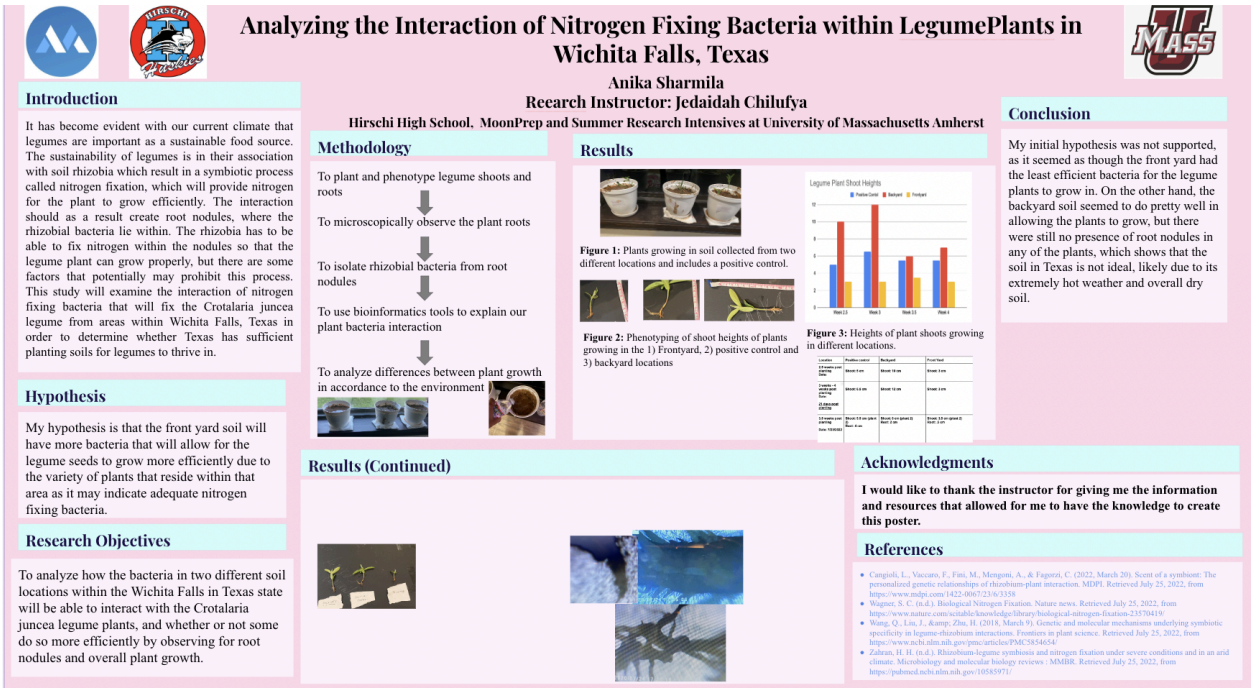
## RESULTS

The pot with the poorest overall plant growth was from the front yard, attaining two sprouts with the tallest plant's shoot growing to approximately 3.5 centimeters, and a root of a half centimeter. The soil within the front yard's pot was also exceedingly dry even after a few hours subsequent to watering. The backyard soil did adequately better, getting two fairly healthy sprouts with the tallest shoot being 12 centimeters and the tallest root being 2 centimeters. The positive control had three plants within its pot, all fairly healthy. The tallest shoot for the positive

control was 6.5 centimeters, but it had the longest and most healthy root of the three pots with it being 4 centimeters. Despite this, there were no root nodules present in any of the legume plants that had grown.

**DISCUSSION**

My initial hypothesis was not supported, as it seemed as though the front yard had the least efficient bacteria for the legume plants to grow in. On the other hand, the backyard soil seemed to do pretty well in allowing the plants to grow, but there were still minimal root nodules. This observation demonstrates that the soil in Texas is not ideal for the interaction of nitrogen fixing bacteria such as rhizobia within the legume plants. This occurs when the initial stage of interchange between the bacteria and the plant does not occur, which in this case the most probable cause being due to the extremely hot weather and overall dry soil that inhibited the attraction the bacteria would have towards the roots in normal conditions.

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Anvitha Arjula

# The Effects of Bacteria on Legume Plants in Monroe Twp, NJ.

**ABSTRACT**
The objective of this experiment was to observe the effects of soil microbes and bacteria on the process of legume-rhizobia symbiosis, as well as to observe the effects of fertilizer on root nodule formation and growth. In this experiment, two different soil locations in Monroe Twp, NJ 08831, were used in order to help analyze the effects of different soil bacterium on the crotalaria juncea-rhizobia symbiosis. The growth and development of these three pots were compared in order to assess the effects of certain soil bacteria, microbes and commercial additives on plant shoot and root growth as well as its effects on legume-rhizobia symbiosis. It was found that an excess of fertilizer or lawn additives can harm the crotalaria juncea-rhizobia symbiosis and inhibit formation of root nodules, while certain soil microbes and bacteria can also affect root nodule formation and growth.

**INTRODUCTION**
Rhizobium-Legume symbiosis is a mutualistic relationship between a legume plant and the bacteria known as rhizobia. On the roots of the legume plants, small white or pink nodules known as “root nodules,” or specialized organs can be found. Within the mutualistic relationship between the two organisms, the legume plant provides nutrients to the rhizobia bacteria that allow it to fix nitrogen into ammonia that the legume plant then uses to foster growth through photosynthesis (Adesemoye & Kloepper, 2009). This study aims to understand the consequences of varying soil locations and microbes, as well as fertilizer and soil/lawn additives on the crotalaria juncea-rhizobia symbiosis.

**MATERIALS AND METHODS**
The soil for all three pots came from various locations in Monroe Twp, NJ, 08831. The first pot contained soil from a backyard that contained soil additives and fertilizers. The second pot contained soil from a local pond and the third pot contained soil from a backyard as well, however this pot was supplemented with a commercial inoculant. The soil was collected using gardening tools, such as a rake and a shovel.
Once the soil for the pots was collected, then the legume seeds were planted. Small holes were dug into the soil in the pots where a seed or two was then placed into the hole. The pots were watered and then monitored in order to observe germination. Weekly photos were taken to document growth.

After three weeks post planting, the legume shoots were phenotype for the first time. The soil in the pot was carefully wetted in order to make it easier for it to break apart, and then, using one's fingers, the legume shoot (along with some of its surrounding soil) was carefully lifted out of the pot in order to ensure minimal damage to the plant roots. Once the plant shoot was taken out of the soil, plant tap water was used in order to rinse it off and remove any excess dirt. The plant was then dried off on a paper towel and placed on a black background alongside a centimeter ruler and a label with its location. Once photos of the plant shoot were taken, and their heights were recorded, the shoot was carefully replanted back into the pot. This process was repeated two more times for one plant shoot from every pot.

Then, around 4 weeks post planting the legume shoots were ready to undergo a second shoot phenotyping, as well as a root microscopy and bacterial isolation. After the plant shoots were carefully removed from the pot and photographed, the plant roots were placed under a microscope stand and observed using the microscope. Magnified photos of the roots were taken and studied.

Once phenotyping of the roots had occurred, a scalpel was used in order to carefully cut away a small section of the roots (preferably containing the root nodules). The small piece of plant roots was placed in a solution of bleach and water (8.25% bleach in a 1:20 ratio of bleach to water) in order to sanitize the roots and remove any unwanted bacteria. Then the root sample was cleaned off in a solution of plain tap water and placed in a new, clean microcentrifuge tool. Using a pestle, the root sample was then crushed up inside of the microcentrifuge tube for 3 minutes to ensure that all of the root bacteria was released. Then, 0.2mL of water was added to the crushed up root sample and mixed up thoroughly. A sterile swab was then used in order to swab the mixture onto new, clean, agar media plates. The media plated were wrapped in aluminum foil and left in a warm location to develop, with daily photos of them being taken. This process (the second phenotyping as well as the root microscopy) was then repeated for a single shoot from every single pot.

**RESULTS**

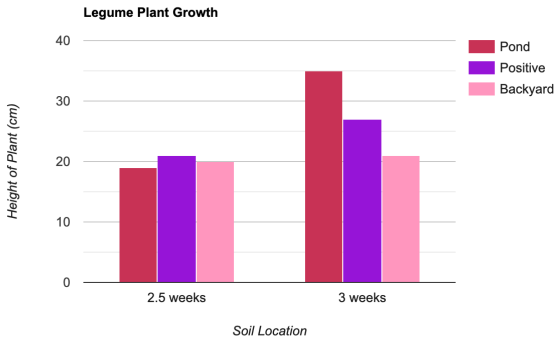
**Collecting the soil samples to plant**

Fig.1: Pond
Fig.2: Positive control
Fig.3 :Backyard

**Results of the first phenotyping:**
Pond shoot length: 19 cm
Backyard shoot length: 20 cm
Positive control shoot length: 21 cm

Fig. 1: Pond Fig. 2 : Backyard Fig. 3: Positive Control

**Second shoot phenotyping results**
Pond shoot length: 35 cm
Backyard shoot length: 21 cm
Positive control shoot length: 27 cm



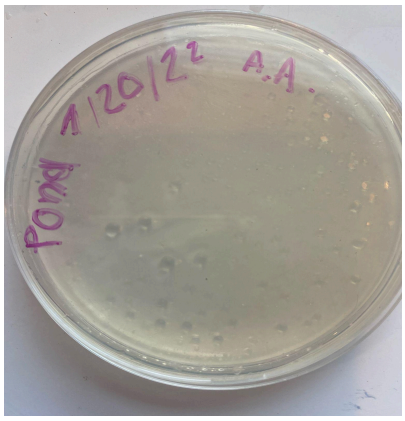

Pond

Positive control

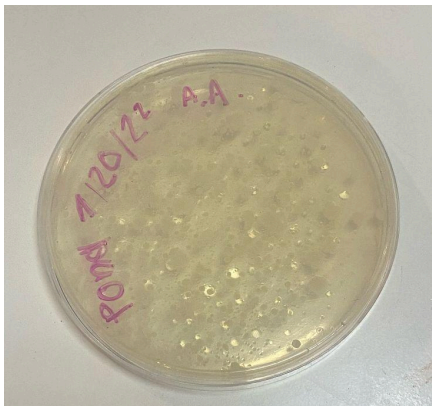
Backyard

**Root microscopy results**
Nodules were found only on the roots of the soil from the pond, neither soil from the backyard or the positive control had roots with developed nodules. Nodules found on the roots of the pond plant were small, with only two in total across the entire plant roots, and they were slightly pinkish in color.

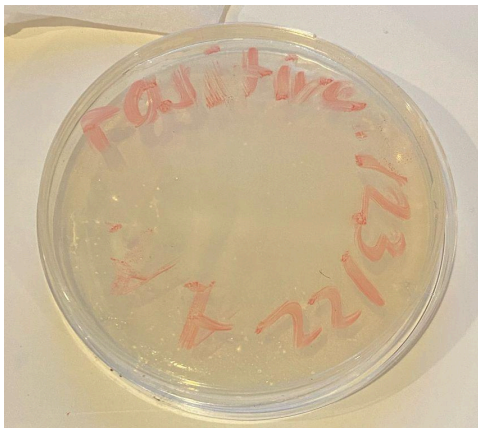
**Isolation of bacteria**



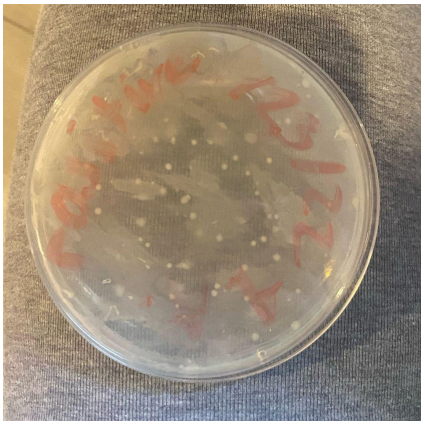

Pond at day 0 after isolation




Pond at day 2 after isolation

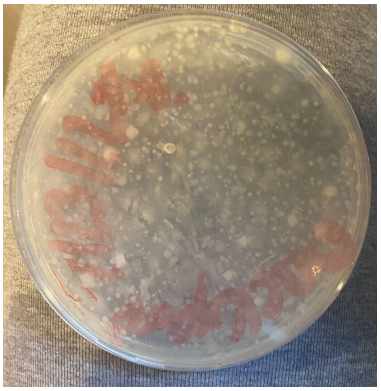


Positive Control day 0



Positive control 2 days after isolation

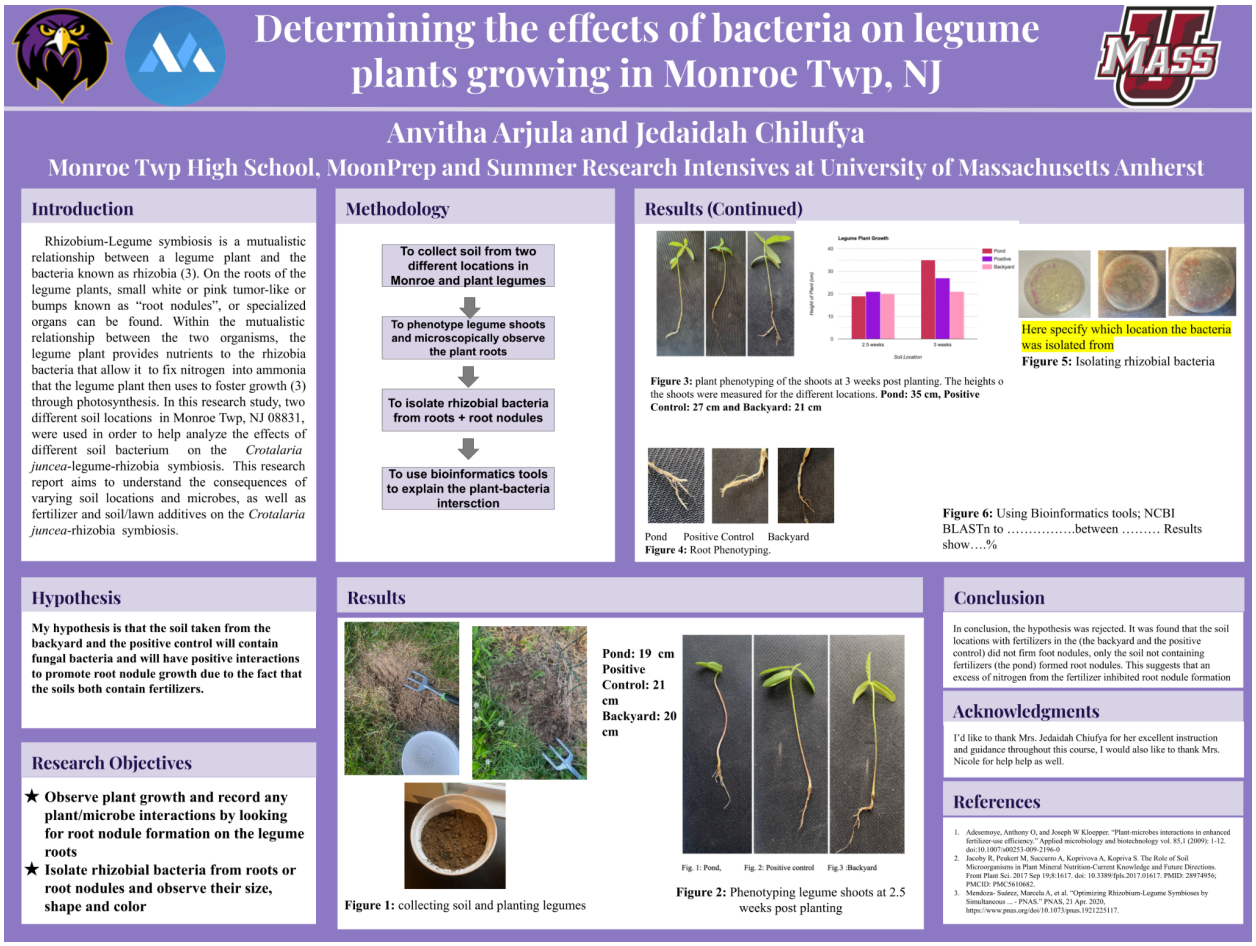
Backyard day 0



Backyard on day 2 after isolation

**DISCUSSION**

Throughout the course of this experiment, it was found that nitrogen fixing root nodules were only found on the roots of the plants that were grown in the soil from the pond. In comparison to the soil from the backyard and the positive control, the soil from the pond was treated with any kind of fertilizers or lawn additives in order to obstruct the growth of weeds or unwanted plants. As most fertilizers are made up of a mixture of N-P-K or nitrogen, phosphorus, and potassium, they are abundant in nitrogen (Townsend et al., 2020). This profuseness of nitrogen already present on the soil may make it pointless for the rhizobia bacteria to form root nodules, as the reason root nodules are formed is to help create nitrogen for the plant to use to create food through photosynthesis and to survive. As there is already nitrogen present in the soil, it can be hypothesized that the reason no root nodules were found on the plants grown in soil that already contained nitrogen through fertilizers previously used in the area is because there was no biological reason for the root nodules to grow. As nitrogen was already present in the soil, the presence of the root nodules would neither have been beneficial or detrimental, as there was no reason that nitrogen had to be “fixed” through the nodules; instead, it could have been used directly from the soil itself, explaining the lack of nodules on the plant roots. This explanation can be used to answer the hypothesis : does fertilizer already present in the soil have an effect on the growth and development of root nodules? It is clear that, yes, it does. These results were unexpected, as it's easy for one to assume that fertilizer, which is usually used in order to help crops thrive, could inhibit the growth and development of the root nodules, leading to a negative plant/soil interaction. However, on a deeper look, it makes sense as to why fertilizer would stop the formation of nodules, as it is actually benefiting the plant.

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Arushi Das

# Observing the Effects of Nitrogen Fixing Microbes on Legume Development in Edison, NJ


## ABSTRACT

Plants require nitrogen fixing bacteria to thrive. Due to plants' inability to absorb nitrogen from the atmosphere, nitrogen-fixing bacteria convert atmospheric nitrogen into usable nitrogen absorbable by plant roots. In legumes, the main nitrogen source comes from Rhizobium. It is unknown whether or not the bacteria required to do such functions is present in Edison, New Jersey. Therefore this research was conducted in order to detect its presence and in what quantity it is present in. This was done by analyzing the growth of legumes from two different locations in Edison and comparing it to the growth of legumes in a positive control already possessing the healthy nitrogen fixing bacteria. If one location's soil allowed for greater legume growth, it can infer that the soil from this location possess more bacteria that allow nitrogen fixation.


## INTRODUCTION

There are several forms of bacteria that are capable of forming mutually beneficial relationships with plants. Such beneficial partnerships are known as symbiotic relationships, a long term interaction between two different species. In the case of this experiment, the symbiotic relationship being studied is the process of nitrogen fixation, between nitrogen fixing bacteria, and legume plants. Nitrogen is a crucial component in the development of plants, as it is the foundation for the production of enzymes and proteins which facilitate several metabolic reactions in plants that keep them alive. Nitrogen is also found in chlorophyll which aids in the production of energy in the form of ATP that keeps plants functioning. Although 78% of the Earth's atmosphere is composed of nitrogen, plants are incapable of accessing nitrogen from the air. Their only form of nitrogen acquisition is through the soil near their roots. Bacteria such as Rhizobium, Azotobacter, Beijerinckia, and Clostridium function as nitrogen fixing bacteria. Rhizobium form symbiotic relationships with legume plants, fixing nitrogen for the roots and aiding in legume growth and development while in return the roots form nodules to house the Rhizobia making this a mutualistic symbiotic relationship. Without Rhizobia, the population of earth would not have legumes such as beans, peas, soy, and clover. As of right now, there is limited information on the impact and importance of Rhizobium in legume development.

This study aims to identify the presence of nitrogen-fixing bacteria in different locations in Edison, NJ. I hypothesize that the front yard location will have a higher concentration of nitrogen fixing bacteria due to the large number of animals that pass through this area on a daily basis dropping nutritional waste as well as the benefits of decomposed plants that once occupied the soil.

## MATERIALS AND METHODS

This experiment was conducted by taking soil from two different locations, the front yard and backyard. This soil was potted into two different pots and labeled. A third pot was filled with extra soil from the front yard and mixed with an inoculant that contained all the necessary bacteria to grow healthy legumes. This third pot was labeled as the positive control. After potting the plants, a rake was used to put fifteen holes in the soil. Legume seeds were planted in these holes. In the positive control pot, an additional pinch of inoculant was added in each hole before the seeds were planted. The pots were then thoroughly watered with filtered water and placed in a sunny area indoors. These plants

were watered daily. The legumes were closely monitored over the course of four weeks post planting. The number of sprouts per pot, shoot height, color, and nodule growth were all taken account of in an e-lab notebook.

During week four post planting, the research included a study of Rhizobia bacteria colony growth. To study this, root nodules from all three pots were extraded and washed in bleach and water to thoroughly clean. They were then crushed into a liquid in a tube with water. Using a cotton swab, this liquid was spread on agar media plates to facfiloiktae colony growth. These plates were closely observed for the next 48-72 hours. Observations of the agar plates were also recorded in the e-lab notebook.

**RESULTS**

Table 1 summarizes the data collected at week 4 of experimentation. Height, development of nodules on the roots, and assessment of the agar plate for growth of rhizobia and non-nitrogen-fixing bacteria were all collected.

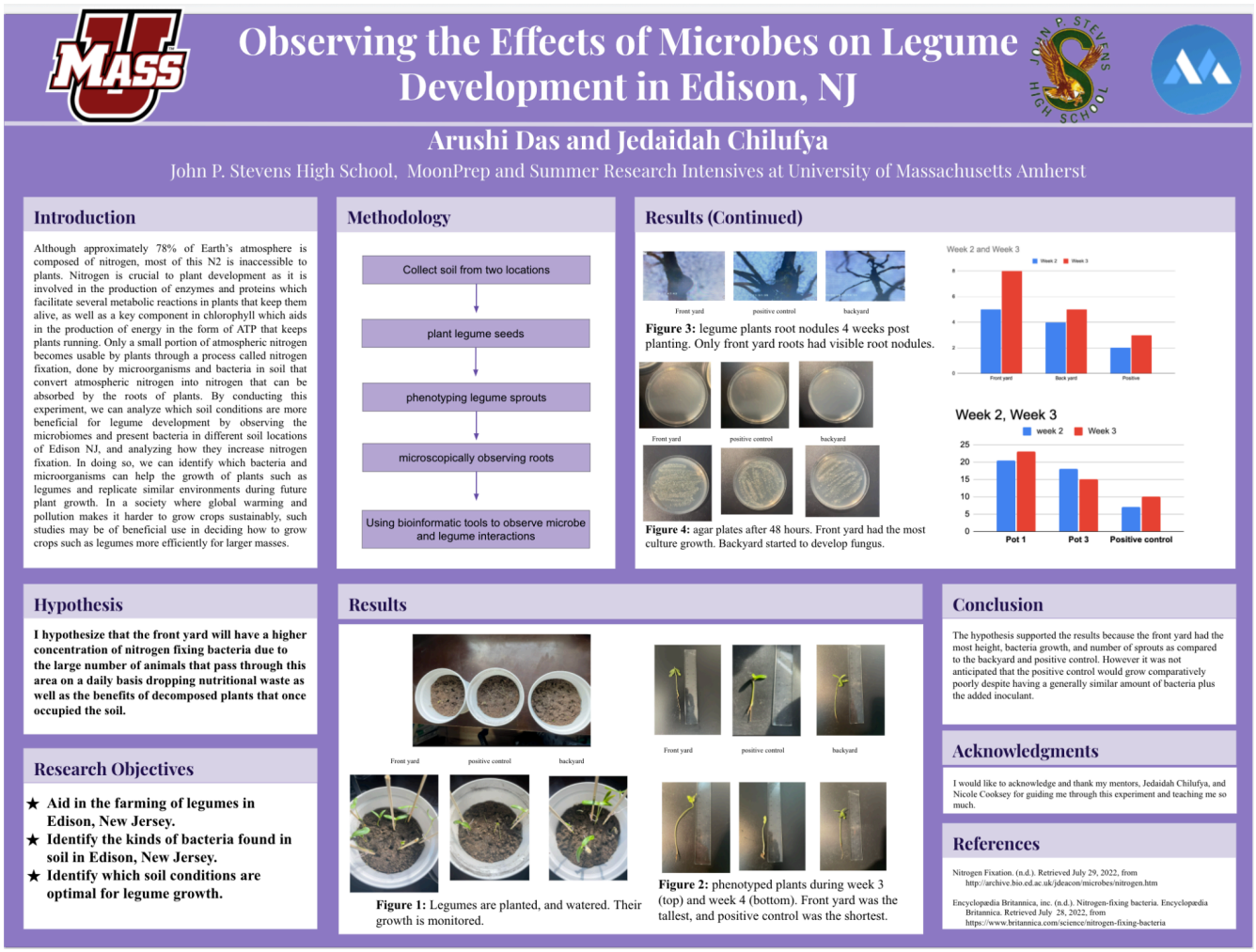
Table 1. Week 4 phenotype comparison of plants grow in backyard, front yard, and positive control soil

| | Plant height (cm) | Nodules Present | Bacteria Colonies Present |
|---|---|---|---|
| Backyard | 11 | no | no |
| Front Yard | 12 | yes | yes |
| Positive Control | 8 | no | no |

**DISCUSSION**

Although the results supported the hypothesis, it was unforeseen that the positive control would have the shortest height and bacteria growth. This demonstrates that even if legume seeds are predisposed to healthy nitrogen fixing bacteria such as those found in the inoculant, they may not thrive as expected leading to the inference that there must be other contributing factors that were not taken into account. To maximize the reliability of this experiment, these other factors need to be considered and controlled to ensure that the presence of bacteria in the soil, with and without the inoculant, is the only variable. Such factors to consider might include providing the exact same amount of water to each pot, ensuring that each pot receives equal sunlight, and making sure that the soil is taken from the same level in the ground to make sure one soil is not more nutrient dense. Other factors that could affect the quality of the experiment include human error during depotting. It is possible that root nodules were not found in the depotted plants from the backyard and positive control pot because they were detached from the roots during extraction.

This experiment should also be repeated several times throughout New Jersey. The outcomes of these experiments should be compared to this one to better understand them and form more dependable results. Such data can aid in the farming of legume plants in New Jersey by identifying what soil conditions and microbes are best suited for legume growth.

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Arya Sreenivas

**Microbial Interactions with Clotalaria Juncea Plants in Flower Mound, TX**

## INTRODUCTION

Microbes reside everywhere that sustains life. They could be in the human body, the water vapor in the air, or they could even live in the soil. Within the soil, microbes seek other organisms to latch onto in the hopes of forming a connection. Legumes are an interesting set of organisms as they need to form a bond with nitrifying bacteria, specifically Rhizobia, in order to thrive. The legume when famished for nitrogen releases signals to draw the rhizobia in and bond with it (Concha & Doerner, 2020). The mutualistic relationship arises as bacteria turns the nitrogen in the soil into ammonia for the plant, and the plant in return supplies carbon to the bacteria (Westhoek et al., 2017). The main goal of this study is to determine which soil in Mound, Texas contains the rhizobia catered to specific legume plants using the observance of nodules, their colors, and their presence in the roots.

I hypothesize that the formation of nodules will be greater in location 2 than in location 1 due to the abundance of shrubbery in the area hinting at the possibilities of rhizobia in the soil. Since the positive control has an inoculant it is reasonable to say that there will be some nodule formation.

## MATERIALS AND METHODS

In the preparation week one, soil was collected and the Clotalaria juncea seeds were added. Three pots were taken and labeled with the location in Flower Mound TX; they were collected in the Backyard (1) and at the High School (2). The third pot was labeled positive control. The third pot was filled with soil from location 1 and commercial inoculant [consisting of Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and R. leguminosarum biovar vicae] was added. Seeds were placed in the soil of each pot.

In preparation week 2, I observed germination of the plants combined with watering the soil. Every other day from the planting day, the pots were watered. Excess water was removed from the bottom. Any signs of growth were noted from day to day. If there was a lack of growth in one of the pots, additional seeds were added to the soil. Commercial inoculant was spread once more in the positive control pot, and then was watered. If there were still no plants, more Crotalaria juncea seeds were grown in a bowl of water, which was cleaned out every day, until they sprouted, and then they were placed in pots that did not have at least one plant.

In week 2, the phenotyping of the plant shoots commenced. Starting with the pot from location 1, the tallest plant was carefully dug out using fingers to push away dirt around the shoots and roots. Once the plant was pulled out of the soil, it was placed in a tupperware container filled with water, and lightly shaken to rid the roots of any soil. The plant was dried with the aid of a paper towel by gently tapping it on the roots. Measurements of the shoot and root were taken, and the plant was returned to the soil, being filled shortly after. The same was done for location 2 and positive control pot.

In week 3, the plants' second shoot phenotyping took place, and there was also root phenotyping by observing them under a digital microscope. Moreover, bacteria from the plants' roots were grown. The tallest plants from each location were dug out. The plants were gently cleaned in the water until there was little to no soil left on the roots. The plant was dried using paper towels. Then the

shoot lengths were measured once more, and the roots were phenotype by identifying any nodules that appeared using a microscope (photos were taken). The plants were set aside and bacteria isolation was prepared. Five microcentrifuge tubes were taken and labeled with: bleach, water, location 1, location 2, and positive control. The bleach tube was filled to 0.1 mL of 7.5% bleach, and filled with water until the 2.0 mL mark. Using the scalpel, a segment of the roots were cut off, preferably with nodules. The roots were placed in the diluted bleach tube, and shaken 5 times. The roots were then taken and placed on a disinfectant wipe. The roots were then moved to the water tube, and shaken 10 times. The roots were again placed on a wipe, and then put inside their respective microcentrifuge tube. Using a plastic pestle, the roots were pushed all the way to the bottom tube and ground up until they became very small particles. The tubes were then filled with water to the 0.5 mL mark. Petri dishes were taken and the contents of the microcentrifuge tubes were emptied onto it. Using a q-tip, the root pieces were spread across the agar. Once the plates were fully dried, they were flipped and a lid was put over each, marked with the location, the date was added, and the initials of the one who did the task. The petri dishes were wrapped in aluminum foil and placed on the window sill.

In week four, the BLAST database was used to compare the prevalent bacteria in my current location with ones found in the inoculant.

**RESULTS**

Two days after the planting, one sprout was observed in the location 2 pot. The next day another sprout emerged from the same pot, and a sprout was beginning to surface in teh location 1 pot . The day after, the 2 sprouts in the location 2 pot were standing tall, and the other sprout had fully emerged. The positive control pot had no signs of emergence, so additional seeds were planted. However, 1 of 2 sprouts in the location 2 pot had snapped at two points. The rest of the plants continued growing. In week one, the germination of the plants was still being observed. By this time, the location 1 pot had three plants, and the location 2 plant had two plants, one of which was the snapped one. There was no emergence of sprouts in the positive control pot, so additional seeds that had already germinated were placed in the soil. In week two, the plant shoots were measured and recorded in a graph as shown in Figure 3. The location 1 pot maintained the three plants it had. The location 2 pot at this point had one plant because the other was decaying. The positive control pot had four new plants emerge. In week 3, the second shoot phenotyping was done as well as root phenotyping under the microscope. The data for the shoot is conveyed in Figure 2 & 3 and the roots in Figure 4. The location 1 root by far had the most prevalent round white nodules. The location 2 did not produce any visible nodules. The positive control had a few round white nodules. Additionally the roots, if not nodules, were isolated and placed in general media to observe bacteria content in the soil. Day 0 had no signs of growth for any of the media. On day 2, however, there were numerous forms of microbes. There were types ranging from round and irregular for typical bacteria, and there were also filamentous hinting at fungi in the media. This was all the same except for the positive control, which consisted of only round and irregular shapes. These petri dishes can be observed in Figure 5. In week four, the bacteria Bacillus subtilis which is common in North Texas and Oklahoma was compared to Rhizobium leguminosarum biovar phaseoli, and according to Figure 6 there was no significant similarities between the two bacteria.

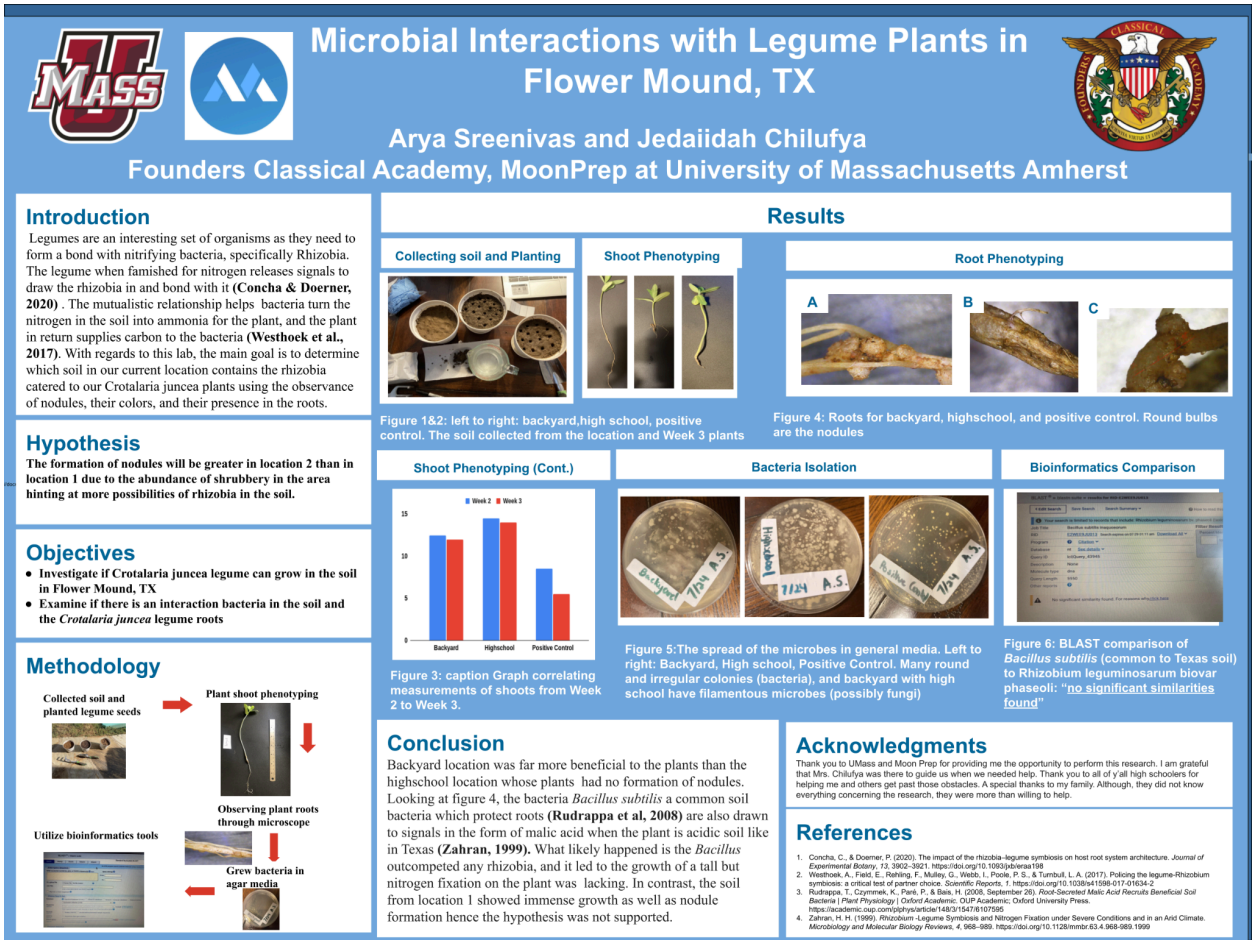
**DISCUSSION**

Unexpectedly, location 2 showed no signs of a mutualistic relationship through nodule formation. Instead location 1 and the positive control showed nodule growth, location 1 more than the positive control. A probable reason is the pH of the soil in Texas; it tends

to be more acidic, which is dangerous for the Crotalaria juncea plants. The bacteria Bacillus subtilis, a common soil bacteria in Texas, protects legume roots (Rudrappa et al., 2008). They are drawn to signals in the form of malic acid when the plant is in acidic soil (Zahran, 1999). The Crotalaria likely produced malic acid due to the high pH, and the incoming Bacillus subtilis likely outcompeted the rhizobia in the location 2 soil. In the location 1 and positive control symbiosis, it seems that there were plenty of rhizobia, which created the nodules. However, the white nodules indicate that the relationship has not been sealed, surely due to the pH; if the legume did indeed send out signals for the Bacillus subtilis, the bacteria must have not been able to reach the Crotalaria having been outcompeted by the rhizobia. Since it was unable to come in contact with the roots, the pH damaged the roots preventing the mutualistic relationship from cementing itself.

It is good to remark on the bacteria growth in the petri dishes. It went as expected since there was always going to be microbes in the roots as long as they lived. The one problem with that is that it is the general media and being so allows microbes other than rhizobia to grow.

In future experiments, it would be helpful to maintain the optimum pH for symbiosis between the rhizobia and the Crotalaria juncea. None of the soils were perfect, but this is something we can work off of. The plants grew, and lived their full lives, so the problem of nitrogen deprivation was not there. It could be interesting to study how long the legumes can go without requiring nitrogen. Are there ways to make the rhizobia that is specific to Crotalaria adapt to the weather in Texas. Improving the pH conditions of the soil in Texas will be better for growing different types of crops, specifically legumes they may not have been able to grow. Texas has clay soil which is great for water retention, but it is very difficult for plants to break their roots through it. It could be applied to a location where the soil is less compact; adding on, what about soil with a higher pH?

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Catherine Campos

# Rhizobial Bacteria Affecting Crotalaria Juncea Seeds Planted in Norwich, CT

**ABSTRACT**
Bacteria, specifically rhizobia, often interact in a symbiotic relationship with plants and their roots. The bacteria reside in nodules, and give the plant nitrogen, while the plant gives the bacteria carbon and sugars. The purpose of this experiment was to observe how the rhizobial bacteria interacted and affected the crotalaria juncea plants and roots, specifically when planted in soil from Norwich, CT. The experiment was conducted by planting seeds in three different pots with soil collected from two locations. One of the three pots contained the commercial inoculant to act as the positive control of the experiment. Once planted, plants were observed daily, watered, and phenotyped when ready.

**INTRODUCTION**
Many bacteria live in the soil, and often interact with a plant's roots (Wang, 1970). Rhizobial bacteria is a common bacteria found on the plant's roots in what are called nodules. From their "homes" in nodules, bacteria take nitrogen that had dissipated from the air and into the soil, and transform it into nitrogen that the plant can use to survive. In return, the plant provides the bacteria with protection, and useful resources such as carbon and sugars (Lindström & Mousavi, 2020). In this experiment, it was asked how rhizobial bacteria, if any, would affect plants and their roots, specifically in soil collected from Norwich, CT. Legumes, specifically crotalaria juncea, were each planted in two pots with soil from two different locations. A third pot was also used, with soil from the same location as one of the other two, but containing the commercial inoculant to act as the positive control. One plant from each pot was phenotyped over the course of two weeks in order to answer the question of whether or not nodules were present on the roots of the plants, and if so, how they affected the growth and life of the plants when planted in soil from Norwich, CT.

**MATERIALS AND METHODS**
This experiment was conducted by first collecting soil from two different locations and putting them in three different pots. Each pot was labeled with the location of the date, and initials. One pot contained the commercial inoculant, and was therefore the positive control. After the soil was collected, about fifteen seeds were planted in each pot. The pots were watered and monitored daily for two weeks. At two weeks post planting, one plant from each pot was carefully removed from the soil, and phenotyped for shoot height. Afterwards, the plants were carefully put back into their pots. Between weeks two and three, plants were monitored and watered as needed. Also, some plants needed to be transferred from one pot to another, and in order to do that, first the soil had to be watered with excess water. Then, the plant was carefully taken out of one pot, and placed in clean water. Once the plant's roots were clean, it was placed in the new location, its roots were covered with soil, and it was watered. At three weeks post planting, the plants were again removed and phenotyped, however this time both shoots and roots were examined under the microscope. After the plants were phenotyped, their roots were cut off using a scalpel. The roots were then sterilized with a bleach and water solution, and then they were cleaned in pure water. Afterwards, the roots were crushed in a tube and were spread over media in petri dishes. The plants were returned to their soil, and the Petri dishes were wrapped in foil and were monitored daily for any bacteria growth.

## RESULTS

Three weeks post planting, the front yard shoot was 11.4 cm tall, and four weeks post planting, it slightly shrunk to 10.5 cm. The root length of the plant from the front yard pot was 1 cm, four weeks post planting, and there were not any nodules apparent on the roots. The plant from the backyard had a shoot height of 11.3 cm three weeks post planting and 11.5 cm four weeks post planting. The backyard's plant root length was 5 cm four weeks after planting, and did have some small, white nodules on the roots. The plant from the positive control pot, containing the commercial inoculant, had a shoot height of 6.3 cm three weeks post planting, and 15.6 cm four weeks post planting. The root length for the positive control plant was 2.9 cm four weeks after planting, and did not have any nodules on the roots. Despite the lack of nodules on the majority of the plants, there were numerous bacteria colonies thriving and growing rapidly in all three of the petri dishes with roots from all three locations.

## TABLES, GRAPHS and PHOTOS

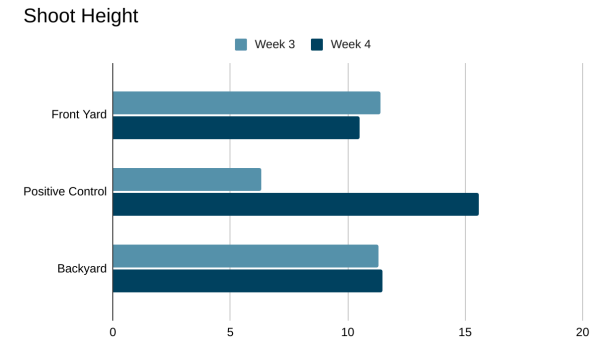



Table 1. Shoot height in all three locations three and four weeks post planting.

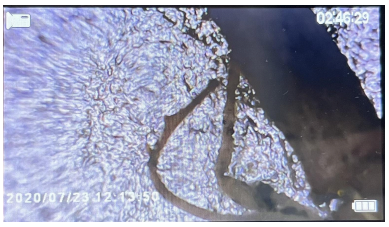


Image 1. Small, white nodules on the roots of the plant from the backyard.

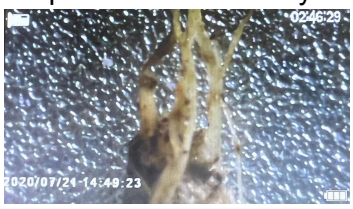


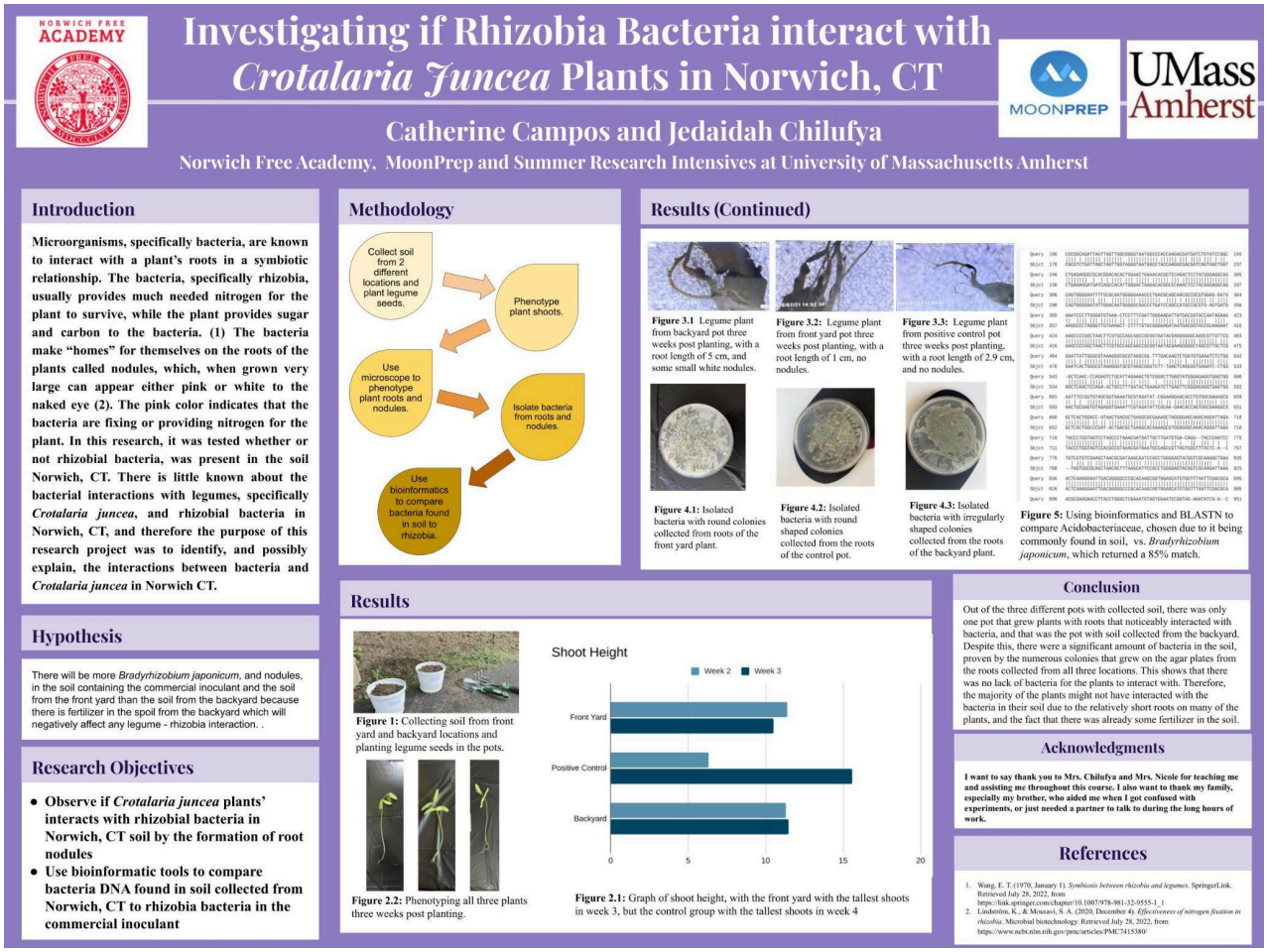
Image 2. Very short roots of the plant from the front yard, with no nodules apparent.


Image 3. Legume plant from positive control pot three weeks post planting, with a shoot height of 15.6 cm.

## DISCUSSION

This experiment was conducted in order to see if rhizobial bacteria, if any, affected the growth and life of crotalaria juncea plants when planted in soil collected from Norwich,CT. Surprisingly, despite growing to a relatively good height, there were no nodules present in the front yard pots. This could be because the roots were relatively short, and they never grew long enough for the bacteria to interact with the plant in order to

form the symbiotic relationship. Furthermore, the plant from the positive control showed no signs of nodules either, despite having the commercial inoculant. The soil for that pot was collected from the front yard as well, and, similar to the plants from the front yard with no commercial inoculant, the roots were very short. For this reason, the initial communication between the bacteria and the plant may have never occurred. Despite these results on the roots, however, the media collected from the roots of the two plants does reflect that there were numerous bacteria living in the soil, and the lack of nodules was not because there was a lack of bacteria. In contrast to the results from the front yard and positive control pots, the backyard roots did show some small white nodules. The color of the nodules reflect that the nitrogen transfer was no longer occurring between bacteria and the plant, and the size shows that there was only a small amount of bacteria living there. The difference between these roots from the backyard is that they did grow to be substantially longer than the front yard roots, and therefore had a higher chance of being able to interact with the bacteria.. Furthermore, despite showing signs of nodules, the growth in bacteria colonies in the media for the backyard roots, was relatively the same with that of the front yard and control plants. These results further provoke the question of bacteria in the soil. Is there a certain length, perhaps, that roots need to reach to communicate with bacteria? Or was there some other cause? Why were the roots so short on the plants with soil collected from the front yard compared to the backyard? Further research will help us understand the nuances of how bacteria interact with plant roots, and how we could possibly mimic those perfect conditions to aid our own plant growth in everyday life.

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Diya Sammanna

# Observing the Interaction Between Legumes and Rhizobia in Cupertino, California

**ABSTRACT**

The purpose of this experiment was to observe the symbiotic relationship between legumes and rhizobia bacteria in the soil. In order to do this, soil was filled up from two different locations and legume seeds were planted in three different pots. From three weeks post planting, the plant shoots and roots were phenotyped using the naked-eye and a digital microscope. The rhizobia were isolated from the root nodules and placed into media plates in order for the bacteria to grow. Bioinformatics was used in order to explain the symbiotic relationship between legumes and rhizobia. It was found that the positive control contained small white nodules and the backyard contained no nodules. In addition, the park contained one medium-sized pink nodule. When observing growth of bacteria on media plates, it was found that all three plates contained colonies that were round, entire, and convex. Additionally, when phenotyping plant shoots, it was observed that the legumes in the positive control and park had growth between the two phenotyping sessions (10.5cm - 18.5cm, 17.5cm - 21cm respectively). But, the legumes from the backyard did not have growth between the two phenotyping sessions, instead there was a small decrease in the height (12.5cm - 11cm).

**INTRODUCTION**

The goal of this experiment was to observe whether the interaction between sunn hemp and rhizobia would occur in Cupertino, California. Rhizobia provide nitrogen in a form that legumes can access and the rhizobia also benefit from interaction with the legumes (Masson-Boivin, 2018). There are millions of microbes present in the soil and in order for symbiosis to happen, the plants and rhizobia The plant was then rinsed in a tupperware of clean water in order to remove any soil from the roots. A paper towel was used to gently placed back and covered up with soil. This was repeated with the other two pots and the results were compared.

At four weeks post planting, the plant shoots and roots were phenotyped once again. The same procedure was followed in order to phenotype the shoots, using one plant per pot. In order to phenotype the plant roots, using a phone, first a photo was taken of the roots zoomed in. The purpose of this was to be able to view the root nodules and the roots of the plant more clearly. Then, the plant was placed under a digital microscope in order to get an even more zoomed in view of the roots and root nodules. The coarse focus knob and fine focus knob were used in order to get a clear image of the roots and root nodules. This process was repeated for the other two legumes. Furthermore, in order to determine which location had the most interaction with rhizobia, we isolated the bacteria in the roots and root nodules onto media plates. In order to do this, bleach with 7% active ingredient was diluted. A microcentrifuge tube was filled up with bleach up to the 0.1 mL mark and labeled with the location name. Another microcentrifuge tube was filled up with water until the 1 mL mark. The two tubes were combined and shaken well in order to dilute the bleach. One more microcentrifuge tube was filled up with water until the 1.5 mL mark. The plant was gently taken out of the soil and excess soil was cleaned off by rinsing the roots in water. Then, a scalpel was used to cut off the roots and root nodules. This was placed in the tube with the diluted bleach and turned upside down five times. Then, the roots and root nodules were patted dry with a paper towel and placed into the tube with 1.5 mL of water. The tube was turned upside down ten times and placed in a new fresh centrifuge tube with a label of the location name. A plastic pestle was used to crush the roots and the root nodules and once this was

complete water was added up to the 0.5 mL mark. This solution was shaken in order to dilute the roots and root nodules with the water.

Finally, a swab was used to spread the mixture of roots and water onto the media plates labeled with location, date, and initials. The solution was poured onto the plate and the swab was used to spread the mixture around. This process of isolating rhizobia bacteria was repeated for the other two locations. An image was taken of the plates which served as day zero. The media plates were wrapped in aluminum foil and placed by the window. Every day, the plates were observed and pictures were taken of them until bacteria had significantly grown. After this, the plates were disposed of safely. Bioinformatics was used in order to explain our results, for instance, the reason that some plants had white nodules, while others had pink nodules, and some had no nodules at all. After obtaining the genetic sequence of the microbe that caused no nodules to form, it was put into a blast search and compared against a bacteria from the commercial inoculant such as bradyrhizobium. The software told us how similar the bacteria was to bradyrhizobium and showed us the sequence alignment for the sequence of the microbe versus bradyrhizobium. This was repeated for the other two situations of white nodules and pink nodules. The results were analyzed and compared with each other in order to understand how this affected our study.

The materials needed in order to complete this whole process are three pots, drainage trays, shovel, gardening fork, gloves, sterilization wipes, tupperware, water, legume seeds, commercial inoculant, soil from two different locations, digital microscope, paper towel, black cloth, labels, iPhone, goggles, ruler, bleach, microcentrifuge tube, scalpel, media plates, swabs, sharpie, aluminum foil, and a computer.

**RESULTS**

The positive control pot had plant roots with several small, white, and round nodules. The park pot had plant roots with one round, medium-sized, and pink nodule. Finally, the backyard pot had plant roots with no visible nodules. At three weeks post planting, there were five healthy plants and two dead plants in the positive control, three healthy plants and one small plant in the backyard soil, and three healthy plants in the park soil. As more time went on, more legumes started to die and the number of plants in each pot started to decrease. In addition, the bacteria on the media plates grew within one day and all the colonies were described as round, entire, and convex. Although, the backyard and positive control had smaller colonies than the park which had more darker and spread out colonies. Bioinformatics was used to explain the results of why some plants had white nodules, pink nodules, or no nodules. By finding a microbe that could potentially cause one of the three cases, we compared its genome against a rhizobia bacteria in order to see how similar the two were. I found that the bacteria that caused no nodules was not very similar to the rhizobia family but the bacteria that caused white nodules or pink nodules was fairly similar to the rhizobia family genome.

**TABLES, GRAPHS and PHOTOS**


Images of plant shoots 3 weeks post planting of positive control (left), backyard (middle), and park (right).

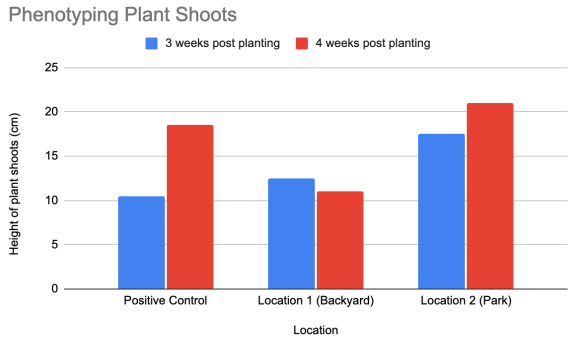
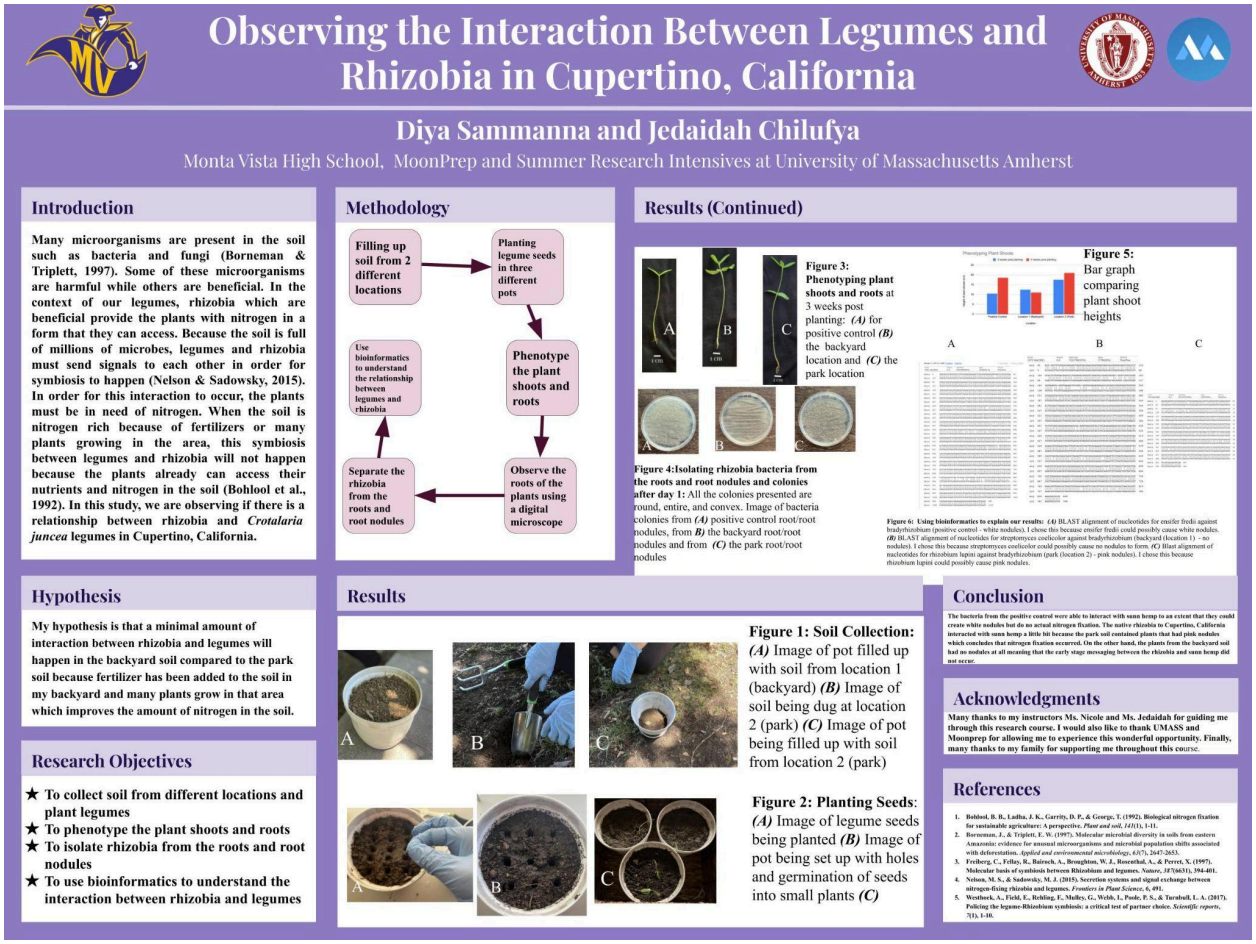



Figure 1. Comparison of plant shoot heights at 3 weeks post planting and 4 weeks post planting

DISCUSSION

Because the positive control had white nodules, the rhizobia in the commercial inoculant interacted with sunn hemp to a minimal extent. The rhizobia were able to enter the roots to create nodules but never were able to fix nitrogen. There are many possible reasons for this, one being that the bacteria are not specific to sunn hemp meaning they cannot complete symbiosis. Another reason is that the soil conditions are not ideal for this interaction to occur, resulting in nitrogen fixation not occurring. The rhizobia native to Cupertino, California are also able to interact with sunn hemp to a small extent as the legume from the park soil had one pink nodule. This shows that the early stage messaging between sunn hemp and the rhizobia did occur. The rhizobia moved into the roots and nodules formed. Once nitrogen fixation occurred, and symbiosis was completed, the nodules of the plant turned pink (Gage, 2004). On the other hand, the legume from the backyard soil had no root nodules. This may be because the soil in the backyard previously had fertilizer added to it, and many plants have grown there over time as predicted in the hypothesis. This results in more nitrogen in the soil causing the soil to become nitrogen rich. When the soil is nitrogen rich, the legumes will not send signals to the rhizobia because they can already access nitrogen from the soil. This discourages symbiosis and causes no interaction to occur between the legumes and rhizobia causing no root nodules to form. Another reason for this may be that the soil is too dry and hard which makes the soil conditions not ideal for the interaction between legumes and rhizobia (Zahran, 1999). In order to explain our results, using bioinformatics, the bacteria that we compared against rhizobia could possibly be in our soil, resulting in either white nodules, pink nodules, or no nodules.

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Grace (Seo hyun) An

# Determining the Presence of Rhizobia in Legume Seeds and their Impacts on Plant Growth in Irvine, California

**ABSTRACT**
A bacteria called rhizobium is found in soil, where they persist until they may infect the roots of legume plants and help in the nitrogen fixation process in leguminous plants. It is attached to the leguminous plant's roots and grows nodules (Wang et al., 2012). These nodules capture nitrogen from the atmosphere and transform it into ammonia, which the plant can use to thrive and grow. The interaction between plants and rhizobia is symbiotic (mutually beneficial), as one organism benefits from the other and provides something in return (Wang, 1970). Rhizobia are thus among microbiology's most important bacteria that should be studied more.

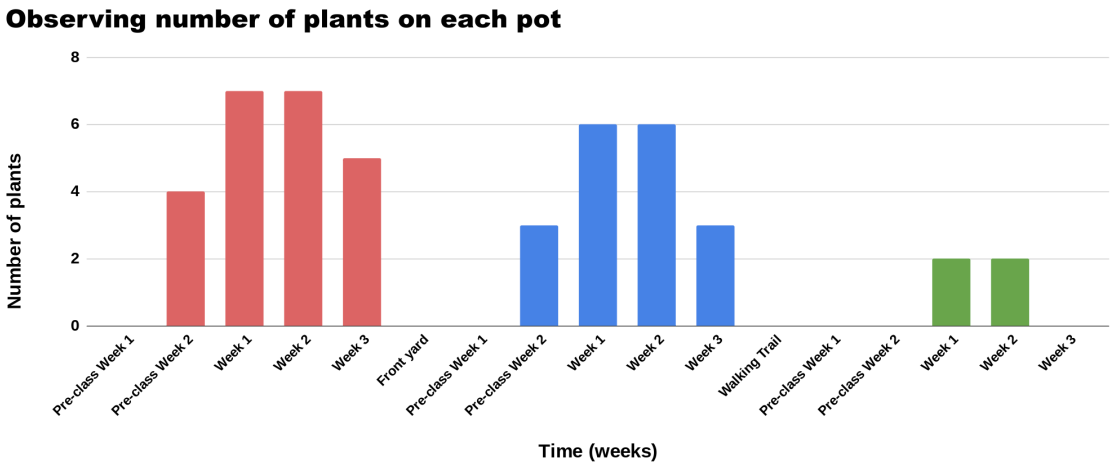
**INTRODUCTION**
In my experiment, my main focus was to determine the presence of rhizobia and its impacts on plant growth on legume seeds by following the methodology below. I hypothesized that the soil in location 1, the front yard will have more bacteria than in location 2, the walking trail, because plants and mushrooms are present in the front yard and the positive control would interact with rhizobia having the most nodules.

**MATERIALS AND METHODS**
This study consisted of a duration of six weeks with the procedure as followed: [1] Collect soil from 2 different locations, [2] Plant legume seeds on 3 pots, [3] Observe plant growth (week 2-3), [4] Phenotype legume plants shoots and roots, [5] Microscopically observe the plant roots, and [6] Isolate rhizobial bacteria from roots and root nodules.

I collected soil from 2 different locations, a front yard and a walking trail in Irvine, California. Then the legume seeds were planted on three pots using digging tools and by making holes and watering the pots. Pot 1(red) was from the front yard and was planted with the commercial inoculant, being the positive control. Pot 2 (blue) was from the front yard soil without added inoculant. Pot 3 (green) was from the walking trail. Next, those three pots were monitored specifically by their plant growth by watering the plants every other day. Then I phenotyped and made observations of the growing plant shoots and roots with the naked eye. The next week, I phenotyped and made observations of plant shoots and roots for the second time but with both the naked eyes and with the digital microscope to check for the presence of root nodules. Lastly, I isolated bacteria from the roots and grew the bacteria on agar media plates.

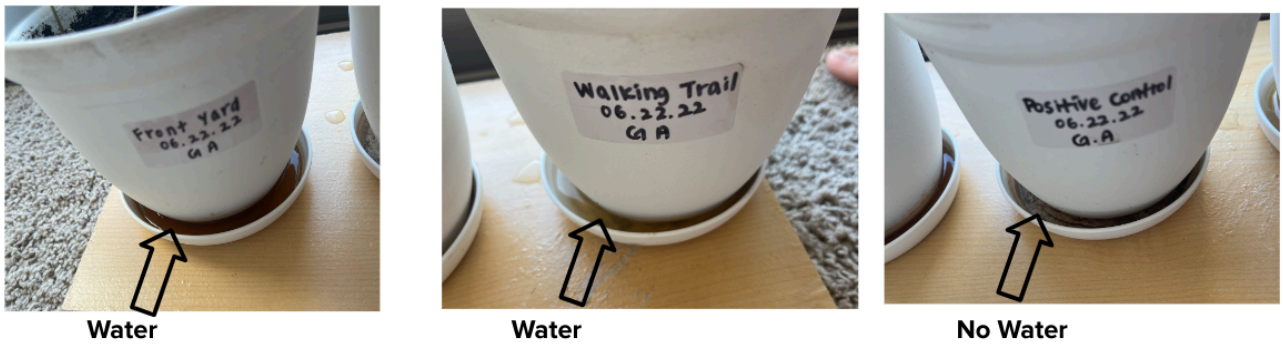
**RESULTS & TABLES, GRAPHS and PHOTOS**
Figure 1 contains growth information, number of plants grown, for each of the three pots over the course of three weeks.

*[Figure 1] Monitoring Plant Growth*
Red = Positive control / Blue = Front yard / Green = Walking Trail




As this bar graph shows, the number of plants on positive control (red) had the most growth throughout the weeks. The walking trail pot (green) had the least amount of growth, initially sprouting last and concluding with all of the plants dying by week 3.

The pictures below show the water absorbent for each pot.




Water | Water | No Water

**Monitoring how fast each pot absorb the water**

I monitored how fast each pot absorbed water. The positive control pot was able to absorb the water most successfully and the walking trail pot was absorbing the water most poorly, repelling the water.

[Figure 2] Height of plants grown in each pot at Week 2

| | Positive Control | Front yard | Walking trail |
|---|---|---|---|
| Height (cm) | 10.6 | 8.2 | 7.7 |

The photos below were from the first phenotyping experiment in Week 2.

Positive control

Front yard, Irvine, CA

Walking Trail, Irvine, CA

They all look healthy and green but can see shoot height differences, with positive control plants being the tallest and walking trail plants being the shortest. However, I was able to examine definite differences in roots. The root for positive control was the longest and thickest while the roots for the front yard and walking trail were still babies, not grown much.

[Figure 3] Height of plants grown in each pot at Week 3

| | Positive Control | Front yard | Walking trail |
|---|---|---|---|
| Height (cm) | 9.6 | 9.7 | N/A |

These photos were from the second phenotyping experiment in Week 3.

Positive control

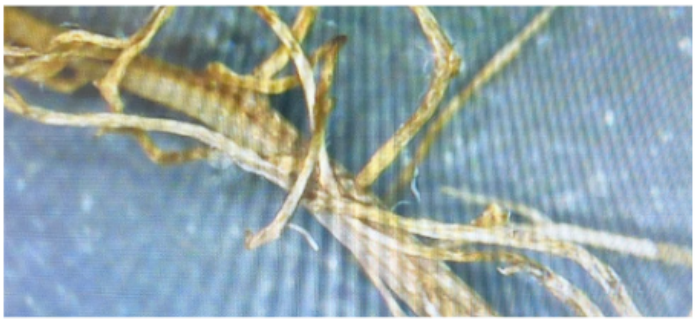
Front Yard, Irvine, CA

Both plants from the positive control and front yard groups looked healthy and green. It is important to note that the plants from the positive control pot had begun to wilt. Additionally, in the walking trail pot all plants were dead so I could not collect data. I was only able to phenotype and make observations for those two pots with the same soil, front yard.

[Figure 4] Microscopically Observe the Plant Roots
Below are photos of the roots for two pots, the positive control and the front yard microscopically phenotyped to check for the presence of root nodules.

Positive control

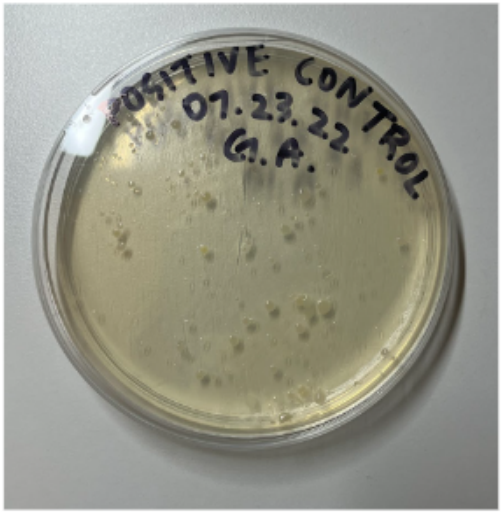
Front Yard, Irvine, CA



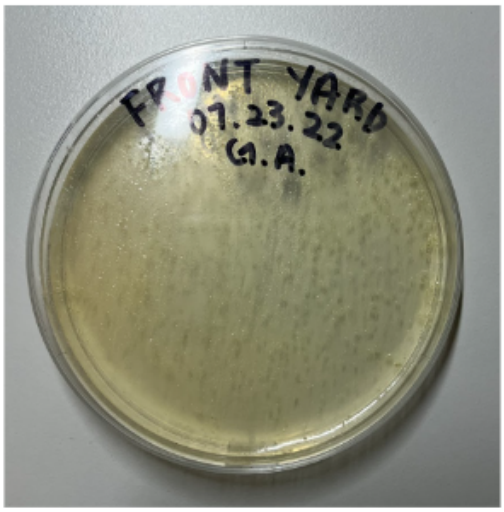
As the pictures show, both pots have no root nodules.

[Figure 5] Isolation of Rhizobia Bacteria from Roots onto Agar Media Plates
These pictures below show the agar media plate Day 1 for the positive control pot and front yard pot.

[Positive control]

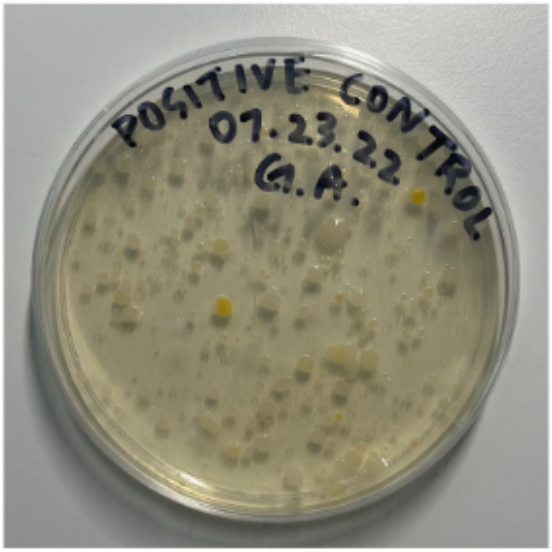



[Front Yard, Irvine, CA]

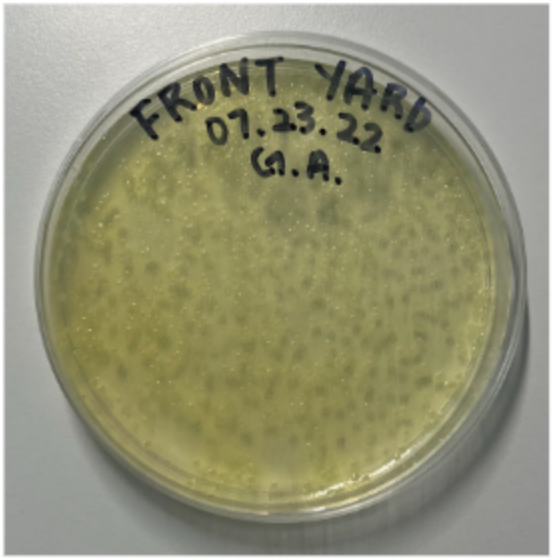



These pictures below show the agar media plate Day 2 for the positive control pot and front yard pot.

[Positive control]



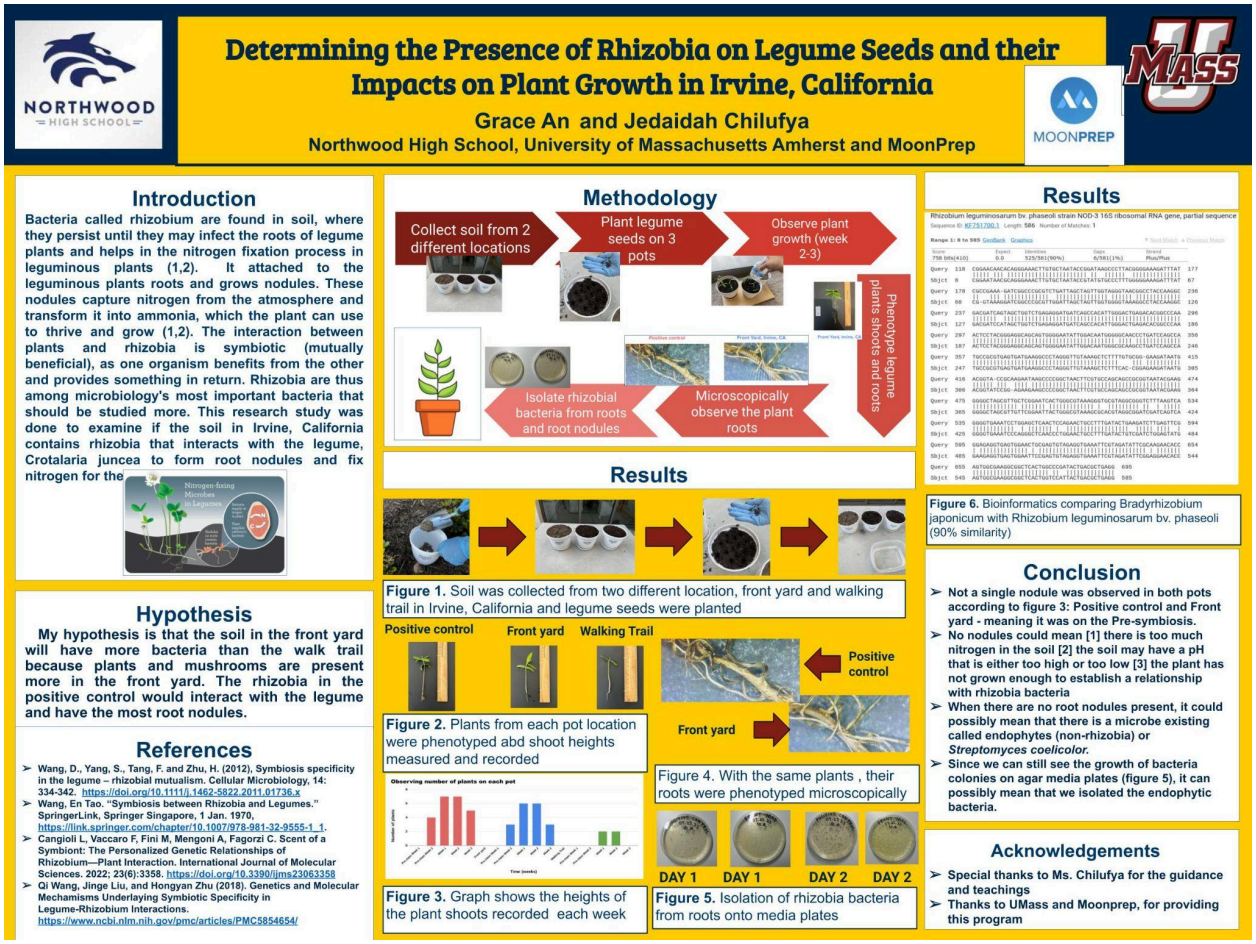

[Front Yard, Irvine, CA]




As the pictures above show, an abundant amount of bacteria colonies were observed on Day 1 and Day 2. For positive control, more bigger-sized, white-colored colonies were shown but with larger spaces from each other, and on Day 2, big-sized yellow colonies were examined. For the front yard, the whole agar media plate was more yellowish but with smaller colonies and more packed.

**DISCUSSION**

As shown in figure 1, the number of plants on the positive control pot had the most and stayed the longest, and the numbers of plants on the walking trail pot had the least and grew the slowest and could not survive longer than other pots since the soil was too heavy, muddy and dried too quickly. Not a single nodule was examined in both pots according to figure 4: Positive control and Front yard - meaning it was on the Pre-symbiosis. No nodules could mean [1] there is too much nitrogen in the soil, [2] the soil may have a pH that is either too high or too low, or [3] the plant has not grown enough to establish a relationship with rhizobia bacteria. When there are no root nodules present, it could possibly mean that there is a microbe existing called endophytes(non-rhizobia) or Streptomyces coelicolor. Since we can still see the growth of bacteria colonies on agar media plates (figure 5), it can possibly mean that we isolated the endophytic bacteria.

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Gyan Malani

# Soils Effect on Legume Seedling Development and Rhizobium Variety Appearance in Beaumont, TX

**ABSTRACT**

There are a variety of microorganisms that live in the soil beneath us. Rhizobia, a type of microbe, interacts with plants in a symbiotic relationship, as they provide plants with nitrogen in the form of ammonia and receive glucose in return. We investigated how soil type affects legume shoot height as well as the growth of rhizobium bacteria. In our study, we planted legumes and phenotyped their shoots and roots. Then, we microscopically observed the legume roots. Finally, we isolated the rhizobial bacteria from the legume root nodules and used bioinformatic tools to explain the plant-microbe interactions. Our results indicated that the Patio soil (devoid of commercial inoculant with organic material present from tomato and pepper plants) had a variety of beneficial rhizobia that assisted with legume height development and with root nodule production, much more than the Backyard soil (devoid of commercial inoculant with grasses and weeds present). Additionally, commercial inoculant, made up of Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and R. leguminosarum biovar viceae, proved to be the most significant factor in plant shoot height. Based on the results, we concluded the production of white root nodules to possibly be as a result of a type of rhizobia within the soil known as Ensifer adhaeren. We recommend that future studies be conducted on much larger scales or with a greater variety of soils, as this research has many future uses in farming practices and crop growth.

**INTRODUCTION**

Sunn hemp, scientifically known as Crotalaria juncea, is a legume variety widely believed to have originated in India and have tropical Asian roots (Narasimhulu, 2018). They, like most other plants, have a respiratory system for growth and sustenance, often relying on the assistance of bacteria. More specifically here, legumes primarily depend on the use of Rhizobium bacteria for the process of nitrogen fixation, where a mutualistic symbiotic relationship is formed between plant and bacterium to assist with the plant's metabolic processes (Blanco, 2016). The legume (Crotalaria juncea) provides carbon in the form of C4-dicarboxylic acids to the rhizobia, and the rhizobium within the root nodules of the sunn hemp in return releases nitrogen so that the plant can use it in the form of amino acids (Wang, 2019). Similar to the legume-rhizobia interactions, a study has been conducted in the past within Beaumont, Texas regarding how soil type affected the amount of ammonium produced. It found partial increases in ammonium levels in Beaumont soils versus Nada soil (Chen, 1985). Another study looked at root nodules in soybean production areas within the United States and quantified the amounts of hydrogen released by rhizobia (Lim, 1981). However, there is little current evidence of plant-microbe interactions within the Beaumont, Texas area. Thus, this research project was carried out to investigate the effect of soil type on legume development and rhizobia appearance in Beaumont. We hypothesized that if soil is collected from an area with various types of plants, such as pepper plants and tomato plants, it will contain a greater number and variety of rhizobia as well as a greater plant shoot height when compared to an area of mushy soil with just weeds, presumably because of the nutritious bacteria present in the former soil.

## MATERIALS AND METHODS

*Week One:*

To begin the experimentation process, soil was collected from two different locations, a backyard and patio located at 5260 Merlot Drive, Beaumont, Texas. Three 6-inch pots and trays were used to collect the soil, and were respectively labeled Backyard, Patio, and Positive Control, which was used as a guaranteed comparison factor for legume and rhizobia growth. Using a shovel and fork, soil was collected from both locations and placed into the pots labeled Backyard and Patio. Soil from the backyard location (chosen at random using a coin toss between the backyard and patio locations) was placed into the Positive Control pot. The pots were placed in a lab space and filled to the brim with water. However, because the soil was already damp from previous precipitations, much of the water seeped through the bottom of the pots. Excess soil and water were removed from each individual pot. Next, came the procedure of planting the sunn hemp. After sterilizing the fork with 70% ethanol, it was used to make fifteen individual holes in the soil of each of the three pots. The Positive Control pot received a small dosage of commercial inoculant within each of the holes before the planting of the legume seeds to help assist with their growth. Finally, the legume seeds were placed into each of the individual holes within each pot, and excess soil and water were removed once again. The pots were watered every other day due to the soil being damp and plant growth was monitored.

*Week Two:*

By seven-days post planting, each pot had less than three germinating sprouts, so seven more seeds were planted in each pot as well as additional commercial inoculant in the Positive Control pot. We observed the wilting of the sprouts eight-days post planting, and decided to use dead sprouts as natural fertilizer and ten-days post planting, the location of the pots was moved to an area with more direct sunlight yet shade over the head. Six more seeds were planted nine-days post planting, and observation of germination continued.

*Week Three:*

Growth of legume sprouts was continually recorded and soil was watered every other day. However, the weight of many of the sprouts began to exceed its own limits, causing the sprout to droop. To help support the weight of the plant, plastic straws were inserted within each pot and tied to the sprouts. Significant growth of the sprouts could be immediately observed after the insertion of the straws.

*Week Four:*

The process of observing the plant shoot (measuring height from roots to leaves) began. First, the soil was softened through the addition of water. Using gloves, the tallest sprout from one of the pots was cautiously dug out with the roots intact. The sprout was placed into a tupperware of water to wash off the soil from the plant roots and stem, and tapped dry with a paper towel. It was then placed onto a black background next to a forty-six centimeter ruler and label indicating the pot it was taken from. A camera was used to take a photo of the sprout, ruler below it, and location label next to it. This process to observe the plant shoot height (phenotyping the plant shoots) was repeated for each of the other pots.

*Week Five:*

Phenotyping of the plant shoots in Week 4 was repeated in the exact same manner during this week. However, additional phenotyping was completed with the roots (recording structure and color of the roots as well as presence of root nodules). After phenotyping each plant shoot, the roots of each sprout were then placed onto the stage of a digital microscope in order to record the presence of root nodules in each plant. The roots were then used for the isolation of bacteria that could be grown onto agar media plates (culture). The roots of a sprout from each pot that were already used in the process of phenotyping were cut off from the

sprout using a scalpel. They were placed individually into a 1.5 mL microcentrifuge tube that held diluted bleach (0.2 ml 7.55% concentrated NaOCl clorox bleach with water dilution up to the 1.0 ml mark). Sterilization of the roots began as the microcentrifuge tube was turned one-hundred eighty degrees five times. The roots were then transferred from the diluted bleach microcentrifuge tube into a tube with just water using a scalpel. The tube was turned one-hundred eighty degrees ten times to assist with washing off the bleach. The roots were transferred again into a new microcentrifuge tube, where a plastic pestle was used to repeatedly crush and grind the roots into a paste-like structure. Pure water was added to the tube (0.5 ml) to create a suspension that was then poured out onto agar, or Yeast Extract Media Colony Red (YEMCR), media plates. Using a cotton swab, the suspension was spread all over the petri dish, and once it was dry, was turned upside down to take a photo for recording purposes. The plate was wrapped in aluminum foil and placed near a window of around 26.7 degrees celsius. This process was completed with one sprout from each pot, and observation of the bacterial colony growth began two-days post isolation.

*Week Six:*
A bioinformatics software was used to explain our results of white nodules only being present. Research was completed to find a specific type of bacteria that may be associated with this specific scenario. Using the BacDive database and Fast Alignment (FASTA) software online, the genetic DNA sequence for each bacteria was found and recorded. We then opened up the Basic Local Alignment Search Tool (BLAST) software online, where the DNA sequence was placed into the nucleotide search bar and one type of rhizobia, from the four types of rhizobia that make up the commercial inoculant, was placed into the organism section of the page. After BLASTing the two organisms, similarities between the organisms' DNA sequences were recorded. This process was completed to understand the microbial interactions that may have occurred to cause white root nodules to form only.

**RESULTS**
The plant shoot heights within the different soil locations had very little variation, with a standard deviation of 1.007 cm 3 weeks post-planting and 1.044 cm 4 weeks post-planting. Additionally, the legumes with the tallest shoot height as shown by Figure 1 appeared to be from the Positive Control pot at 16.7 and 17.5 cm, contrary to our prediction of the legumes with the tallest heights being from the Patio pot (due to the predicted nutritious bacteria present there). Nevertheless, the legumes grown in the patio soil still had an overall greater mean shoot height than the backyard plants: 15.7 cm vs 15.35 cm respectively (Fig. 1). Accordingly, the Patio pot held 57.1% of the total white root nodules (pink nodules were not observed) seen through the LCD microscope, which matches our prediction of a greater number of rhizobia present within the patio soil because root nodules only form through the compatible interactions between plants and microbes (Fig. 2). When looking at the YEMCR media plates themselves, the Patio petri dish holds a variety of bacteria colonies. These include large colonies with a round and opaque structure, orange pigmented colonies that are also round and opaque in structure, and irregular colonies with an undulated-like structure. Compared to the Backyard agar plate, which only held filamentous and irregular-opaque colonies, and Positive Control dish, which held one filamentous colony, the Patio media plate holds many more colonies in variety and number.

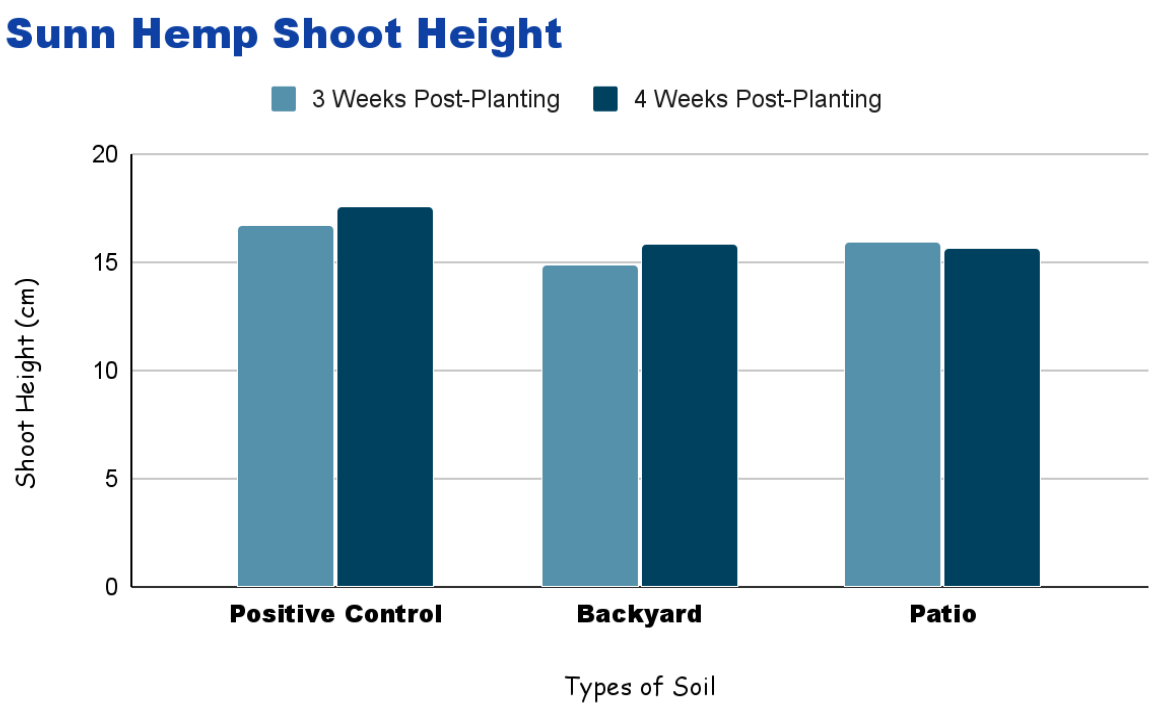



**Figure 1. Bar Graph displaying legume shoot height in three pots at two different time periods (post-planting)**

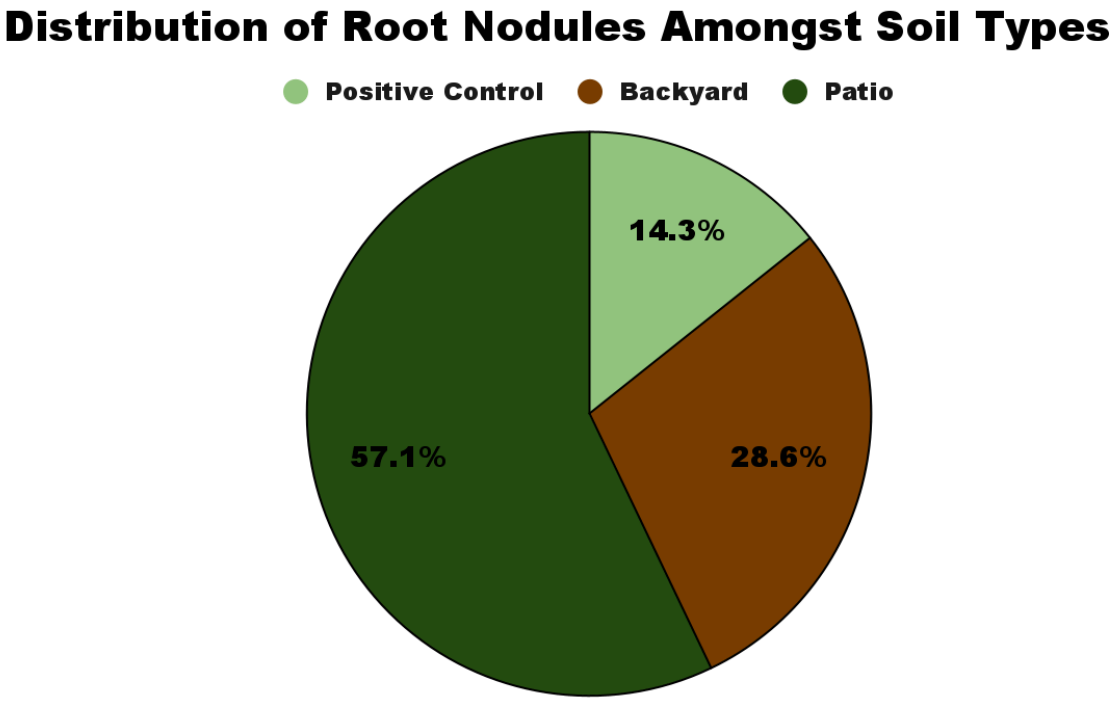


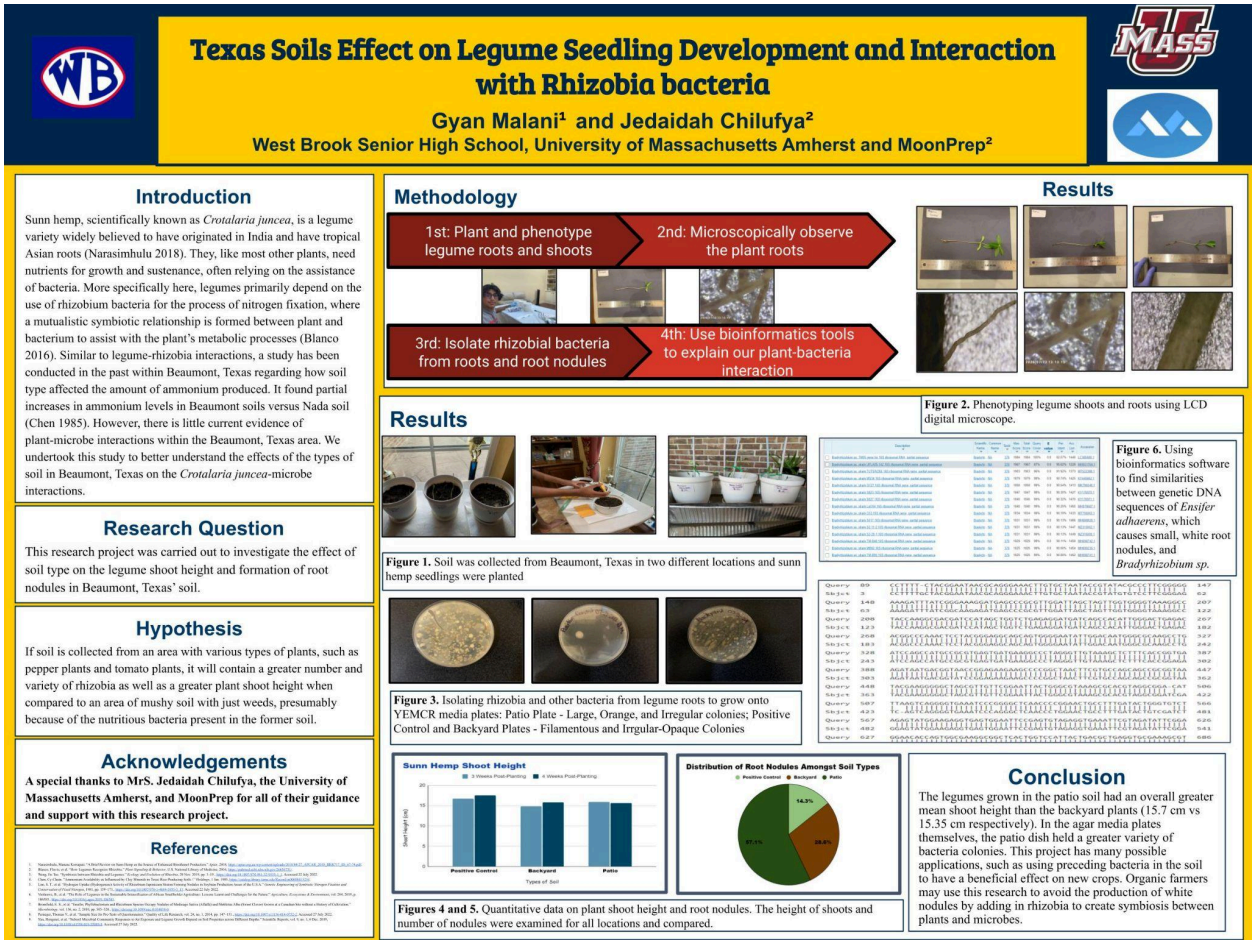

**Figure 2. Pie Chart portraying percentage of total white root nodules held by each pot**

**DISCUSSION**

Our results were contradictory to our expectations. Primarily, commercial inoculant present in the Positive Control pot substantially improves legume growth and development, possibly due to the there being four types of rhizobia (Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and R. leguminosarum biovar viceae) in the inoculant that can all interact with the sunn hemp. Although native bacterium is often believed to be more effective for plant growth, this isn't always the case. The inoculant here presumably provides more nitrogen to the plant, which is essential for its respiratory processes and thus physical growth (Vanlauwe, 2019). There is still the confounding result of only white nodules being present on the roots of the Crotalaria juncea. Root nodules form through the symbiotic relationship created between a microbe and plant. Accordingly, white root nodules are formed when bacteria are present on the roots of the plant through nodular infection but the host is not compatible with that rhizobia for a mutually beneficial interaction. We explored the possible types of bacteria that could have had this effect, coming upon Ensifer adhaerens, a rhizobia that has been proved to cause many white nodules on the roots of plants such as alfalfa and sweet clovers (Bromfield, 2010). To confirm its possible effect on the pots, we used the BLAST bioinformatics software to see the percent match between Ensifer adhaerens and a type of rhizobia present in the commercial inoculant and legumes, Bradyrhizobium sp. (Vigna). It was found to be a 95.62% identity match with an expect value of 0.0, meaning there was a very high likelihood that the Ensifer adhaerens was present alongside the rhizobia from the inoculant and plants. Thus, it can be determined that a rhizobia, likely Ensifer adhaerens, caused the formation of only white root nodules on the roots of the Crotalaria juncea in this research project.

There were some confounding variables that may have affected the results. One such variable is a small sample size. Our experiment included only three samples, yet for the results to be considered statistically significant, a sample size of at least thirty is needed (Perneger, 2014). Additionally, the amount of sunlight was not equally distributed throughout the entirety of the research process, as the soils received little daylight until ten days post-planting (pots were transported from inside shelter to outside shade). Another possible variable is randomness, as the locations for this experiment were specifically chosen and not out of chance from a designated region. Lastly, only two soil types were tested, yet there are a variety of soils within Texas that may have contrasting results on the legumes with respect to shoot height and bacteria growth (Yan, 2019).

Researchers looking at the effects of rhizobia may now understand how preceding bacteria in the soils can have a beneficial effect on new crops. Additionally, inoculants can be used for substantial plant development. Organic farmers may use this research to avoid the production of white nodules, adding in beneficial rhizobia to form a symbiotic relationship with their plants, thereby increasing growth of the plants through additional nitrogen.

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Jose Cayetano

# Observing Legume-Rhizobia Bacteria Relationship in Los Angeles, CA


## ABSTRACT

This study aims to see how rhizobia bacteria, a type of bacteria that can be found in soil, would affect the growth of the Sunn Hemp legume plant. Note that rhizobia bacteria works by supplying a plant with usable nitrogen to help it grow (Lindstrom K, 2020). In return, the legumes allow the bacteria to live within it in the form of a tumor-like structure called a root nodule (Burghardt L, 2020) This experiment tested this symbiotic relationship over the course of 5 weeks. In the end it seemed like the relationship was not symbiotic as it should be, since the rhizobia managed to nodulate the roots and live on it, but the nodules they made were all white in color, meaning that the rhizobia did not supply the Sunn Hemp plant with nitrogen for growth.


## INTRODUCTION

Varying climate has a large impact on plant growth and survival. The environment plays a key role for the growth of plants (Samiksha S, 2014) Los Angeles is a good example of an environment with varying types of climates, all for different types of plants. However climate alone is not the only factor that affects plant growth; soil type and even the bacteria within the soil have an impact. The experiment aimed to see how rhizobia bacteria would interact with the plant, and answer whether premade soil contains microbes dedicated towards plant growth, since it was described as “specifically for planting.” I hypothesized that the premade soil would result in the most legume growth compared to naturally occurring soil.

## MATERIALS AND METHODS

The materials for this experiment consisted of a bag of premade soil, pots, legume seeds, commercial inoculant, containers for water, gloves, black paper, black binder, labels, a ruler, a scalpel, 1.5ml microcentrifuge tubes, plastic pestles, and agar media dishes. During week 1 I filled all three pots with the premade soil, along with some commercial inoculant for 1 pot, making it the positive control, and then moving the soil to allow the legume seeds to be planted. After that, it was a matter of watering the plants and observing growth. This would go on until Week 4 and 5, which is when I removed 1 plant from all three pots for phenotyping. For phenotyping I measured the shoots of the plants with a ruler in centimeters. During week 5 I measured the roots again with the same ruler, observing the roots for root nodules, and isolating the rhizobia bacteria and spreading them on agar media plates to observe colony growth for any isolated bacteria if any.

## RESULTS

After Week 1, there were 3 sprouts in premade soil pot 1 (Pot 1) no sprouts in the positive control (Pot 2), and 1 sprout in premade soil pot 2 (Pot 3). At the end of Week 2, there were 5 small plants in premade soil pot 1, 5 small plants in the positive control pot, and 8 small plants in the premade soil pot 2. At the end of Week 3, there are 8 plants in the premade soil pot 1, 5 plants in the positive control, and 8 plants in the premade soil pot 2. At the end of Week 4 there were 8 plants in the premade soil pot 1, 6 plants in the positive control, and 9 plants in the premade soil pot 2. The heights of the shoots of the plants from pots 1-3 are as follows: 20.5 centimeters, 17.5 centimeters, and 24 centimeters. At the end of Week 5, the premade soil pot 1 still had 8 plants, but the positive control and premade soil pot 2 lost a plant. The heights of the shoots of different plants from pots 1-3 are as follows: 23 centimeters, 20 centimeters, and 15

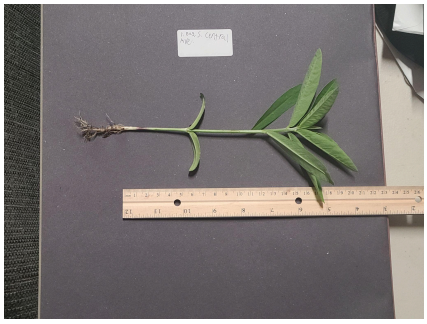
centimeters. The height of the roots of the plants from pots 1-3 are as follows: 7 centimeters, 6 centimeters, and 6 centimeters. All of the plants except for the plants of premade soil pot 2 had white nodules, premade soil pot 2 had some pink nodules.  At the end of Week 5, media plates that contained isolated bacteria had developed bacteria colonies. Premade soil pots 1 and 2 had spread out colonies that looked like dots of varying sizes and thickness, while positive control developed lines of bacteria colonies that constantly overlapped.

**TABLES, GRAPHS and PHOTOS**

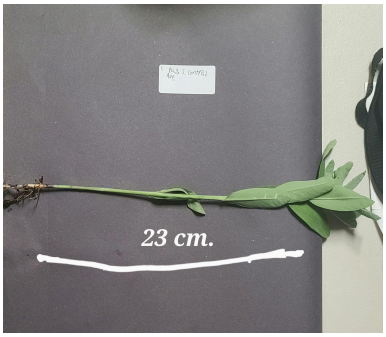
Collected soil for Week 1

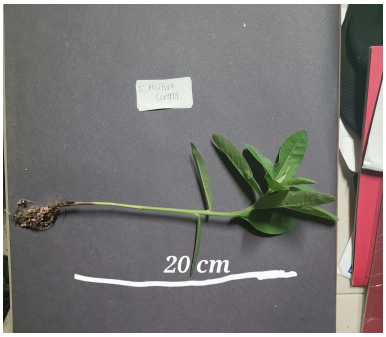
All pots on Week 2, there are some plants in all of the pots.

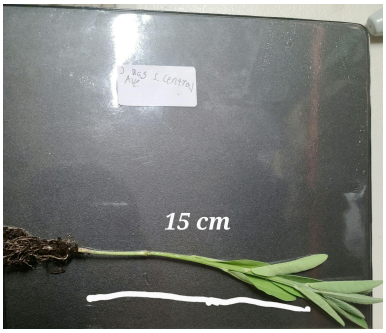

**Top view of the pots on Week 3.**



Phenotyping of plants from all 3 pots, Week 4
Height of shoots in order: 20.5 cm, 17.5 cm, and 24 cm.

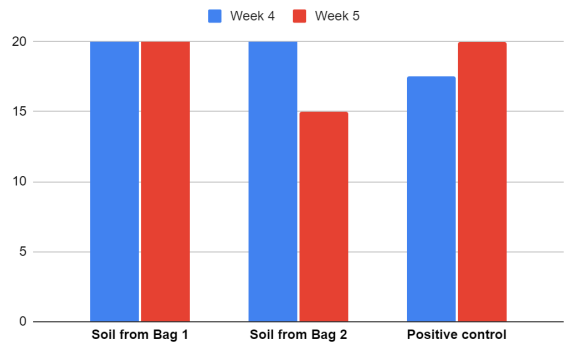



New plants for phenotyping, Week 5

Roots from all three Week 5 plants in order from 1 to 3.




Graph comparing plants from Week 4 to Week 5.

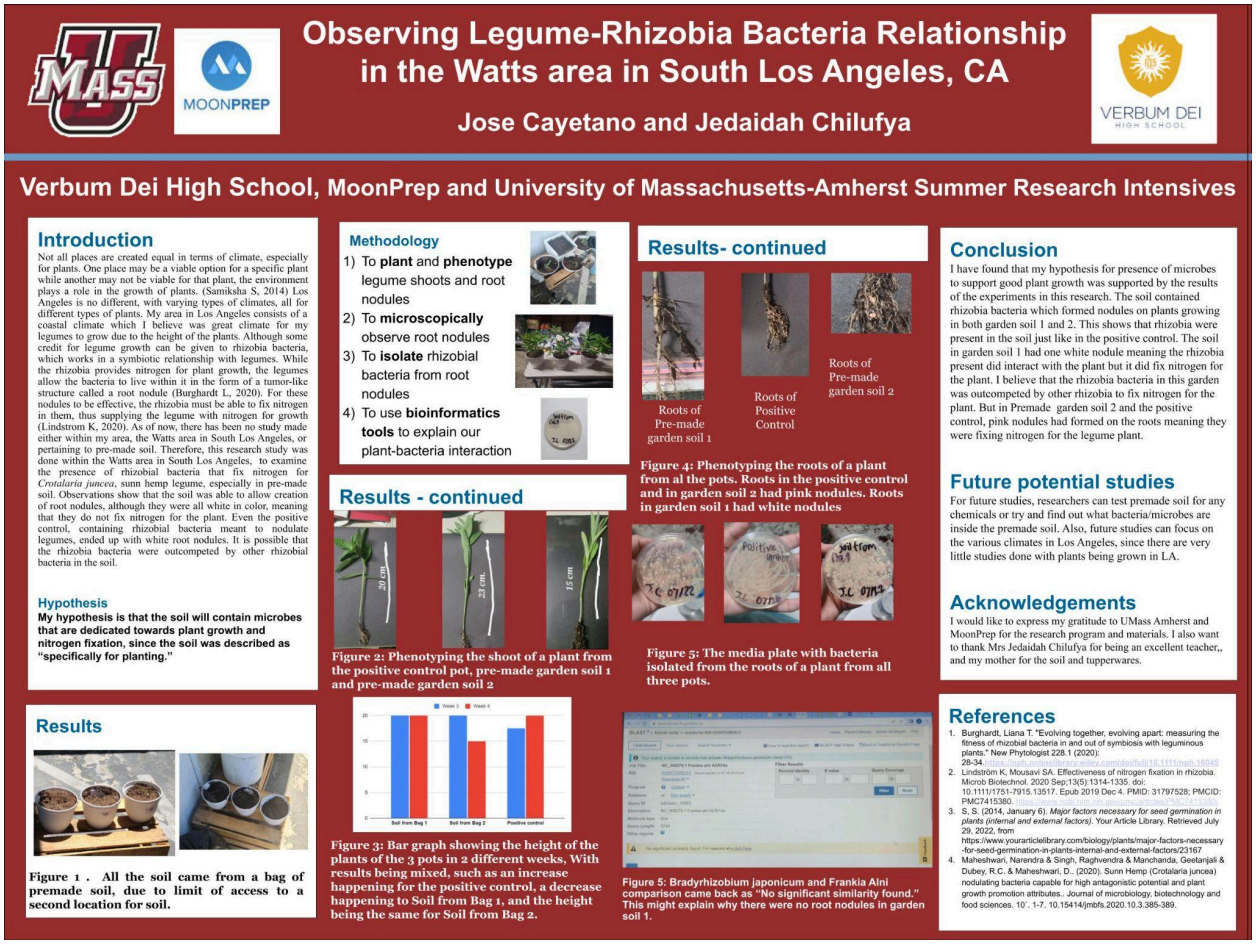
Shoot heights in order: 23 cm, 20 cm, and 15 cm




Bacteria colonies from all three pots.

**DISCUSSION**

At first, having white root nodules surprised me since I believed that because the soil would contain bacteria to help stimulate plant growth, meaning that the root nodules would be pink (especially in the positive control), or that further plant growth should have been provided by the rhizobia bacteria providing the plants with nitrogen. I then realized that perhaps the reason why there were white root nodules was because the rhizobia bacteria had to compete with other nitrogen fixing bacteria for the roots, and that whatever ended nodulating could not properly respond to the signals of supplying the plant with nitrogen.

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Khushie Shelat

# Observing the Effects of Bacteria in the Soil of Jericho, NY


## ABSTRACT

The purpose of this experiment is to observe the growth of the legume seeds in soil from different areas of Jericho, NY. Some methods that were utilized in this experiment were collecting soil from the two locations, planting the legume seeds into the soil, observing the plants with the naked eye and under the microscope, and phenotyping the legume plants. The main research objectives of this experiment was to grow the Crotalaria Juncea legume in the soil of two locations in Jericho, NY and to observe and determine if nodules were present in the roots of the legume plant which indicates nitrogen fixation.


## INTRODUCTION

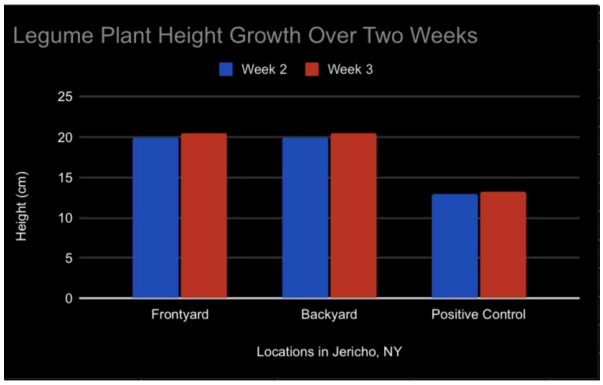
This experiment sought to understand the relationship and interaction between microbes and the growth of plants. The interactions between rhizobial bacteria inside of the roots/root nodules of the Crotalaria Juncea legume may drive and affect the growth and development of the plant itself (Etesami, 2021). This study also allowed for observation of N2 fixation systems under environmental conditions which may also affect the behavior and fitness of the legume plant. The environmental issues include: salt stress, heavy metals, drought stress, acidity or alkalinity in soil, nutrient deficiency in soil, fertilizers, and pesticides. Nitrogen fixation is extremely important for the growth of the rhizobia. As such the prevention of nitrogen fixation as well as the presence of environmental stressors may prevent the rhizobia from building certain characteristics in the roots of the legume plant (Zahran, 1999).

In order to study the symbiotic relationship of rhizobial bacteria in the Crotalaria Juncea legume plant and Sunn Hemp legume, the legume seeds were planted in the soil taken from Jericho, New York. To further observe this relationship, the plants were phenotyped and the bacteria from the roots of the plants were isolated. My hypothesis was that the front yard pot would contain more plants meaning that the front yard would have more bacteria than the other pots.

## MATERIALS AND METHODS

The methods that were utilized in this study include: collecting soil from two locations, planting the legume seeds into the soil, observing the plants with the naked eye as well as using the microscope, and phenotyping the legume plants. The materials used to conduct this experiment included: three pots, digging tools, personal protective equipment (PPE), ethanol wipes, household bleach, rulers, microcentrifuge tubes, plastic pestles, petri dishes, cotton swabs, aluminum foil, and commercial inoculant.

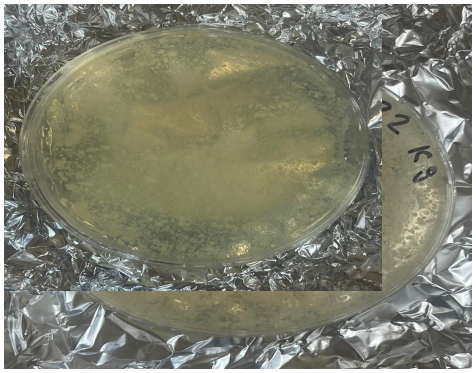
## RESULTS/PHOTOS



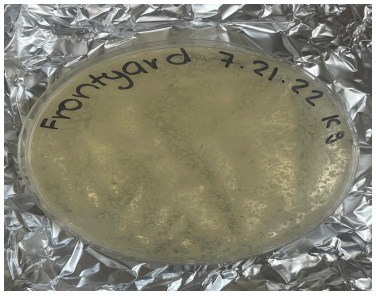

*Shoot height growth over the course of week two and three.* The plant shoots did not have much growth over two weeks. The shoots from the backyard and frontyard were approximately the same height and the positive control shoots were 7 cm shorter.

However, the positive control demonstrated greater growth compared with the other two pots. The backyard had the least amount of shoots in its pot.

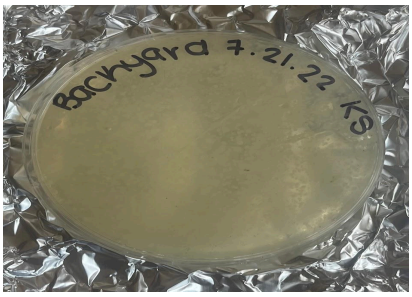
*Bacterial growth after two days of isolating bacteria from the root.*



Positive Control




Front Yard




Back Yard

*Bacterial growth after two days of isolating bacteria from the root.* All agar plates demonstrated bacteria growth from the roots of the legume plants. Minimal bacteria growth had been seen in the agar plates in 24 hours. Bacterial growth at 48 hours is shown above.

**Blast-n search results Rhizobium leguminosarum biovar phaseoli (bacteria in inoculant in positive control)** and pseudomonasdota (one of the many bacteria found in soil). The gaps presented in this search demonstrate that both the Rhizobium leguminosarum biovar phaseoli and the pseudomonasdota would not be able to interact directly. The lack of this interaction would prevent nitrogen fixation thereby precluding the development of root nodules.

**DISCUSSION/CONCLUSION**

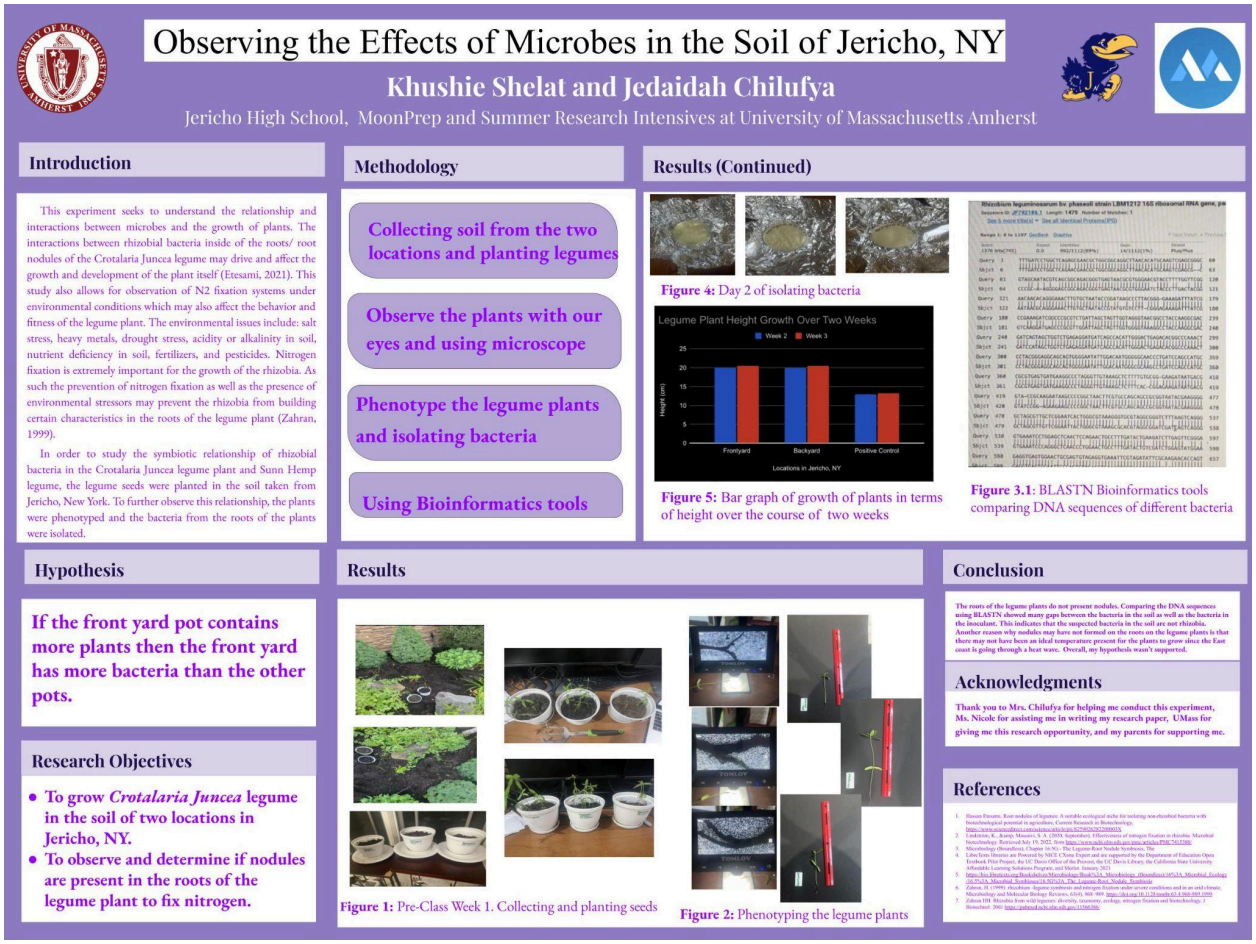
My hypothesis was not supported since the agar plates revealed that all three pots presented an equal amount of colonies of bacteria. I had expected for at least one of the plants to conduct nitrogen fixation resulting in the creation of nodules on its roots. However, this did not occur since the bacteria from the inoculant as well as the bacteria from the soil demonstrated many gaps in the ribosomal RNA demonstrating an absence of symbiotic relation resulting in an absence of root nodules. The lack of root nodules prevents nitrogen fixation from taking place in the legume plant roots from bacterial symbiosis.

In addition to the lack of symbiosis between the bacteria and the plant, other reasons to explain the lack of root nodule formation include environmental stressors. In the case of this experiment, another important factor affecting root nodule formation is temperature. With the recent heat wave in the northeast, the texture of the soil may have been significantly affected. Additionally, the high temperatures may have decreased the soil pH resulting in a negative impact on both the growth of the legume plants as well as the formation of root nodules. Furthermore, the soil pH may have also adversely affected the bacterial growth needed to cause nitrogen fixation. It has been previously shown by research that in nitrogen poor soil, legumes release flavonoids which signal Rhizobia that the plant is seeking a symbiotic relationship (Lindström & Mousavi, 2020). The flavonoids cause the Rhizobia to release a nodulation

factor that stimulates the plant to create deformed root hairs. Rhizobia then forms an infection thread which allows it to enter the root cells from the root hairs. The root cells divide rapidly forming a nodule (Hassan, 2022). In the current case, since all of the pots demonstrated growth and none of shoots showed evidence of root nodule formation, there may have been adequate nitrogen already present in the soil obviating the need for a symbiotic relationship and the need for root nodule formation.

Since roots from all of the pots were separately cultured to assess bacterial growth and they all demonstrated bacterial growth at 48 hours, it can be established that bacterial growth was present in the nerve roots in all three pots. However, less than ideal pH secondary to the dryness of the soil may have inhibited but not fully prevented bacterial growth thereby preventing timely growth of nodules. As a result of stunted bacterial growth, root nodule formation may have been delayed. Since the current study only assessed growth within 2 weeks, it is possible that with continued observation for an additional 2 weeks root nodule formation may have been observed.

This study demonstrates the importance of assessing the idiosyncrasies of the soil including salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, and the possibility of inadequate photosynthesis prior to utilizing a bacterial inoculant. It is also important to note that even after such idiosyncrasies are understood and the soil has been made ideal for the use of a bacterial inoculant, sufficient time needs to be given for root nodule formation to occur such that maximum possible growth of the legume plants can be achieved.

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Lance Yang

# Determining the Effects of Pre-existing Legume Microbiomes on Crotalaria juncea Root Nodule Symbiosis in Upper Dublin, PA


## ABSTRACT

This experiment attempts to determine the effect of legume soil microbiomes on other species of legumes. Soil from regions near bean and white clover plants in Upper Dublin, PA were gathered and used to grow Crotalaria juncea (Sun hemp) seedlings. A positive control with commercial inoculant was also included in the experiment. The seedlings were watered and observed for 5 weeks. Overall, through weekly phenotyping and root inspection, all three groups of seedlings had root nodules present. In addition, samples from all three seedling groups grew Rhizobia colonies on agar.


## INTRODUCTION

Many organisms enter into symbiotic relationships for mutual benefit. One specific group of plants, known as legumes, commonly enters symbiosis with rhizobia soil bacteria. In a symbiotic relationship with legumes, rhizobia form nodules on plant roots and fix atmospheric nitrogen into a usable form for plants (Concha & Doerner, 2020). Prior research has shown that legumes benefit other plants and provide them with nitrogen (Brophy et al., 1987). While there has been much research on interactions between legumes and other plants (Martínez-Romero, 2003), much less research has been performed to investigate the interactions between different species of legumes. The purpose of this experiment is to determine how legume species' microbiomes interact by growing Crotalaria juncea in soil originally adjacent to Trifolium repens (beans) and Phaseolus vulgaris (white clover), two other species of legumes. In order to perform this experiment, Crotalaria juncea seeds were planted in soil from different locations, with different legumes originally nearby. Phenotyping, root observations, and agar bacteria cultivation were used to obtain results.

## MATERIALS AND METHODS

The experiment was performed using Crotalaria juncea seeds. Tools and personal protective equipment were sterilized with 70% ethanol wipes between activities to minimize cross-contamination. Soil from 2 different regions in Upper Dublin, PA, one in a patch of bean sprouts (garden) and another in a patch of white clovers (yard), was used to fill 2 identical pots. A third pot was filled with soil from the second region, yard, and then inoculated with commercial inoculant. This pot acted as the positive control. 15 seeds were planted in each of the three pots. Seedlings were watered every other day for three weeks. From then on, plant shoots and roots were phenotyped every week. Finally, the roots and root nodules of one plant from each pot were crushed and the bacteria within grown on agar plates.

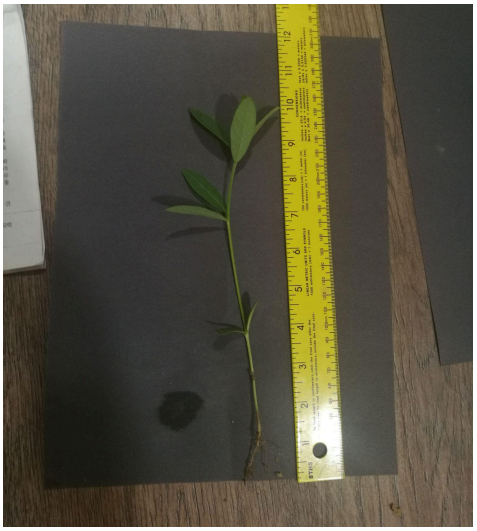
## RESULTS

At the first phenotyping during week 3, the garden and positive control pot had 3 visible nodules each, and the yard pot had 2 nodules. The next week, the yard pot had 4 nodules, the positive control pot had 3 nodules, and the garden pot had 2 nodules. Every time the plants were phenotyped, there were at least 2 root nodules on the roots of each plant. Overall, the "yard" pot (from near the white clovers) had the most root nodules at the end of the experiment, with a cluster of 4 nodules. Nodules were reddish gray and typically appeared in round clusters. Samples of the roots from all three pots grew into dotted rhizobia bacteria colonies on agar.

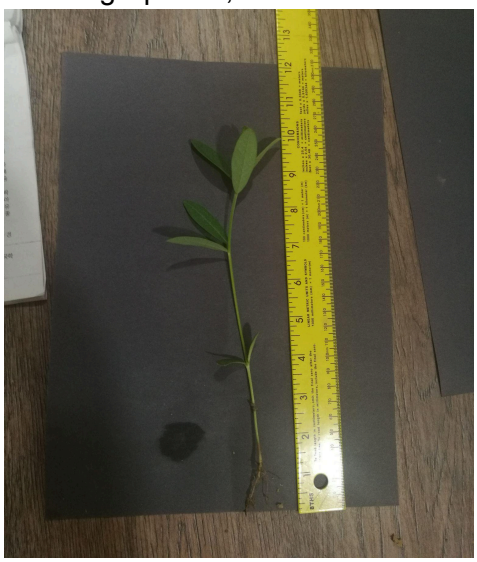
**TABLES, GRAPHS and PHOTOS:**

Filling Pot with Soil

Watering Sprouts, Week 2

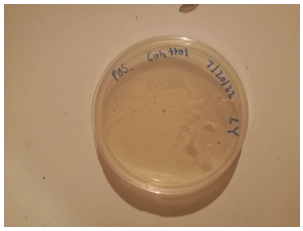


Watering Sprouts, Week 2

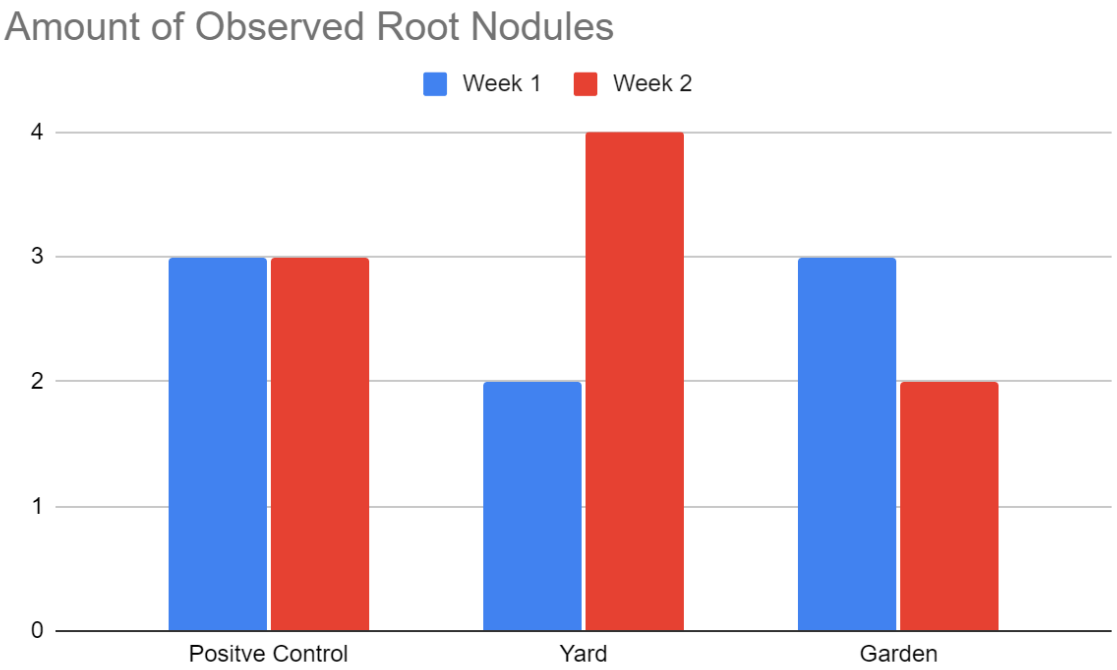


Phenotyping


Root Imaging

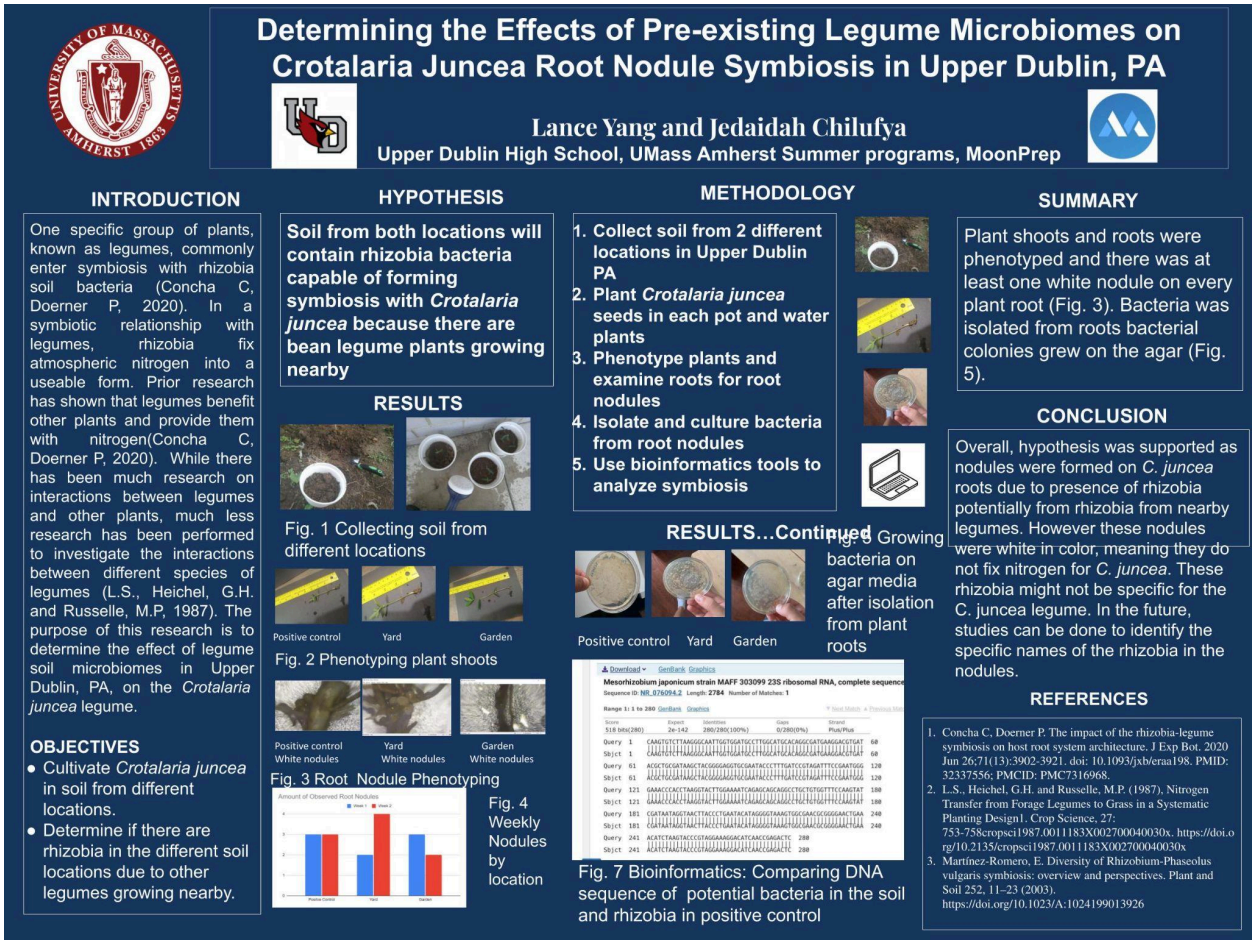


Growing Bacteria on Agar




**DISCUSSION**

Surprisingly, seedlings from all three pots grew some root nodules with rhizobium bacteria. In addition, petri dish colonies show rhizobia bacteria were present in the root nodules of plants from all three pots. This shows that commercial inoculant is not necessarily needed to promote rhizobia symbiosis in legumes. In fact, samples from the yard area (near white clover) actually grew more nodules than the positive control. Instead, just planting near previous legumes of a similar kind could provide benefits This could benefit legume agriculture. Further avenues of research include testing other species of legumes to see how they interact with each other. Finally, a comparison of different soil types to see how well they retain rhizobia is another future avenue of research.

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Lauren Szeto

# Observing the Crotalaria Juncea-Rhizobia Relationships In Soil From Brighton and Weston, MA

**ABSTRACT**
This research aimed to see how soils that undergo different activities affect the Crotalaria juncea-rhizobia bacteria relationship. Crotalaria juncea is a type of legume that forms symbiotic relationships with rhizobia. Soils from Brighton and Weston, MA, were collected in three white pots to start this scientific investigation. Soon after, legumes were planted and grown, and pictures of the legumes' growth process were taken. Once the legumes grew to a certain height, they were removed from their pots to be phenotyped. Their heights were measured, and their roots were examined under the microscope. In the end, the positive control group had the most root nodules and the strongest legume-rhizobia relationship, followed by the Weston legumes and the Brighton ones, respectively.

**INTRODUCTION AND HYPOTHESIS**
Legume plants and rhizobia bacteria engage in a symbiotic relationship (Wang et al., 2012). This mutualism contributes to forming root nodules with rhizobia inside (Wang et al., 2012). In this relationship, the rhizobia can convert nitrogen from the air into ammonia, a molecular form of nitrogen that the legume plant can use (Via et al., 2016).

This research project is essential because it will help us better understand the crotalaria juncea-rhizobia relationship in two different locations, Brighton and Weston, MA. Learning about this relationship is crucial as the soil microbiota from these locations differ, affecting the crotalaria juncea-rhizobia relationship.

In a research experiment recently conducted in 2022, scientists analyzed strain-specific and host genotype-specific interactions between rhizobia and legumes and their role in establishing symbiosis. These researchers highlighted the need for more research on the effects soil microbiotas have in establishing symbiosis. Gaining a greater understanding of the effects soil microbiotas have in starting symbiosis can help develop strategies for bioinoculants and synthetic communities' assemblage (Cangioli et al., 2022).

There are not very many researchers who have specifically studied the crotalaria juncea-rhizobia relationship. This project researches crotalaria juncea specifically. Additionally, there have been no studies done on the legume-rhizobia relationship with soil from Brighton and Weston, MA, and little is known about the microbiotas of the soils from these locations.

Soils from Brighton and Weston contain different bacteria and fungi because they have various activities. The soil from Brighton was laced with fertilizer, but the dirt from Weston was covered with mulch. Despite the difference in soil activity, both areas have plant growth. Bacteria growth is accentuated in soil with plants because as plants decompose, bacteria in the soil consume the plant remains. Fertilizer provides plants with essential nutrients, while mulch helps block evaporation. Moisture is kept in the soil, keeping plants hydrated. Mulch also helps control weed growth, preventing weeds from consuming nutrients in the soil that are essential to plant growth.

This research aims to understand how soil from various places, possibly with different microbes, can affect the legume-rhizobia

relationship. This research project is crucial because we can better understand how the microbiotas in Brighton and Weston soils affect the crotalaria juncea-rhizobia relationship.

**METHODOLOGY**

The first step was to gather Brighton and Weston soil for the three identical white plastic pots. One pot was the positive control group. This soil was from Weston but was laced with a commercial inoculant. A small gardening shovel was used to dig and scoop soil into the pots.

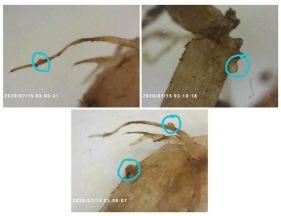
A gardening fork was used to insert fifteen holes in each pot of soil. The soil was wetted with water, and one legume seed was placed in each hole. Once this was complete, the soil was smoothed over, and the holes were covered. The soil was watered once again. Photos were taken every week with a smartphone. After three weeks, the legume shoots were ready to be phenotyped. The scientist's finger was inserted deep into the soil close to the legume plant. Then, the finger was angled inward toward the legume plant. Finally, the finger was lifted, and the soil came up along with the legume plant and its roots. The plant's roots were submerged in water to remove the excess soil and patted dry with a paper towel. The plant was laid on a black surface, and a thirty-five-centimeter ruler was placed beside it. The zero-centimeter line was put next to the legume stem's start, and the legume's height was measured. After that, the legumes' roots were observed with a digital microscope. The roots were placed on top of the microscope's base and underneath the lens. The lens was moved vertically, and the focus knob was turned until the roots were visible on the microscope's screen. After four weeks post-planting, rhizobia bacteria were isolated from the legume plants' roots. To begin this process, a diluted household bleach solution was created and poured into a plastic tube. Some clean water was then poured into another plastic tube. After phenotyping a legume plant's shoot and roots, their roots (and root nodules) were cut with a scalpel and placed into the tube with the bleach solution. The tube was closed and inverted five times. Once that was completed, the bleach solution and roots were poured onto a paper towel. The roots were placed into the tube with clean water, the tube was closed, and it was inverted ten times. The water and roots were poured onto another set of clean paper towels. Then, the roots and root nodules were put into another plastic tube and crushed with a plastic pestle. The tube was filled with water and shaken. The mixture was poured onto an agar media plate and spread with a cotton swab. The media plate was wrapped in aluminum foil and placed in front of a sunny window. Pictures of the media plates were taken every day until bacteria started forming.

**RESULTS**

The results are listed below in two tables. The table 1 contains the results of phenotyping legume shoots and roots. The table 2 displays the results of the rhizobia bacteria growth.

**Table 1. Phenotyping Legume Shoots and Roots**

| | Grandma's yard (Planted 11 days later than positive control and front yard due to soil issues.) | Positive control | My front yard |
|---|---|---|---|
| 7/12/2022 | Ten days post-planting: 8 cm | Three weeks post-planting: 16 cm | Three weeks post-planting: 17 cm |

| | | | |
|---|---|---|---|
| | No root microscopy yet | No root microscopy yet | No root microscopy yet |
| 7/14/2022 | 12 days post-planting: 8 cm<br><br>0 root nodules | 3.5 weeks post-planting: 20 cm<br><br>2 root nodules (one pink, one white) | 3.5 weeks post-planting: 17 cm<br><br>4 root nodules (all white)<br>  |
| 7/20/2022-7/21/2022 | 18 days post planting: 5.5 cm (different plant, original one died)<br><br>0 root nodules<br>  | 4 weeks post-planting: 20 cm<br><br>8 root nodules (one pink, seven white)<br>  | 4 weeks post-planting: 16.5 cm (different plant, original one died).<br><br>0 root nodules (may have accidentally been pulled off the roots while the plant was being pulled out of the soil). |

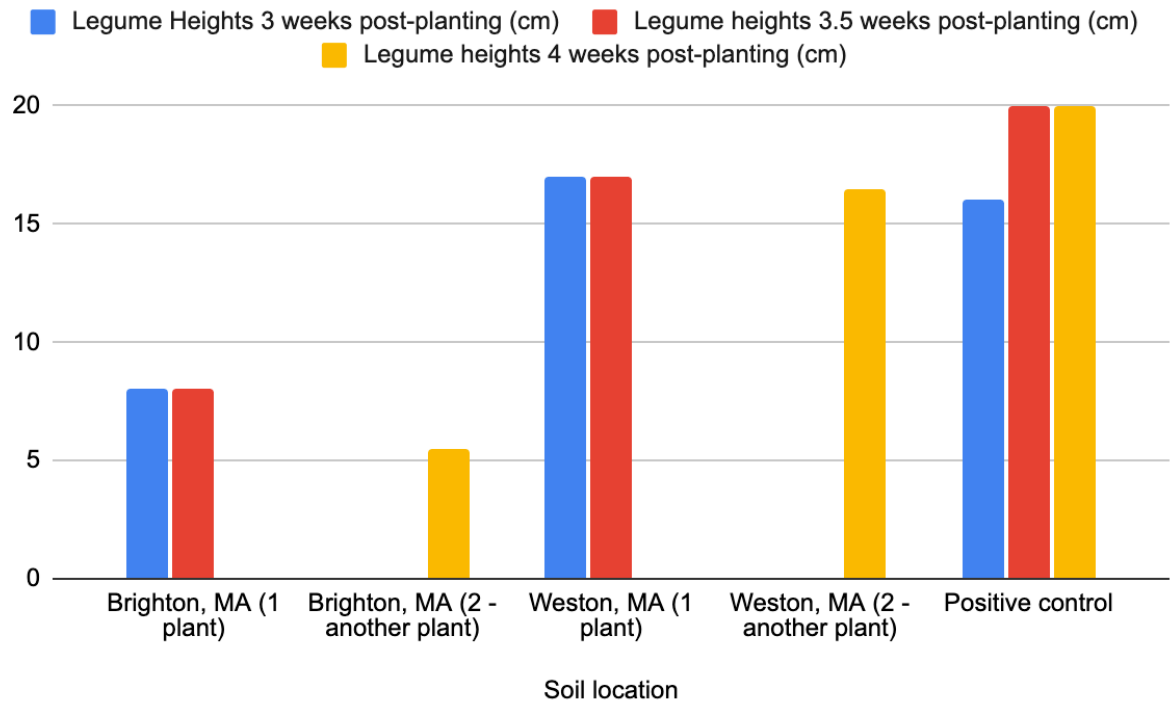
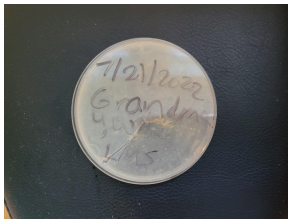
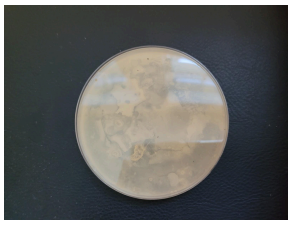



**Table 2. Observing Bacteria Growth on Agar Media Plates**

| | 7/21/2022 | 7/22/2022 | Images (Days three/four) |
|---|---|---|---|
| Grandma's yard | Rhizobia bacteria are isolated. | Bacteria began flourishing on the "grandma's yard" agar media plate. |  Small, opaque, entire, round, raised bacterial colonies. |
| Positive control | Bacteria started growing on the "positive control" agar media plate. | Bacteria growth is well underway. |  Large, opaque, |

| | | | |
|---|---|---|---|
| | | | irregular, raised bacterial colonies. |
| My front yard | Rhizobia bacteria are isolated. | Bacteria started blossoming on the “my front yard” agar media plate. |  Small, opaque, round, irregular, raised bacterial colonies. |

**Table 3. Using Bioinformatics (BacDive and NCBI BLAST Technologies) to Better Understand the Legume-Rhizobia Relationship**

| Bacteria | Description | Comparison | Similarity percentage |
|---|---|---|---|
| Dyella japonica | General soil bacteria (incompatible with crotalaria juncea) that does not help form root nodules. | Compared to Bradyrhizobium japonicum, which is a type of rhizobia in the commercial inoculant used for the positive control group. | 84% similar |
| Variovorax paradoxus | General soil bacteria (incompatible with crotalaria juncea) that can help form white root nodules. | Compared to Bradyrhizobium japonicum, which is a type of rhizobia in the commercial inoculant used for the positive control group. | 79% similar |
| Rhizobium leguminosarum | A type of rhizobia that is compatible with crotalaria juncea and can help form pink root nodules. | Compared to Bradyrhizobium japonicum, which is a type of rhizobia in the commercial inoculant used for the positive control group. | 75% similar |

**DISCUSSION**

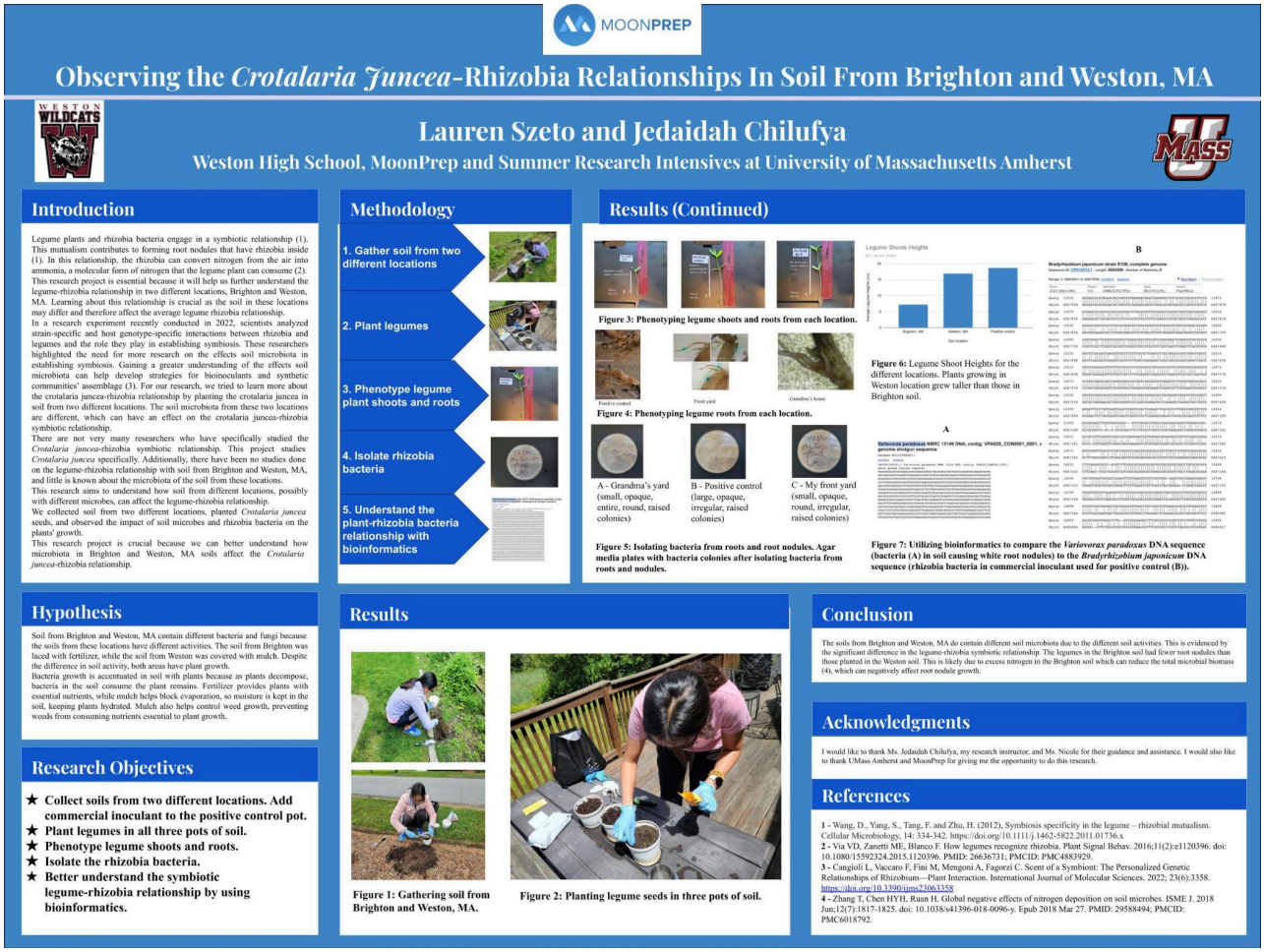
This experiment aimed to answer the following question: "How do different soil activities affect the soil microbiota and the sunn hemp-rhizobia (crotalaria juncea-rhizobia) relationship?"

The most significant results of this experiment were that the positive control legumes had the most root nodules and were the tallest, followed by the Weston legumes and then the Brighton plants. Because the Brighton legumes were the shortest and had the fewest root nodules, the Brighton soil contained the weakest legume-rhizobia relationship. These results were expected because the Brighton soil has been fertilized numerous times, and fertilizers generally contain nitrogen. Therefore, there could be excess nitrogen in the Brighton soil, reducing the total microbial biomass (Zhan et al., 2018). A reduced total microbial biomass can negatively affect root nodule growth.

The legumes in the Weston soil had more root nodules than those in the Brighton soil but fewer root nodules than the positive control legumes. The Weston legumes grew moderately well and had an average sunn hemp-rhizobia relationship. These results were expected, as the Weston soil was laced with mulch, but it was not fertilized like the Brighton soil. Mulch does not affect the amount of nitrogen in the soil but has many benefits. It contains moisture, prevents weed growth, and aerates the soil.

Lastly, the positive control group had the most root nodules, and the legumes were the tallest. The soil the legumes were planted in was from Weston, but it was laced with a commercial inoculant. The commercial inoculant used housed various rhizobia, which enhanced the sunn hemp rhizobia relationship and therefore optimized the legumes' growth.

In conclusion, different soil activities can affect the soil microbiota and thus affect sunn hemp-legume relationships. More data and a better understanding of what components in the soil can optimize the legume-rhizobia relationship will benefit us in the future. The compatible legume-rhizobia relationships are essential in stimulating plant growth and enhancing plant development (Concha & Doerner, 2020). This knowledge is beneficial to the farming industry, for instance. If farmers know about the compatible legume-rhizobia relationships, they could grow taller, stronger, and healthier legumes than they did before. The inoculation of mutual bacteria as biofertilizers can improve crop production and soil health (Sindhu, et al., 2019).

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Mahil Selvakumar

# Identifying rhizobial bacteria on legume roots in Chesterfield, NJ

**ABSTRACT**
The purpose of this experiment was to identify rhizobial bacteria on legume roots and see the effects on legume plant growth in different types of soils collected from different locations in Chesterfield, NJ. The goal was to compare the results of the plant roots and its root nodules at the end as well as comparing their growth rates. During the process, I phenotyped and looked at roots under the microscope, looked at the plant shoots with naked eye, and isolated rhizobia bacteria from the roots.

**INTRODUCTION**
Nitrogen fixation in rhizobia happens in the roots and is induced by the bacteria in legumes. Researchers are fascinated by this symbiotic process and aim to see how nitrogen fixation adds benefits to legumes by its interaction between rhizobia and legumes (Lindström & Mousavi, 2020). We can also see how rhizobia is tolerant for such severe environmental conditions and why rhizobium-legume symbiosis could be helpful to improve soil conditions, better replacement for inorganic fertilizer, and for important future research (Aroney, 1970). Bacteria from rhizobia could also be used in biotechnology by analyzing its specific traits and using it in industrially important compounds (Zahran, 2001). Finally, there are many aspects to rhizobia overall such as how its mechanisms of non-rhizobial bacteria improves legume-rhizobia symbiosis (Westhoek et al., 2017).

This experiment aimed to identify what soil was the most beneficial to plant growth. Samples were collected from two different locations (Backyard, Positive control, Creekside). I isolated and found root nodules under a microscope to distinguish which legume plants in what soil locations had the most microbial bacteria. I hypothesized that the creekside pot would have the most benefit with the presence of bigger plants and more microbial bacteria such as rhizobia. This was based on the fact that the Creekside soil had an abundance of decomposed matter, fungi, and microbial bacteria.

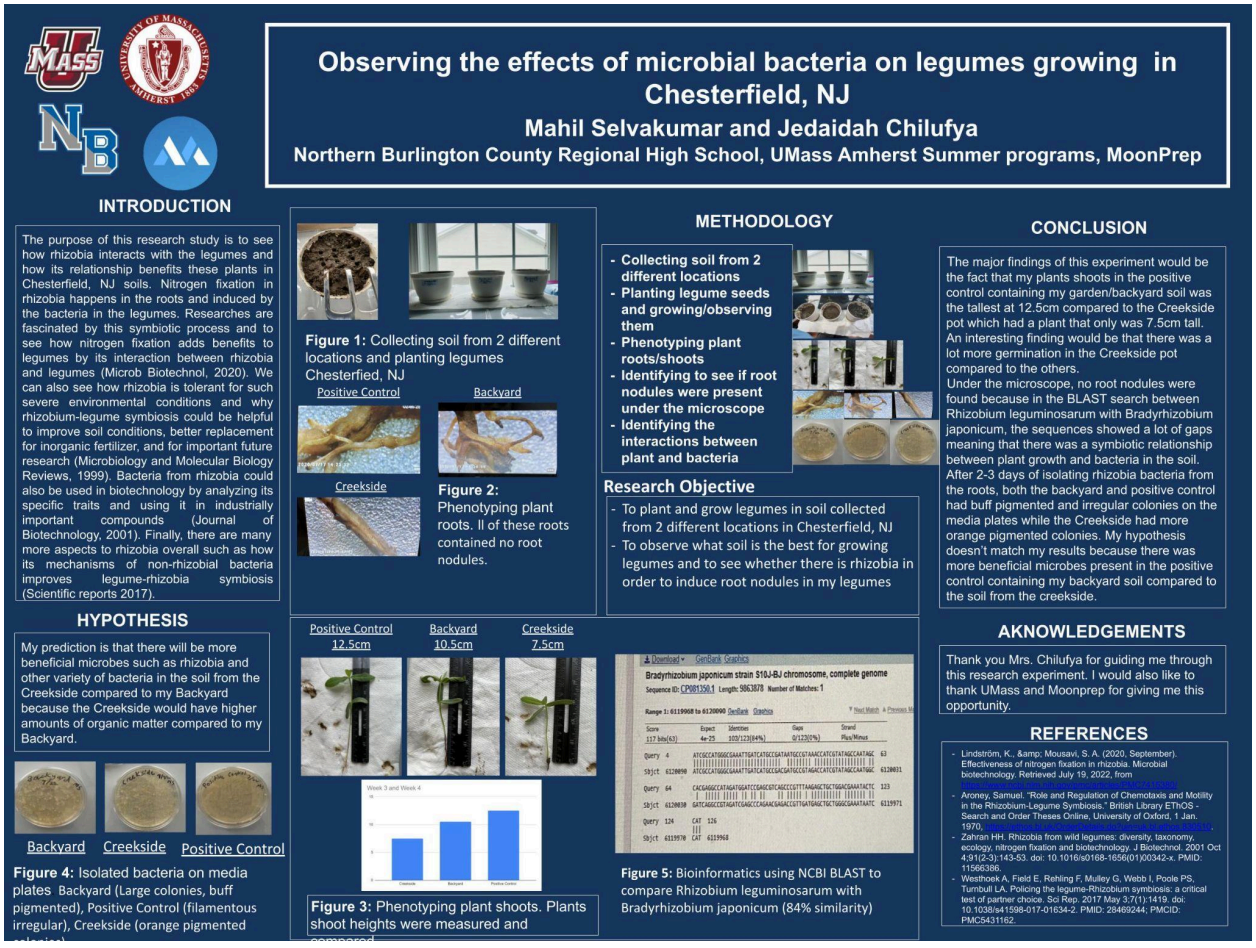
**MATERIAL AND METHODS**
For this experiment, I noted the procedures of what I did each week in my e-lab notebook as well as took pictures of the plants to document progress. I observed the roots under the microscope to identify root nodules and microbial bacteria that benefit the plants. In three different pots, I planted legume seeds including adding an inoculant to the positive control, watering plants every other day in order to keep them growing, checking soil conditions, and keeping track of the amount of plants present. The materials I used included gloves, safety goggles, legume plants, shovel, cup of water, paper towels, microscope, inoculant, paper towels, pots, soil from 2 different locations, fork, agar media plates for isolating rhizobia bacteria from legumes' roots, 3 tubes, crusher, and cotton swabs in order to spread the bacteria onto the media plates.

**RESULTS**
The results of my experiment show 3 different results from my pots in terms of plant shoots. In week 2.5, the Positive Control shoot was the tallest at 12.5cm. The Backyard soil resulted in the second tallest plants at 10.5cm and the Creekside was the shortest at 7.5cm. Under the microscope, the photos show that there were no root nodules for any of the plants. The Positive Control had a bigger root structure compared to the Creekside. After 2-3 days of isolating rhizobia bacteria from the roots, both the Backyard and Positive Control had buff pigmented and irregular

colonies on the media plates while the Creekside had more orange pigmented colonies. In coloration of the agar media plates, the Positive Control was the darkest as it was more orange compared to the Creekside, meaning there had been more microbes in the Positive Control soil probably due to rhizobia added from the inoculant.

## DISCUSSION

The surprising part about my results would be that although I expected, based on my hypothesis, the Creekside plants to grow the most, they actually grew less than the plants from the Backyard. The Positive Control's soil was from the backyard and had the tallest plant at 12.5cm. What I also found interesting was that the Creekside pot had up to 8-10 germinating plants while the other pots had fewer. The Positive Control contained fertilizer, added inoculant containing rhizobia, and other nutrients from the backyard, creating the most impact on the legumes in this pot. Based on my knowledge, the soil from the backyard was the most beneficial and nutritious because a few years ago, my dad composted vegetable peelings and other composts to add more beneficial microbes in the soil. Therefore, this experiment showed that out of the soil types, the backyard soil present in the Positive Control pot was the most beneficial for plant growth.

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Michael Zhu

# Observing Legume Plants's Growth Due To Rhizobia Bacteria In Brookline, MA


## ABSTRACT

The reason for conducting this experiment was to compare soil that was lush with a variety of plants to dry and barren soil and see their effect on plant growth. The locations used were Baker Field which had the dry soil and a backyard which had many different plants. There were three types of soil used in this experiment. The three types of soil included: Positive Control, Baker Field, and Backyard. In the Positive Control, commercial inoculant, which contained Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and R. leguminosarum biovar viceae, was added. The plants used in the experiment were Crotalaria juncea. There were five major methods used to go through this experiment: Soil collection, legume seed growth, shoot and root observation, microscopic root analysis, isolation of rhizobia bacteria, and rhizobia analysis. The results of this experiment deemed that my hypothesis was not supported.


## INTRODUCTION

This study aimed to show if a more developed plant implies more rhizobia bacteria present in the soil. Nitrogen is an essential tool in the process of germination and growth of a plant. However, most plants cannot process the natural nitrogen that is in the air and instead can only use nitrogen in the soil (Leghari et al., 2016). This means that plants have to rely on some other method to take in nitrogen which comes from rhizobia bacteria. Rhizobia bacteria is unique in the fact that it has a mutualistic symbiotic relationship with legume seeds (Concha C and Doerner P, 2020). Rhizobia bacteria and legume seeds have an exchange where the legume seeds provide the Rhizobia with carbon-heavy substances such as glucose and amino acids while the Rhizobia bacteria provides nitrogen in a useful form to the legume plant through a process called nitrogen fixation (Cooper, J., 2007). Since nitrogen contributes such an important factor in plant growth and Rhizobia bacteria provides the plant with nitrogen, we hypothesize that the fertility of each soil correlates with the amount of Rhizobia bacteria present.

## MATERIALS AND METHODS

Materials used included a digital microscope, a scalpel, clean water, a trowel, a garden fork, three pots, legume seeds, commercial inoculant, safety goggles, latex free gloves, 75% alcohol wipes, a ruler, labels, agar media plates, microcentrifuge tubes, plastic pestles, 4.5% sodium hypochlorite bleach, Q-tips, and tinfoil.

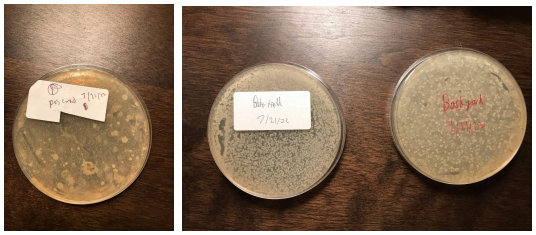
In order to complete this experiment, soil was collected from two different locations using a trowel and the three pots were filled with that soil. After rehydrating the possibly dry soil, a garden fork was used in order to make holes within the soil. Each pot had 15-18 holes made by the garden fork and in each hole, a legume seed would be inserted. In the positive control, a commercial inoculant containing rhizobia bacteria was added within the holes. After nurturing the legume plants for 3 weeks, a phenotyping of the shoot was conducted using a ruler. The steps to phenotyping included carefully removing the tallest legume plant in each pot and rinsing the dirt off with a fresh bowl of water. Afterwards, a photo was taken of each shoot height. After one week, another phenotyping of the shoots was done. However, in addition to the shoots, the roots were phenotyped using a digital microscope. After the phenotyping, a 1:11 ratio of water to the bleach solution was mixed in a microcentrifuge tube. Using a scalpel, the

roots and nodules were cut off and separately placed into the solution. The roots were washed off using clean water and then dried. After the roots were dried, they were put into an empty microcentrifuge tube where they were crushed to release the bacteria from the roots and nodules. Finally 0.75 mL of clean water was added and mixed. In total, there were 3 different microcentrifuge tubes of different locations' legume plants. These microcentrifuge tubes were poured into 3 separate agar media plates and then spread out using a Q-tip. They were wrapped completely in tinfoil and were incubated for 3 days.

**RESULTS**

All three soils contained some form of bacteria. For the positive control soil there was a tint of brown with several large patches of bacteria colonies. The backyard and Baker Field soils both had the same appearance in terms of type of bacteria. However, Baker Field had much more bacteria overall compared to Backyard.

**Photo of media plates of all 3 locations:**



The positive control legume at 3 weeks post planting, had a shoot height of 11 centimeters and at 4 weeks had 12.5 centimeters. It appeared to have pink roots with 2 root nodules. The backyard legume at 3 weeks post planting had a height of 12 centimeters and at 4 weeks post planting, it wilted and thus a new plant was phenotype which was 10 centimeters. It had no nodules and white roots. The Baker Field soil had a height of 11 centimeters at week 3 and at week 4 it had a height of 13 centimeters. The roots were completely white and had 0 nodules.

**Table describing phenotyping:**

| Location | Positive control | Backyard | Baker Field |
|---|---|---|---|
| 3 weeks - 4 weeks post planting<br>Date: 7/12/22<br><br>21 days post planting | Shoot height: 11 cm<br># of roots: 10 (6 very large) | Shoot height: 12 cm<br># of roots: 8 (all very small) | shoot height: 11 cm<br># of roots: 5 (all very small) |
| 4 weeks post planting<br><br>Date: 7/20/22<br><br>28 days post planting | Positive Control:<br>12.5 cm<br># of root nodules: 2<br>Bottom of roots pink | Backyard:<br>(previous plant died so using new one: growing for same time)<br>10 cm<br># of root nodules: 0<br>Bottom of the roots are completely white. | Baker Field:<br>13 cm<br># of root nodules: 0<br>Bottom of roots whites |

## DISCUSSION

The most significant results were the plant germination, the phenotyping, and the bacteria growth. These elements of the plant helped distinguish how fertile each soil was. I was surprised as I thought the barren and dry soil would contribute to a slower growth. However, my hypothesis was unsupported. I found that the positive control plant grew slower than the Baker Field plant even though it had the commercial inoculant. I can use this data by understanding that there is most likely another type of bacteria that is promoting growth or possibly even fixing nitrogen. People who could benefit from this would be botanists or farmers. This data could be used to define certain factors that need to be studied more to gain information on plant growth and better supplements for germination and nitrogen fixation.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Mira Bhatia

# Exploring the Impact of Bacteria and Microbes on Legume Seed Growth in Soil From Lake Forest, IL

**ABSTRACT**
Microbes, specifically Rhizobia bacteria, have a tremendous impact on the growth and development of legume plants and roots. The bacteria create root nodules that allow for nitrogen from the air to be turned into ammonia, a compound that the plants can absorb and use for growth (Jones, 2007). In this experiment, three different soil locations were compared to determine the best soil conditions to grow legume plants in Lake Forest, Illinois. Determining growing conditions benefits many industries, including agriculture, climate change, and food manufacturing. In knowing which soils and microbes benefit plants, measures can be taken to create sustainable growing environments for crops. In this research, legumes were planted and observed over three weeks to phenotype plant shoots and roots and isolate Rhizobia bacteria from the plant roots to determine what soil is the most effective for plant growth. This research found that soil around the pond had the pinkest nodules, suggesting a healthy amount of nitrogen fixation occurs.

**INTRODUCTION**
Rhizobia bacteria are microbes that exist in soil and play a significant role in the health and well-being of legume plants (Concha, 2022). The bacteria interact in a mutualistic relationship with the plant, assisting the legume plant in accessing and processing nitrogen for growth while the bacteria, in exchange, receive carbon. While rhizobia bacteria play a significant role in legume plant growth, they are not the only bacteria that exist in the soil and interact with the plant. (Ledermann, 2021). A study found that rhizobia and non-rhizobia bacteria are essential for plant growth. Currently, more research is needed to fully understand the role of the non-rhizobia bacteria on plant growth. However, when non-rhizobia bacteria are coupled with rhizobia bacteria, plants can survive under stressful conditions such as salinity and drought (Etesami, 2022).

This experiment observed the interaction between the bacteria in soil and legume plant roots. Results were used to determine which soil the rhizobia bacteria was interacting positively with the roots of the legume plants. Furthermore, in this experiment, bacteria was isolated from the roots in order to determine whether any bacteria was interacting with the roots.

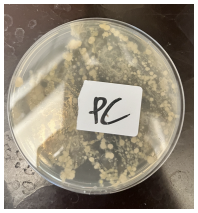
*Methods:*
The following materials were used in this experiment: 3 plastic pots, legume seeds, water bottle, water, garden trowel, garden fork, digital microscope, ruler, gloves, eye glasses, agar plates, and cotton swabs.
To plant the legume seeds, all necessary materials were gathered, then the seeds were planted in three different pots making sure each one had soil from a different location, and one had commercial inoculant added for a positive control. The plants were watered daily using a spray bottle until the soil was fully saturated. The legumes were observed for seed growth over two weeks. To phenotype the legume shoots, water was added to the soil in the pots, then fingers were used to dig out the plant, making sure not to rip the roots, then the plant was rinsed in tap water to remove excess soil, and then the plant was laid on a black surface and a ruler was used to measure shoot height, lastly the plant was replanted into the soil. Next to phenotype plant roots, the plant was dug up again, the roots were rinsed in warm water to remove excess dirt. Next, eyes were used to observe whether there was a

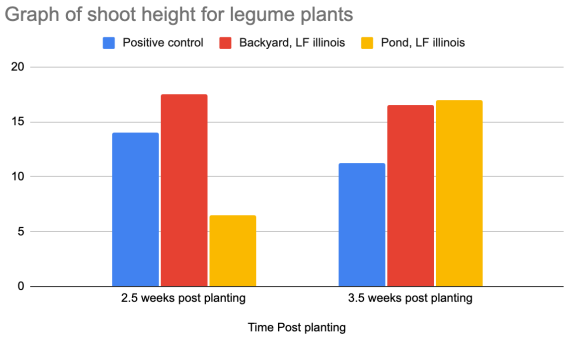
presence of root nodules and their color, then the root nodules were photographed, next, the plant was placed under the microscope and examined closely for root nodules. Lastly, the bacteria was isolated from the plant nodules and roots. First, the plant roots were rinsed with water, then 1:20 parts of bleach to water was mixed to create a cleaning solution in a microcentrifuge tube. Then the root nodules were cut from the plant and placed into the cleaning solution to sterilize them. Then the roots were rinsed in water and placed in a tube with 0.5ml of water. Next, the roots were crushed into the water until it became cloudy. Lastly, using a cotton swab, the solution was swabbed onto an agar plate. The agar plate was set to grow in a dark place and observed for bacterial colony growth.

## RESULTS

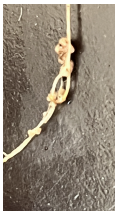
All three soil locations sprouted healthy, tall legume plants. The soil by the pond was the only one that interacted with the bacteria to form pink root nodules on the roots of the legume plants. Neither the backyard location nor the positive control location grew root nodules. The sprout from the pond grew the most from 2.5 weeks post planting to 3.5 weeks post planting, with it being 6.5cm on week 2.5 and 17cm on week 3.5 post planting. The plants grown in the soil from the backyard had the least change, with 17.5cm on week 2.5 and 16.5cm on week 3.5. All three soil locations had many bacteria colonies growing by 3 days post bacteria extraction.

## TABLES, GRAPHS, AND PHOTOS





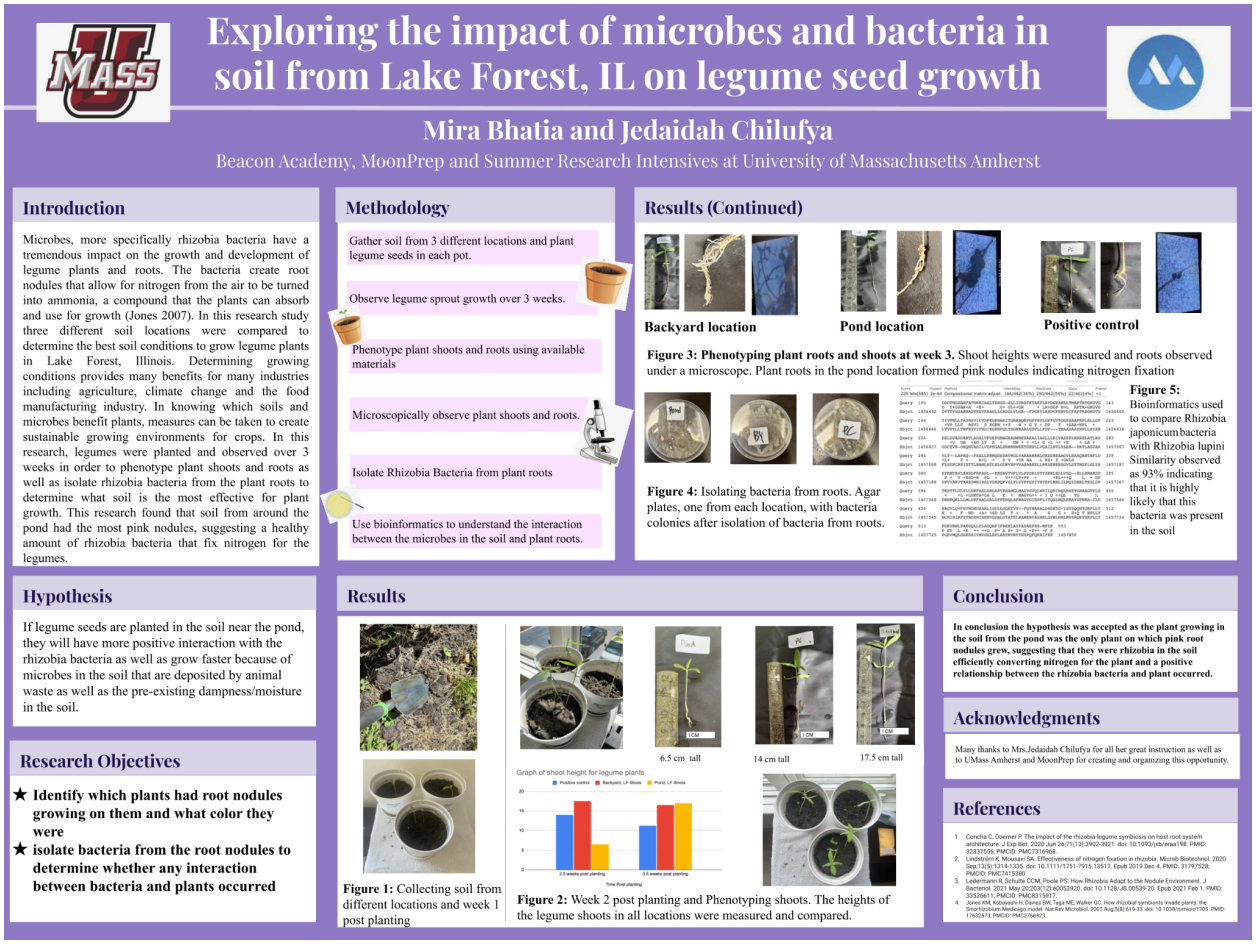
| Location | Positive control | Backyard, LF illinois | Pond, LF illinois |
|---|---|---|---|
| 2.5 weeks post planting | 14 | 17.5 | 6.5 |
| 3.5 weeks post planting | 11.25 | 16.5 | 17 |

## DISCUSSION

The most significant results observed were the pink nodules that grew on the roots of the legume plants from the soil by the pond. The formation of root nodules indicates a healthy, beneficial relationship between the nitrogen in the soil and the plant root. The pink color of the root nodules also indicates that leghemoglobin is present. The pink nodules also indicate that nitrogen is being supplied to that plant and that the bacteria in the soil are receiving carbon in return. The lack of root nodules in the soil and positive control could

likely be due to an excess of nitrogen in the soil, or other imbalances in the soil, such as an overabundance of water or over acidic soil. It is also important to note that the legume plant from the pond soil had the most growth over the one-week period and had the longest shoot height of 17cm at week 3. The pond soil and microbes in the soil most likely contributed to the fast and tall growth of the plant. It could be inferred that because of the root nodules which indicate nitrogen fixation occurring in the soil, the plant was able to use the nitrogen efficiently in order to promote growth. The other legume plants from the other soil locations did not grow as rapidly and did not have any nodules growing on them, this demonstrates how important interactions with the soil and roots in order to form nodules are critical to plant development.

Through this experiment, the hypothesis that the soil by the pond would yield the most positive interactions between the bacteria and the soil by the pond would yield the most positive interactions between the bacteria and plant roots was supported. While all three soil locations had interactions with bacteria, it is most likely that the plant in the soil from the pond had an interaction with Rhizobium bacteria. I was not surprised by the finding of root nodules on the pond plant but was surprised that there were no nodules formed on the positive control plant. This could be because of human error when removing the roots of the plant or as a result of unfit soil conditions. In the future, this data can be used to determine what kinds of growing conditions are optimal for bacteria root interactions which can lead to faster growing, healthier plants. Future research that could be done on rhizobia bacteria and legume plants could include what other bacteria benefit plant growth, do different types of plants interact with rhizobia differently and how can nitrogen be utilized in an artificial way to promote plant growth.

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Mohammed Sharar

**Legume Plant Microbe Project in Wichita Falls, TX**

**ABSTRACT**
The purpose of this experiment was to determine the different types of rhizoids, other bacteria, fungi, or any other types of microbes present on legume plants from two different sites in Wichita, Texas. Soil was collected from two sites and 2 pots were filled with soil from one site and the 1 pot was filled with soil from the other site. After soil collection, holes were made so that seeds could be planted. For the positive control pot, commercial inoculant was used when planting the legume seeds. After initial planting, plants were watered every other day. After the legumes sprouted, they were tested for different types of microbes.

**INTRODUCTION**
I hypothesized that the microbes present in the 3 pots would be bacteria and possibly fungi. I expected bacteria to be on the roots and the stem of the legume plants.

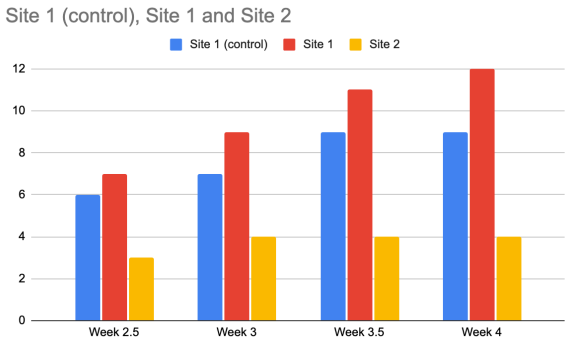
**MATERIALS AND METHODS**
The materials for this experiment were gloves, goggles, shovels, trowels, water, pots, soil, legume seeds, commercial inoculant, microscope, and cups.

Multiple factors were considered when identifying soil collection locations. One factor was the density of the soil. If the soil was very dense then the legumes would not be able to sprout from the soil properly. The next factor considered was the retention of water in the soil. This is how moist the soil stays after watering it. This is important because legumes require damp and frequently watered soil to thrive. Additionally, the sites needed to be far enough apart so they weren't the same soil. Because of these requirements I used soil from my backyard for site 1 including control. For site 2 I used soil from across the street from my house. After gathering the soil, I planted 15 seeds into each pot and watered them. For the first couple of days I watered every other day. However, site 2 seemed to dry out very quickly so I watered every day after realizing. The legumes sprouted in each pot besides site 2. Both site 1 and the positive control, which had added commercial inoculant, has at least 3 plants with the positive control having 4 plants. Because site 2 had no plants, I decided to transfer the extra one in the positive control to site 2. Since then all the legumes in each pot were thriving.
The next step included phenotyping 1 legume from each plant and observing their shoots. A pinkish shoot shows that proper nitrogen fixation occurred and having root nodules showed that beneficial bacteria was in the soil. To phenotype the plant, I took out 1 plant from each pot carefully without breaking any roots. After they were taken out I rinsed them with water and then measured their lengths. After that I used the microscope to get a closer look at the roots and shoots. All of the plants besides the ones from site 2 had pink roots. After this, another experiment took place to determine the presence of bacteria in the plants. Using diluted bleach and a petri dish, I observed the bacteria from each plant.

**RESULTS**
The positive control and the site 1 plants had root nodules form and had bacteria present. Site 2 on the other hand did not have any formation of root nodules and no bacteria. The shoots were also pink-purple in color for the positive control and site 1 unlike any of the legume plants from site 2. From these observations, it can be determined that there were beneficial rhizoids in the soil of site 1. However, if site 2 had any, they were not

beneficial to the plant and did not promote the growth of the legumes despite many being planted. From this, it can be concluded that site 2 is inadequate for the growth of legumes. Site 1 had legumes up to the length of 12 centimeters in both the positive control and the pot without the commercial inoculant. In the case of site 2 however, the legumes all stopped growing at an early stage and the tallest legume from site 2 was 4 centimeters.

**Graph of Growth in Weeks**




**DISCUSSION**

In this experiment, I realized that the soil near our homes was better for growing plants for many reasons. The soil from site 2 was a lot worse in quality because of its inability to retain water in the soil. Legumes could barely grow in the pot for site 2 because of harsh conditions. This was unexpected because of one reason in particular: there was plentiful grass growing in soil from site 2 so I assumed that legumes would grow. However, this assumption was wrong. Only site 1 was able to grow plants without any need for transferring an already grown legume.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Renee Jia

# The Effects Of Previously Added Fertilizers on Rhizobium-Legume Symbiosis in Johns Creek, GA

**ABSTRACT**

The rhizobium-legume symbiosis has received increased attention as it is largely regarded as one of the best ways to improve the quality of soil. The goal of this research is investigating how environmental conditions can affect this symbiosis. The effect of previously added fertilizer in soil on the Rhizobium-Legume system is tested, and reveals that the added fertilizer is detrimental to the development of nodules on the plant roots. The experiment conducted took soil from two different locations in Johns Creek Georgia: the front yard where there was frequent gardening and many fertilizers, and the backyard where there were no fertilizers. The plants were grown under stable conditions and watered frequently. After 4 weeks the roots were compared, and the plants that were grown in soil from the backyard had many more nodules than plants that were grown in soil from the front yard.

**INTRODUCTION**

Rhizobia is the term used for the nitrogen-fixing soil bacteria that produce nodules on the stems of legume plants (Wang, 1970). Inside these nodules is a structure called the symbiosome that is made up of polymorphic cells called bacteroids. Symbiosome allows for the nitrogenase enzyme to reduce elemental nitrogen to ammonia in the bacteroids. This relationship benefits both the legumes and the rhizobia. The legume plant supplies the rhizobia with carbon and energy, and in turn the rhizobia gives the plant nitrogen nutrients that are made by taking nitrogen from the atmosphere and adding it to amino acids (Wang, 1970).

These nitrogen fixing systems play an important role in improving the quality of low-nitrogen soils. One of the most studied systems is the Rhizobium-legume symbiosis. Understanding this symbiosis and how it can be affected by outside conditions is significant because this symbiosis is believed to be one of the best solutions to improving soil quality. Previous research has shown that fertilizers can be harmful to the Rhizobium-Legume system in arid and extreme climates (Zahran, 1999), however there is little known about the effects of fertilizers on the system in a temperate climate such as Johns Creek, Georgia. Therefore, the purpose of this research project is to fill this gap and identify if the fertilizers are still detrimental outside of extreme weather conditions.

We examined the effect of fertilizer on Rhizobium-legume symbiosis by planting legume seeds in fertilized soil frequently used for gardening, as well as soil from a different location that did not have fertilizers added. Both locations were in Johns Creek, Georgia.

**MATERIALS AND METHODS**

Three pots were filled using soil from different places in Johns Creek, Georgia. In pot 1, soil was collected from the front yard using a shovel. This soil was fertilized and had undergone frequent gardening. Pot 2 was filled with soil collected from the backyard. The soil from the backyard had no fertilizers added. Pot 3 was the positive control. It was filled with soil from the backyard (non-fertilized) and commercial inoculants were added.

Lastly, crotalaria juncea (legume) seeds were added to all 3 pots. The pots were placed in the sun and watered daily.

At 3 weeks post-planting, shovels were used to dig out the sprouts and measure them.Then, they were replanted into the soil.

At 4 weeks post-planting, the plants were dug out again using a shovel, and this time after

measuring the plants, the roots were magnified using a camera and examined. In order to isolate and examine the rhizobial bacteria, the root nodules were then sliced off with a scalpel and sterilized with common household bleach. They were crushed and using a q-tip spread onto a petri dish. These petri dishes were wrapped in aluminum foil and placed on a window sill. Over the course of the next 3 days, these petri dishes were examined and photographed.

**RESULTS**

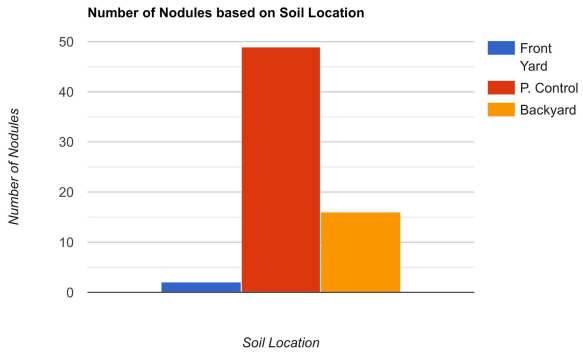
Plants at 4 weeks post-planting:

Positive control at 4 weeks post-planting: 25 cm

Plant from backyard at 4 weeks post-planting: 26.3 cm

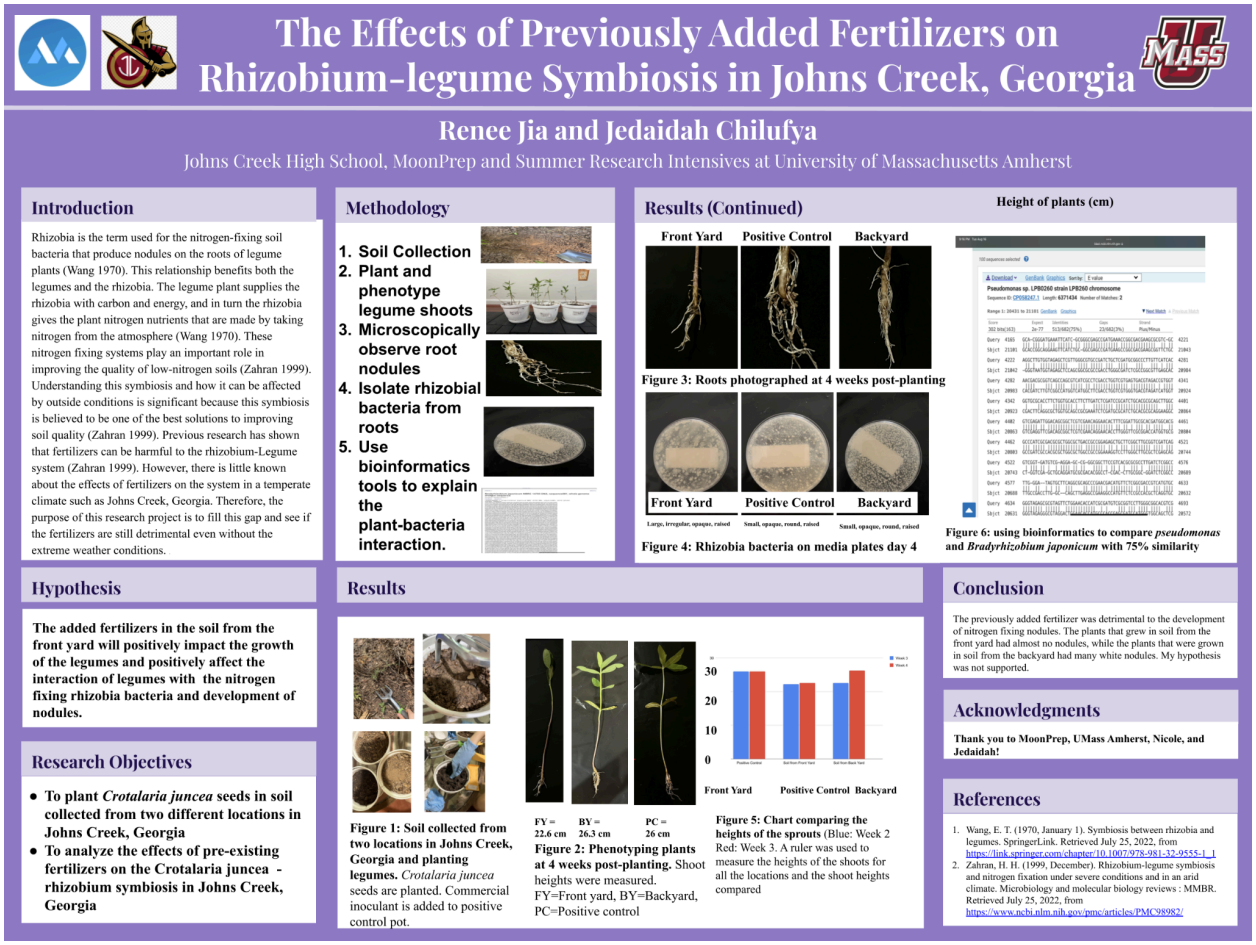
Plant from front yard at 4 weeks post-planting: 21 cm

Out of the three pots, the positive control had the most nodules (49), while the plants grown in soil from the front yard had the least (2). The plants grown in soil from the backyard had 14 nodules. This suggests that the previously added fertilizer was detrimental to the development of nodules.
Additionally, the agar media plates showed that the plants grown in soil from the backyard had the most rhizobia bacteria while the plants grown in soil from the front yard had the least.




## DISCUSSION

The results show that the pre-existing fertilizer in the soil was detrimental to the rhizobium-legume symbiosis and the development of nodules on the roots of the legume plants. These results support similar studies in this field. Further research is necessary to test other environmental factors that are potentially harmful to the Rhizobium-Legume symbiosis. I was very surprised by the results of the experiments as I believed the pre-existing fertilizers would aid in the development of the nodules. I was also surprised when the pot with soil from the backyard (non-fertilized) had significantly more sprouts compared to the pot with soil from the front yard (fertilized), because the backyard soil is very dry and sandy. This research adds to existing knowledge on factors that affect the nitrogen fixing system's ability to improve the quality of soil.



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Ria Narang

**Determining the Microbiome Effects on Legume Plants in Troy, MI**

**ABSTRACT**

In order for plants to thrive in different environments, they must form beneficial relationships with the bacteria in the soil. The purpose of this research was to determine the presence of the nitrogen-fixing bacteria, rhizobia, in the soil, and its interaction with crotalaria juncea legume plants in Troy, Michigan. Rhizobia, a group of bacteria that aids legume plants to obtain nitrogen, is a vital element for the growth of plants. In this research project, soil was picked in three pots from two different locations, with one of the pots kept as a positive control. The positive control pot had commercial inoculant added to it, which contained Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and Rhizobium leguminosarum biovar viceae. Fifteen crotalaria juncea legume seeds were added to each pot, and the growth of the plants in each pot was monitored throughout the research course. Several procedures were done, including monitoring plant growth, phenotyping plant roots and shoots, microscopically observing plant roots, isolating bacteria from legume roots, and using bioinformatics tools to explain the legume-rhizobia relationship. At the end of the course, none of the plants contained any nodules. This may have been the case if the plants were taken out too early, and there wasn't enough time for a relationship to form. Other potential factors include the type of soil, temperature, other bacterias present in the soil, nitrogen levels in the soil (either too much or not enough nitrogen), and a lack of nutrient content. Aside from the positive control pot, the soil from the front yard in Troy, Michigan, proved to be the most beneficial in the growth of crotalaria juncea legume plants as seen by the shoot heights of the plants, as well as the bacteria on the agar media plates.

**INTRODUCTION**

The microbiome consists of both beneficial and potentially harmful bacteria, which is essential for plants as it can enhance or decrease co-existing species and influence complete plant ecosystems (Wang et al., 2012). Microbiomes directly impact plant growth by their ability to improve plant nutrient uptake through multiple biochemical processes, such as nitrogen fixation (Berg, 2014). Legumes are able to form a symbiotic relationship with the nitrogen-fixing bacteria called rhizobia, which results in nodules on the plant root (Via et al., 2016). These nodules capture nitrogen from the atmosphere and transform it into ammonia, which the plant then uses to grow and thrive in different environments (Flynn, 2015). This study aimed to address what the effect of the microbiome was on legume plants in Troy, Michigan. My hypothesis was that damper soil from a pond in Troy will contain more rhizobia in comparison to drier soil. Through monitoring plant growth, phenotyping plant roots and shoots, microscopically observing plant roots, isolating bacteria from legume roots, and using bioinformatics tools to explain the legume-rhizobia relationship, we can effectively answer if rhizobia are present in soil in Troy, Michigan.

**MATERIALS AND METHODS**

*Collecting soil, planting legume seeds and monitoring plant growth*

Lab space was stationed in a window to have even light exposure. Gloves were worn for each experiment and the station was sterilized between steps. First, soil was collected from two different locations in Troy, Michigan, by digging a hole six inches deep. Three pots were filled with soil; Pot 1 from the front yard, and Pot 2 and 3 from the backyard near the pond. All three pots were filled to

about ½ an inch below the brim of the pot. Commercial inoculant was added to pot 3 for the positive control pot.
Before switching locations, I sterilized the digging tools using 75% ethanol wipes. Sterilization between locations was kept consistent throughout the experiments in this course to obtain the most accurate results.
In each pot, 15 holes were made in the soil and crotalaria juncea legume seeds were inserted into each hole. In the positive control pot, a pinch of commercial inoculant was added on top of the seed in each hole. Each hole was then covered, and each plant was watered. By post-planting day five, there was at least one sprout in each pot.
There was continuous observation of germination and the number of sprouts in each pot was recorded. At two weeks post-planting, additional legume seeds were planted to ensure that each pot had at least three plants.

*Phenotyping plant shoots and roots*
The plant shoots of the tallest plant from each pot were phenotyped and recorded for heights (in cm). Excess water was added to each of the pots to make the soil softer. One plant from each of the pots was removed and placed next to a 30 cm ruler on black construction paper. This was to visualize the plant's height. Microscopic observation of the roots of the plants was done to check for any root nodules.

*Isolating bacteria from plant roots*
Agar media plates were used to isolate bacteria from legume roots. Materials used included 70% ethanol wipes, a scalpel, tweezers, paper towels, five microcentrifuge tubes, five plastic pestles, three cotton swabs, water, bleach, and three agar media plates. Three microcentrifuge tubes were filled with 2 mL of water each. One additional tube was filled with one drop of bleach and 1.5 mL of water (for sterilization). The root of the first plant was cut using a scalpel, placed into the bleach and water mixture, and shook five times. Using tweezers, the root was then transferred into the tube with water, and shook 10 times. Lastly, the root was placed into a microcentrifuge tube, and crushed with a plastic pestle. 0.5 mL of water was added to the tube which was transferred onto an agar media plate. With a cotton swab, the root and water mixture was spread over the agar media plate, and air dried for 10 minutes. The process was repeated with all three plants, and each agar media plate was wrapped in aluminum foil and placed outside down near a window.

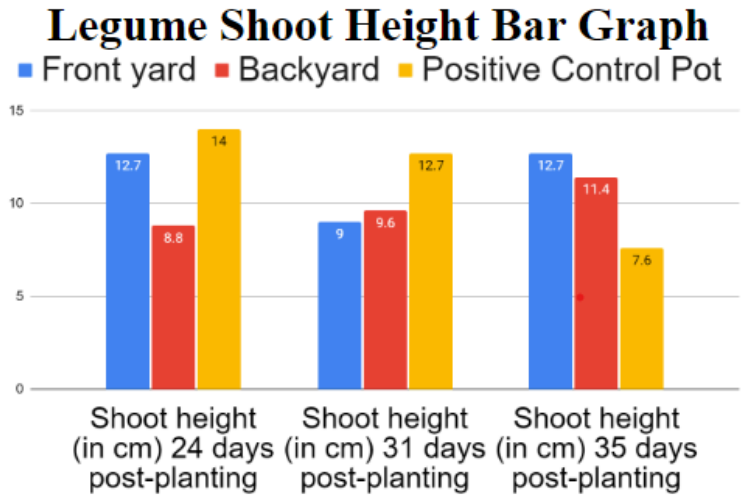
*Using bioinformatics tools*
In the final week, bioinformatic tools were used to do DNA sequencing, and determine the similarities and differences of the bacteria we suspected to be present to the bacteria in the commercial inoculant.

**RESULTS**

Table 1: Phenotyping plant shoots and roots and isolating bacteria from root

| | Shoot Height | Microscopically observing roots | Data from agar media plates |
|---|---|---|---|
| Front yard | 24 days post-planting: 12.7 cm<br>31 days post-planting: 9 cm | No nodules are present on any of the plants throughout the weeks<br>The roots of the plants are primarily white | Day 1: Several flat, circular, white colonies are present<br>Day 2: More flat, circular white colonies have appeared and are more visible |

| | | | |
|---|---|---|---|
| | 35 days post-planting: 12.7 cm | | Day 3: Numerous flat, circular, white colonies are shown |
| Backyard | 24 days post-planting: 8.8 cm<br>31 days post-planting: 9.6 cm<br>35 days post-planting: 11.4 cm | No nodules are present on any of the plants throughout the weeks<br>The roots of the plants are primarily a light pink | Day 1: No colonies visible on the agar media plate<br>Day 2: No colonies visible on the agar media plate<br>Day 3: No colonies visible on the agar media plate |
| Positive control | 24 days post-planting: 14 cm<br>31 days post-planting: 12.7 cm<br>35 days post-planting: 7.6 cm | No nodules are present on any of the plants throughout the weeks<br>The roots of the plants are primarily a light pink | Day 1: Several flat, circular and irregular, white colonies are present<br>Day 2: More flat, circular and irregular, white colonies are present<br>Day 3: Numerous flat, circular and irregular, white colonies are present |




Shoot height differences (in cm) 24 days post-planting, 31 days post-planting, and 35 days post-planting

**Recording Phenotyping plant shoots and roots**

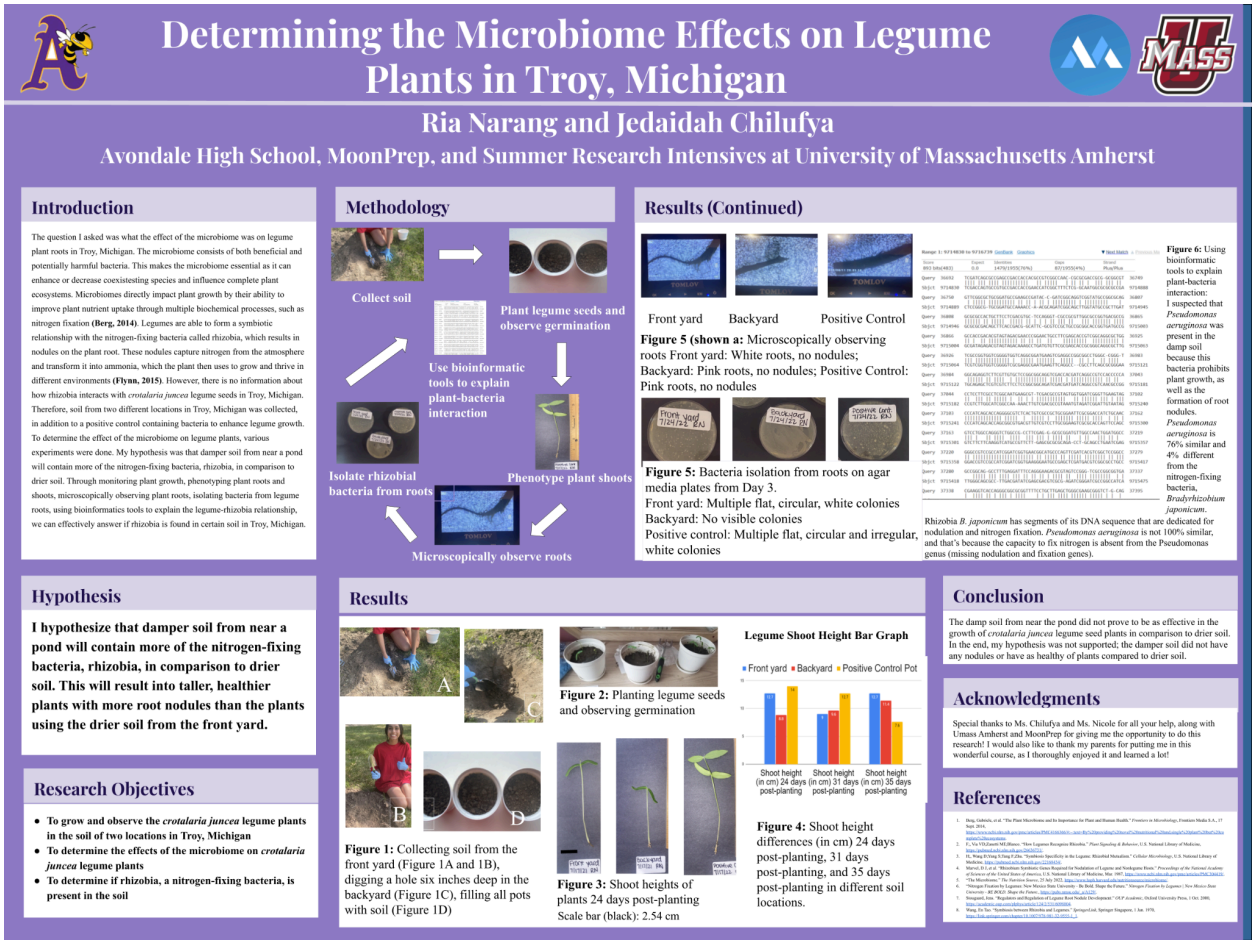
| Location | Front yard | Backyard near a pond | Positive control (soil from back yard near a pond) |
|---|---|---|---|
| **3.5 weeks post planting**<br>**Date: 7/17/22**<br><br>**24 days post-planting** | Four green healthy sprouts, one wilting sprout, and many incoming sprouts<br><br>**Shoot height: 12.7 cm** | Four sprouts and many incoming sprouts<br><br>**Shoot height: 8.8 cm** | Three green healthy plants<br><br>**Shoot height: 14 cm** |
| **4.5 weeks post- planting**<br>**Date: 7/24/22**<br><br>**31 days post-planting** | Many plants in this pot, some are starting to wilt but the rest are healthy and green<br><br>**Shoot height: 9 cm** | Three plants in this pot, the leaves are falling and they are starting to wilt<br><br>**Shoot height: 9.6 cm** | Four healthy plants and some incoming sprouts, along with some wilting sprouts<br><br>**Shoot height: 12.7 cm** |
| **5 weeks post-planting**<br><br>**Date: 7/28/22**<br><br>**35 days post-planting** | Few plants remaining of decent size, plants starting to wilt and die<br><br>**Shoot height: 12.7 cm** | Few remaining plants, some plants starting to wilt and die<br><br>**Shoot height: 11.4 cm** | One remaining plant, many were taken out/died<br><br>**Shoot height: 7.6 cm** |

**DISCUSSION**

The soil from the front yard yielded the best results (apart from the positive control pot), as seen by the shoot heights and agar media plates. While none of the plants had any nodules, the soil from the front yard still had the overall best plant growth as shown by the shoot height of the front yard (12.7 cm) compared with the backyard (which had shoot height of 8.8 cm).

Additionally, after isolating bacteria from plant roots growing in the different locations, the soil from the front yard had several flat, circular, white colonies within a day. This shows that bacteria in this soil entered the plant roots. However, these bacteria did not cause the formation of root nodules. This result suggests that the bacteria found isn't rhizobia. If the bacteria is rhizobia, then some factors in the soil or in the plant stopped them from making root nodules. In contrast, the backyard did not show any colonies even after three days. This shows that no bacteria was isolated from the roots growing in this soil. The backyard soil also had the least number of plants, with the least amount of overall growth. I was surprised by the results as I had initially thought the soil from the backyard would give the best results. The backyard soil was collected from near a pond, so I felt that plants would thrive in the damper soil. However, my hypothesis was not supported by the results, and the drier soil from the front yard had a better outcome.

In summary, the soil in the front yard proved to be more beneficial for crotalaria juncea legume plants in comparison to the soil in the backyard near a pond in Troy, Michigan.

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Rucha Bhat

# Microbes Affecting the Soil in Portland, OR

**ABSTRACT**
The purpose of this project was to see how microbes from different soils affected the growth of legume seeds. Using soil from two different locations, it was placed in three different pots, one pot being positive control and the other two pots being regular soil. We put 15 legume seeds in each pot and observed the growth of those plants in each pot. The soil from the forest grew the legume seeds the best.

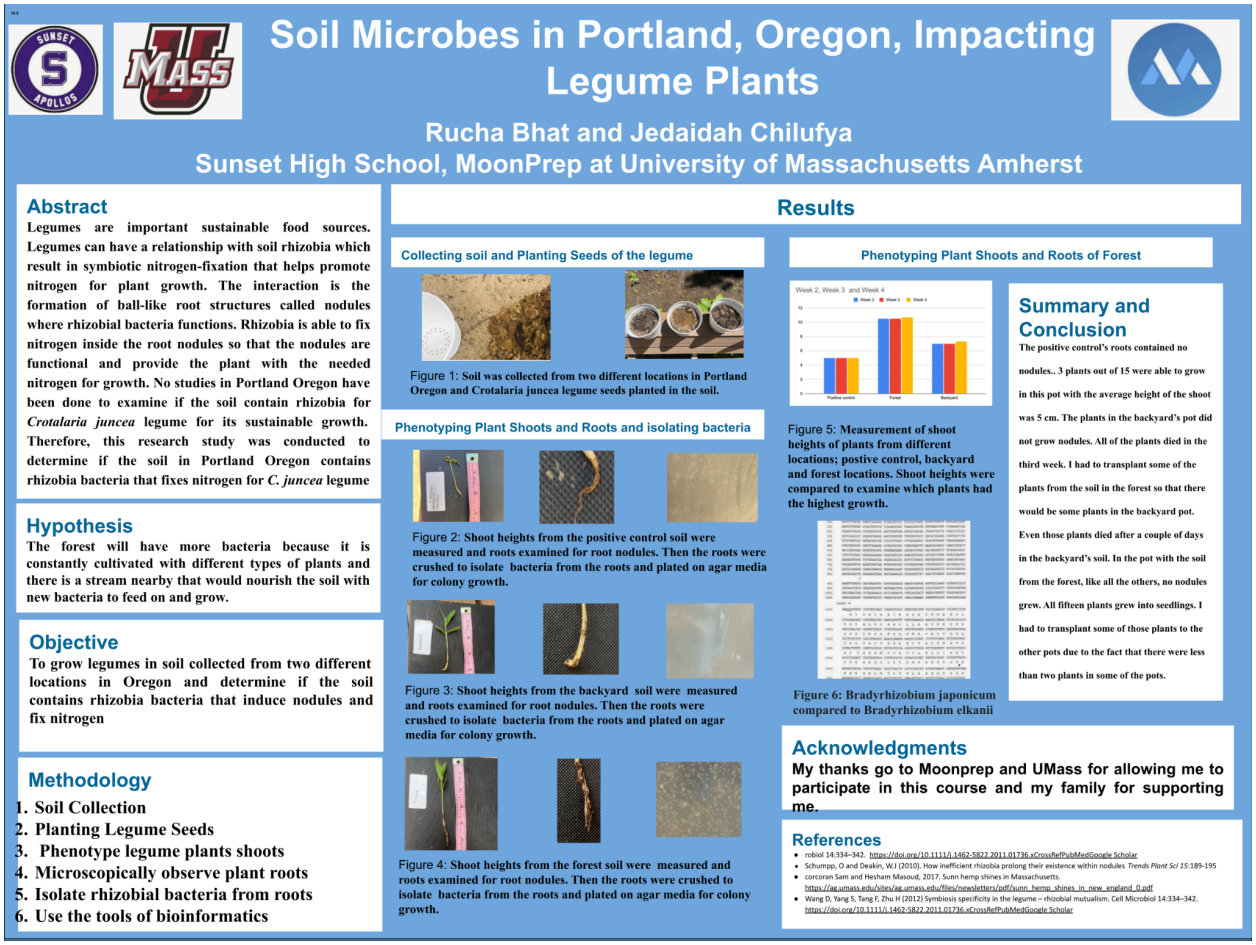
**INTRODUCTION**
Water is becoming more scarce and food insecurities are rising because of global warming and CO2 emissions. Legumes are important sustainable food sources. Legumes have a relationship with soil rhizobia which result in symbiotic nitrogen-fixation that helps promote nitrogen for plant growth. The interaction is the formation of ball-like root structures called nodules where rhizobial bacteria functions. Rhizobia is able to fix nitrogen inside the root nodules so that the nodules are functional and provide the plant with the needed nitrogen for growth in Portland Oregon, for its sustainable growth. Observations show that soil in Portland Oregon shows rhizobia that creates nodules on the new crop, but the nodules are white and therefore do not fix nitrogen for the legume. The positive control (commercial inoculant) involves rhizobial bacteria known to create nodules in other legumes, creating pink nitrogen fixing nodules on these legumes.

**MATERIALS AND METHODS**
First, we collected soil from 2 different locations and placed them in 3 identical pots. In one pot, we put a positive control and we didn't do anything in the other two pots. Next, we placed 15 legume seeds in each pot and we watered them every other day and monitored the process by taking photos. In the third week of monitoring the plant growth we planted and phenotyped legume plants shoots and roots, and microscopically investigated the plant roots using a digital microscope. We also isolated rhizobial bacteria from roots and root nodules of the legume and observed how the roots differed from each of the pots by using the tools of bioinformatics to explain plant-bacteria interactions to analyze the root nodules and shoots.

**RESULTS**
The positive control's roots contained no nodules. Three plants out of fifteen were able to grow in this pot with the average height of the shoot was 5 cm. The plants in the backyard's pot did not grow nodules. All of the plants died in the third week. I had to transplant some of the plants from the soil in the forest so that there would be some plants in the backyard pot. Even those plants died after a couple of days in the backyard's soil. In the pot with the soil from the forest, like all the others, no nodules grew. All fifteen plants grew into seedlings. I had to transplant some of those plants to the other pots due to the fact that there were less than two plants in some of the pots.

**DISCUSSION**
There were some unforeseen circumstances where it was more difficult to grow the plants. Oregon is known for their plentiful birds and animals. In my backyard, where I kept the pots to grow, there are usually a lot of birds flying about. For a week, I didn't see any seedlings growing out of the pots. When I checked the pots, all of the seeds were gone. I had to replant everything and start all over again. If I did this experiment over again, I would purchase a net where I could cover my pots so that the sun could get in but the birds would stay out. I couldn't change the location of the pots because my house doesn't get a

lot of sunlight and the backyard is one of the only places where there is constant sunlight. When I transplanted the plants from the forest soil to the backyard soil, I might have not rinsed off all of the soil well enough so there might have been some cross contamination

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Ryma Saha

# Determining Relationship Between Legume Seeds and Rhizobia Bacteria in Different Locations from Mechanicsburg, PA

**ABSTRACT**

The farming industry continues to change in order to improve crop productivity and maintain soil quality. Rhizobia is a nitrogen-fixing bacteria that interacts with the legume seed, Crotalaria juncea, and improves soil quality, prevents soil erosion, and most importantly, recycles the plant nutrients by converting atmospheric nitrogen into ammonia. This nitrogen-fixing process produces important nutrients in the soil for plant growth. This experiment investigates the ecological symbiotic relationship between legume seeds and Rhizobia bacteria in different soil from Mechanicsburg, Pennsylvania. This study began with collecting three pots of soil from two different locations. Legume seeds were planted and commercial inoculant was added to one of the pots for positive control. Each pot was observed weekly for germination and plant growth. The success of the symbiosis was measured by observing the number of the plant grown in each pot, root nodules and height of shoots, and bacteria isolation from the nodules and roots was observed. Our findings aim to improve the understanding of the relationship between Rhizobia and Crotalaria juncea to increase the productivity of legume growth in colder climates, and thus potentially yielding more robust crops. The result showed that the location of soil made a great impact. Finally, the Rhizobia DNA sequences were compared with the pseudomonas DNA sequences and found an eighty-nine percent similarity which means this bacteria also played a role in the nitrogen fixing relationship.

**INTRODUCTION**

Soil is the most diverse microbial ecosystem which contains millions of microorganisms like bacteria, viruses, fungi, protozoa, and nematodes. These microbial communities are essential to plant health and our environment. Legumes account for 26% of global crop production and are an important part of the nitrogen cycle. Legume crop productivity often relies on a symbiotic relationship between the roots of legumes and nitrogen fixing bacteria known as Rhizobia (Via, 2016). This allows legume plants to prosper, even if the soil is not rich in nitrogen. This symbiotic relationship can improve agricultural production by decreasing the use of chemical fertilizer and thus increasing global food protection.

The Crotalaria juncea, or Sunn hemp, is a green manure which adds nutrients to the soil and improves the long-term soil quality unlike the other chemical fertilizer used for short term growth. It is important to understand this mutualistic relationship between plants and microbes because it is very host specific and gene specific of soil bacteria. Root nodules are formed when legume plants allow Rhizobia bacteria to infect their roots. Inside the root nodules where nitrogen converts into ammonia for better plant growth in the absence of nitrogen in the soil (Lindström, 2019). On the other hand, Rhizobia receives carbohydrates for energy from plants. There is limited research on the interaction between Rhizobia bacteria and the legume plants in the soil of Mechanicsburg, PA. Also it is still unclear to the farmers which Rhizobia strains should be inoculated in legumes for the highest growth rates. This research is about the observation of the relationship between rhizobia bacteria and Crotalaria Juncea in different soil in mechanicsburg, PA. This study will encourage local Mechanicsburg farmers to inoculate Sunn hemp with commercial Rhizobia in order to achieve high crop productivity with less usage of chemical

fertilizer to maintain soil quality in colder climates.

**HYPOTHESIS**

It is hypothesized that more bacteria will be present in my high school location than my neighborhood due to better quality soil and less plants in my newly built neighborhood. Also, soil in positive control would interact best with legume seeds due to inoculation and have most germination and nodules.

**MATERIALS AND METHODS**

The materials that were used for this experiment were legume seeds, soil, water, commercial inoculant, shovel, fork, gloves, sanitizing wipes, tupperware, digital microscope, ruler, a black clipboard for phenotyping. Agar plates, scalpel, microcentrifuge tubes, and plastic pestels were used to isolate and grow the bacteria. Bleach and water was used to sterilize the root. First, soil was collected from two different locations and put into pots. Next, three pots were labeled with their location. One pot was a positive control where commercial inoculant was added to the soil from neighborhood location while the highschool and other neighborhood pot were experimental. Fifteen legume seeds were planted, watered daily, and germination was observed. During the second week, Phenotyping of the shoots and roots was done by using a ruler and a digital microscope. The root nodules were observed microscopically in the third and fourth week. The bacteria was isolated after crushing the segment of the root and the root nodules into the microcentrifuge tube by a plastic pestle after sterilizing the root. Suspension was made by adding water into the tube and adding it to the general solid agar plate. The agar plate was placed by the window to grow at room temperature. Finally bioinformatics tools, NCBI blast search, was used to explain plant bacteria interaction

**RESULTS**

The positive control pot had the most surviving plants compared to the other two location pots. At the end of week 4, the positive control pot had the most plants remaining: six. There were three plants remaining at the end of week four for both the neighborhood and highschool location pots. The control pot had the tallest plant and most healthiest looking plant in comparison to the other two pots. The positive control's tallest plant was 18.5 cm, the neighborhood's tallest plant was 17 cm, and the highschool's plant was 17.5 cm at the end of the four weeks. Finally, the root nodules were observed in the third week and fourth week. In the third week, the positive control pot had three nodules, the neighborhood pot had three nodules, and the highschool pot had two nodules. In the fourth week, the positive control had seven nodules, the neighborhood pot had two nodules, and the highschool pot had zero nodules. All the nodules were white. Finally, bacteria grew from the agar plate (figure 5) in all three locations. The highschool location had the most bacterial growth and the least number of colonies were in the positive control. Although the bacteria is unknown, there were many circular shaped bacteria which were very similar to the positive control agar plate. The neighborhood soil had better quality than the highschool soil but overall the positive control did the best. NCBI blast search tool was used for comparing the Rhizobium japonicum DNA sequences with the pseudomonas aeruginosa DNA sequences and found an eighty-nine percent similarity which means this bacteria also played a role in the nitrogen fixing relationship.

| **Location** | **Positive control** | **Neighborhood** | **Mechanicsburg High School** |
|---|---|---|---|
| **2 weeks post planting**<br>**Date: 7/12/22** | Shoot: 15.5 cm<br>Root: 5 cm<br>Plants: 7 plants | Shoot: 16 cm<br>Root: 8 cm<br>Plants: 8 plants | Shoot: 14 cm<br>Root: 9 cm<br>Plants: 5 plants |

| | | | |
|---|---|---|---|
| **3 weeks post planting**<br>**Date: 07/20/22** | Shoot: 17 cm<br>Root: 8 cm<br>Nodules: 3 nodules green looking<br>Plants: 8 plants | Shoot: 16.5 cm<br>Root: 2 cm<br>Nodules: 3 yellowish<br>Plants: 7 plants | Shoot: 15.5 cm<br>Root: 4 cm<br>Nodules: 2 white nodules<br>Plants: 4 plants |
| **4 weeks post planting**<br><br>**Date: 07/25/2022** | Shoot: 18.5cm<br>Nodules: 7<br>Plants: 6 plants | Shoot: 17cm<br>Nodules: 2 white color<br>Plants : 3 plants | Shoot: 17.5cm<br>Nodules: 0<br>Plants: 3 plants |
| **Media Plate** | No growth | No growth | No growth |

**DISCUSSION**

The highest number of plants was noted in the neighborhood location in comparison to the other two locations. The plants survived longer in the positive control pot, most likely because it was inoculated with Rhizobia. The tallest plant observed was 18.5 cm in the positive control. This could be because of the rhizobia in the inoculant which stimulated plant growth. The highest number of nodules measured in the positive control pot was 10.

All nodules were white meaning the nitrogen fixation did not occur.

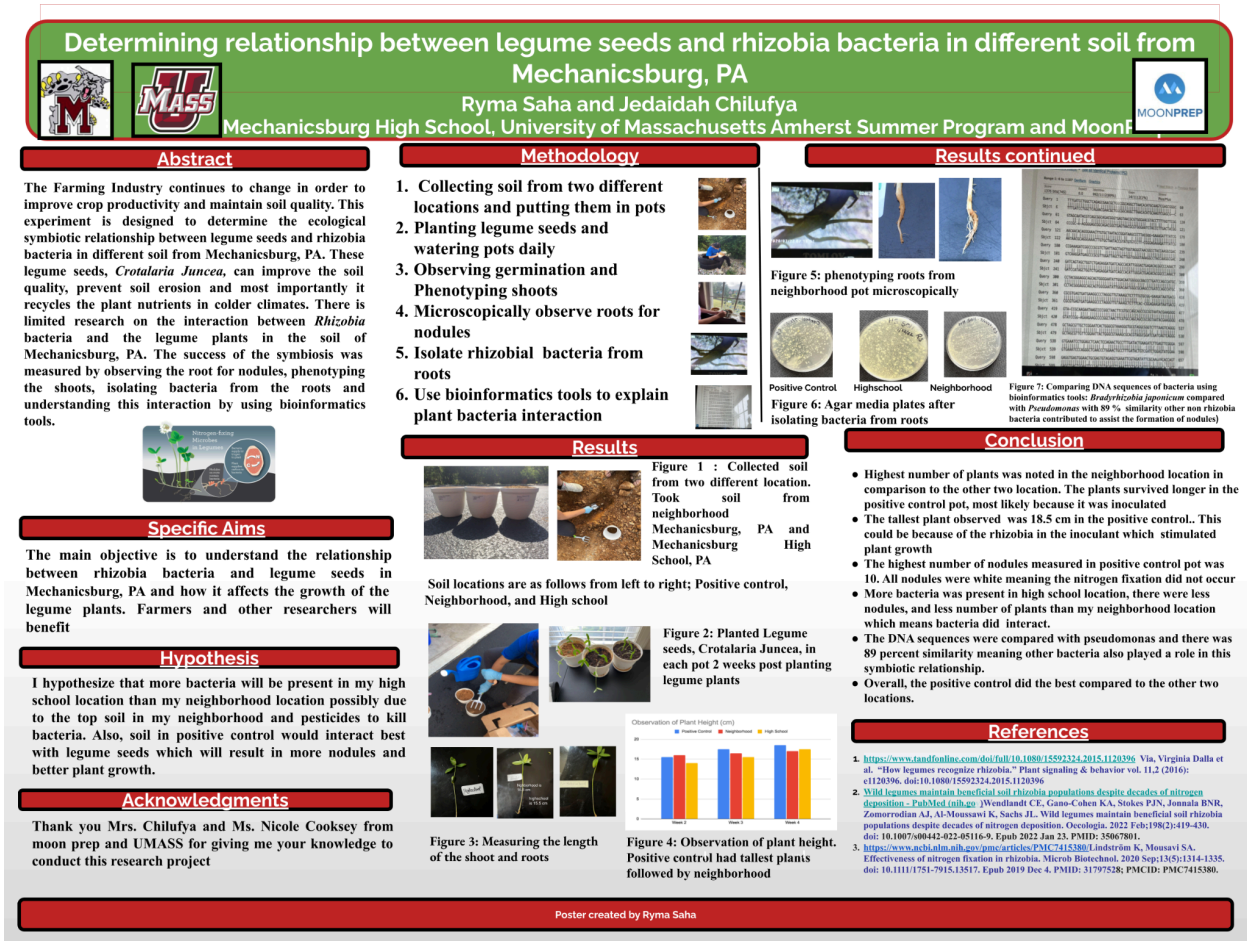
The most bacteria was present in the high school location but there were less nodules and a less number of plants compared to the neighborhood location. This means bacteria interacted with the legume plants but did not go through the nitrogen fixing process resulting in white nodules or most likely the soil had enough nitrogen. The size, shape, and color of the colonies were very similar to the positive control agar plate which indicates that both soil had rhizobia bacteria ( figure 5). As a result, the neighborhood soil was better quality than the highschool location soil. This is because there was a larger number of nodules, taller shoot height, and had a longer survival but overall, the positive control did the best.

The Rhizobia DNA sequences were compared with the pseudomonas DNA sequences (another common bacteria in soil) and found an eighty-nine percent similarity which means this bacteria also played a role in the nitrogen fixing relationship.

The result could possibly be skewed because two different locations, the Mechanicsburg High school and neighborhood Mechanicsburg PA, had a different quality of soil, dried soil condition, and difference in sunlight exposure. Another reason could be measuring error because some plants were bent. During the process of digging the plant out, the root may have gotten damaged which could have resulted in a less number of nodules.

The hypothesis was supported because adding rhizobia to the soil did promote the growth of the plant and more nodules in comparison to the other two experimental pots. The location of the soil plays an important role in planting and farming. Farmers should consider planting crops with specific strains of Rhizobia bacteria for better

plant growth, maintaining soil nutrient for future plants, and less use of chemical fertilizer in order to continue this nitrogen fixing cycle. This research project is important because farmers and other researchers could use this information about the nitrogen system to promote crop production. This experiment could be continued further by looking for rhizobia vs non rhizobia bacteria in different soil, presence of other accelerating and inhibiting factors of this symbiotic relationship in the soil, and understanding the molecular level of this symbiotic relationship.

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Satya Vasan

# Microbial Composition Analysis in Manalapan, NJ

**ABSTRACT**
The aim of this experiment was to conduct an analysis of the microbial interaction between crotalaria juncea legumes and rhizobia bacteria in Manalapan, New Jersey. Soil was collected from two different locations: location A (front yard with fertilizer) and location B (woods). A positive control sample with commercial inoculant was included. Approximately three weeks after initial planting, the roots and shoots of the plants were phenotyped. Additionally, through microscopy rhizobia bacteria were isolated. The original hypothesis was that the location of the soil sample B contained the most beneficial microbes.

**INTRODUCTION**
For an ecosystem to function at its utmost productivity, it needs to be blooming with diversity. According to the BEF theory, diversity is a powerful tool that increases selection effects, functional redundancy effects, and contributes to efficient usage of environmental resources (Benton et al., 2020). It is known that legumes, which have a symbiotic relationship with rhizobia, add to the net productivity by improving soil nitrogen level and soil food web complexity (Yang et al., 2021). Crotalaria juncea legumes are a tropical asian plant, thought to originate in India, and are a part of the Fabaceae family. In the world of agriculture, its primary purpose is to add organic matter to the soil. Rhizobia bacteria is a genus of Gram negative nitrogen-fixing soil bacteria that have a symbiotic relationship with legumes (Yang et al., 2021). The purpose of this is to aid in the formation of root nodules to convert nitrogen gas from the atmosphere into a readily used nitrogen by converting atmospheric nitrogen into soluble nitrates, nitrites, and ammonium compounds. However, much was not known about this endosymbiotic interaction in New Jersey; and more specifically, which soil type would have the most beneficial bacteria.

**MATERIALS AND METHODS**
Over the time-span of four weeks, four primary goals were achieved.

*(1) Plant and phenotype legume shoots and root nodules.*

Materials for this step included a shovel and fork, 3 planting pots, soil/dirt, legume seeds, commercial inoculant, tupperware with water, gloves , safety goggles, black construction paper, centimeter ruler, shovel, and stainless steel cup. The 3 pots were labeled with the soil location names; woods, and front yard in Manalapan, New Jersey 07726. To collect soil for the positive control, soil was collected from location 1, the front yard. To plant legume seeds, the fork was used to make about 15 holes into the soil. For the positive control, about a pinch of commercial inoculant was added into all the holes before planting the legume seeds in the holes. To phenotype of the plant shoots, first extra water was added to the first pot to soften the soil. Then one plant was selected - tallest plant, to phenotype. The plant was carefully removed and soil particles rinsed off by dipping the plant into a large tupperware with water. The roots were then tapped-dry using a paper towel and the plant placed on a black surface. A 30 cm ruler was then placed next to the plant and a label with the location name placed on the other side of the plant. After measurements were taken, the plant was then put back in the soil by digging a hole and placing the root section in the hole and then burying the root with soil.

*(2) Microscopic observation of root nodules*

A microscope was used to examine the root nodules after the phenotyping process was complete.

*(3) Isolation of rhizobia bacteria from root nodules*

Materials for this step included plants, digging tools, paper towels, beaker of water, flat surface with a dark background, ruler, labels and a pen or sharpie, microcentrifuge tubes, plastic pestles (blue), dropper, microscope slide, scalpel, sterile water in the falcon tube, household bleach, and agar media plates. A root segment was removed with scalpel and then placed in a microcentrifuge tube containing diluted bleach (0.2 ml bleach and water up to the 1.5 ml mark). The tube was shaken 5 times to sterilize the root. The root segment was placed in a microcentrifuge tube filled with water, which was shaken 10 times, to rinse off the bleach. The washed root segment was placed in a new microcentrifuge tube and a plastic pestle used to crush the root segment. After fully crushing the root segment, clean water up to the 0.5 ml mark was added to the tube and the crushed-root-water mixture or suspension, poured on an agar media plate. A cotton swab was then used to spread the suspension and the plate was left to dry. The agar plate was then wrapped in aluminum foil with the plate upside down and placed by the window. The steps were repeated for the other 2 pots (1 plant per pot). The plates were checked everyday and to check for bacterial growth.

*(4) The use of bioinformatics tools to explain our plant-bacteria interaction*

**RESULTS**

*Shoot Height*

At approximately 2.5 weeks post planting the legumes planted in the woods' soil were the tallest, with a maximum height of 16.0 cm. The legumes grown in the front yard soil proved to be the second tallest, with a maximum height of 11.4 cm; and the legumes from the positive control sample presented with a maximum height of 11.0 cm. However, at approximately 3.5 weeks post planting, the legumes planted in the front yard soil proved to be the tallest with the maximum height being 27.0 cm; the legumes planted in the woods' soil followed with a maximum height of 24.6 cm; the legumes planted in the positive control sample had a maximum height of 17.2 cm.

*Number of Sprouts*

From post-planting day 11, the germination output was closely monitored. The front yard soil produced the most sprouts: 6 sprouts, with one needing additional support to stand up straight. The positive control sample produced 5 sprouts, and the sample from the woods produced 4 sprouts. On post-planting day 23, the germination output was again recorded. However, only one sample experienced an increase in the amount of sprouts: the positive control sample gained one more sprout. On the other hand, the front yard soil now presented with one more plant that needed additional support to stand still. The woods sample still remained with 4 sprouts.

*Nodules Present*

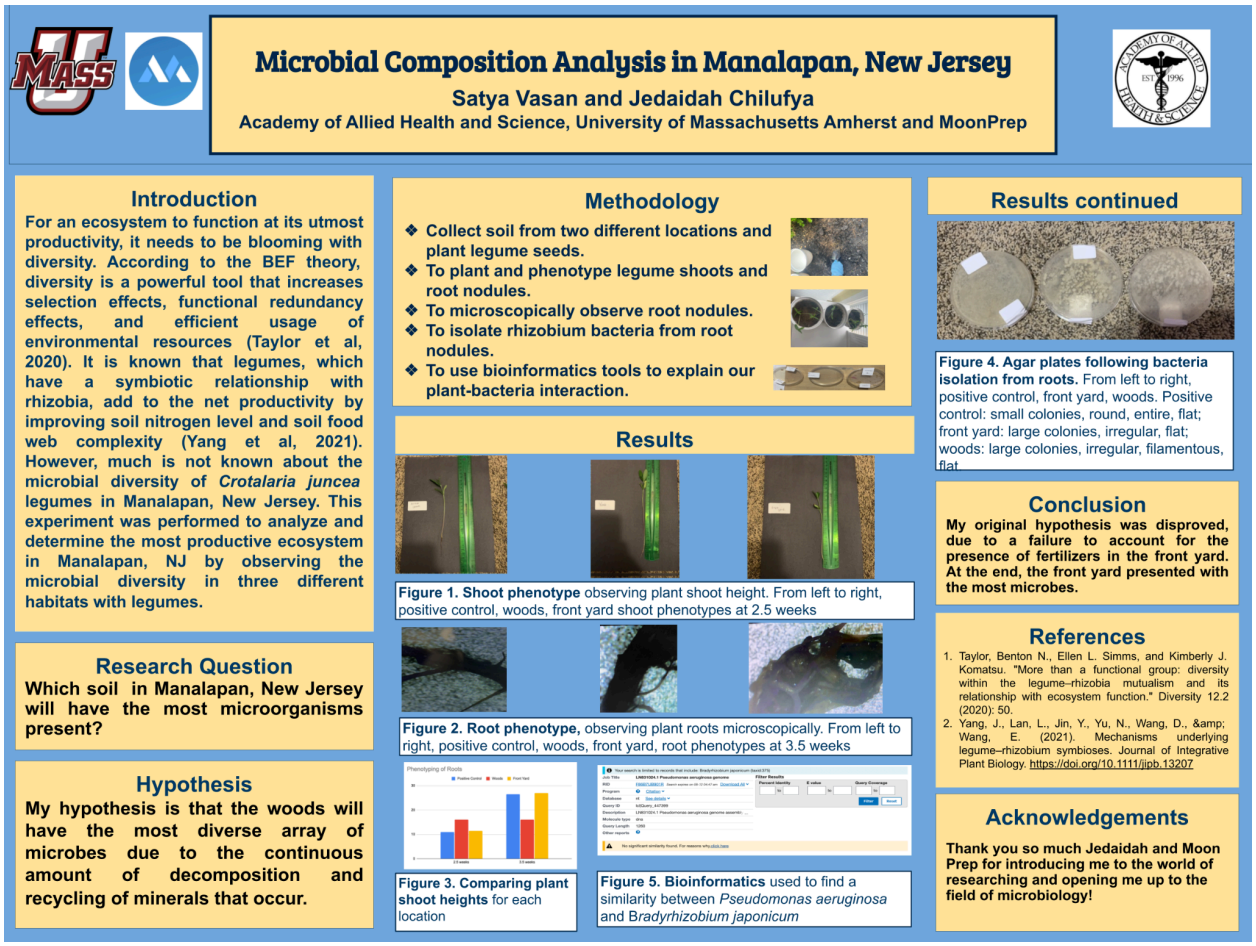
On day 26 post-planting, plants from each pot were phenotyped under the microscope. The samples from the woods and the positive control did not present with any nodules. The sample from the front yard presented about two nodules.

*Bacteria Present*

On day 30 of post planting, bacteria were isolated from the roots of one sprout in each plant. By day 1, all agar dishes were presented with bacteria. The bacteria from the front yard sample was the most numerous, clustered, and diverse. The bacteria was spherical in shape with different sizes. The bacteria from the positive control had the least bacteria present. These bacteria were much smaller in size compared to the bacteria from the front yard sample. The sample from the woods contained spherical and one rod shaped bacteria.

## DISCUSSION

The original hypothesis was that the soil sample that was from the woods would have the most beneficial bacteria. The reasoning behind this was that this ecosystem bloomed with the activities and processes of many living organisms. The woods is a home for decomposition and the recycling of essential minerals, such as carbon, phosphorus, and nitrogen. The soil from the front yard, in fact, had the most beneficial bacteria because this soil allowed for the formation of root nodules which allows the rhizobia bacteria to interact with the roots of the plant. This result can be accounted for by the presence of lawn fertilizer. Fertilizer labels include NPK ratios which include ratios of nitrogen, phosphorus, and potassium. This helps address the issue of the soil being too low in one nutrient. The soil in the front yard used a 39% nitrogen fertilizer, allowing for nodules to form more easily. In the future, this experiment can be altered by still working with three soil samples, but having one rich in nitrogen, one rich in phosphorus, and one rich in potassium to further observe the soil interaction with specific minerals and how that contributes to nodule formation.

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Shreya Sai Sunkara

# Identification and Impact of Microbes on Legume Plants in Plano, TX

**ABSTRACT**
The purpose of this experiment is to determine the impact microbes found in North Texas soil and soil conditions have on the growth of legume plants and the legume plant-rhizobia symbiotic relationship. 15 legume seeds were planted in three different pots each with soil from a different location. One pot served as a positive control and has commercial inoculant that contained different strains of rhizobia. The plants were watered every other day for 3 weeks and were kept in an area with sunlight to allow for the legumes to grow. After three weeks when the legumes became mature, the tallest legume plant from each pot was removed in order to measure the shoot of the legume. After phenotyping the shoots, the roots were then analyzed to determine the presence of root nodules which were then cut off and treated to isolate rhizobia bacteria to determine the amount of rhizobia present. It was determined that the soil acquired from the backyard lacked rhizobia whereas soil from the walkway and the soil with the commercial inoculant did have a significant amount of rhizobia.

**INTRODUCTION**
Plants are dependent on nutrients such as phosphorus, nitrogen, and sulfur in a bioavailable form. To facilitate nutrient uptake, mutualistic symbioses between plants and root-associated microorganisms such as bacteria have evolved. (Ledermann et al., 2021). The most common known symbiosis involves rhizobia bacteria which is known for fixing nitrogen, the process in which bacteria use solar energy to transform inert nitrogen gas to ammonia used by plants(Lindström et al., 2020). Rhizobia bacteria interact with legume roots by secreting signaling molecules called Nod factors that are perceived by LysM receptor kinase of root cells, thus indicating the presence of rhizobia in soil (Via et al, 2016). Various other microbes and soil conditions such as the presence of excess nitrogen, however, can impact the symbiosis between legumes and rhizobia, thus impacting the process of nitrogen fixation and the growth of the plant. There is little information about the effectiveness of rhizobia bacteria in regards to nitrogen fixation in Plano, Texas soil specifically. Therefore, this experiment will determine the impact various soil conditions, including the presence of other microbes and/or excess nutrients, have on plant symbiosis with rhizobia and therefore the effectiveness of nitrogen fixation in such soil conditions. It was hypothesized that if the soil the plants grow in is near a walkway, then there will be less rhizobia bacteria in that soil compared to rhizobia bacteria in the soil from the background due to the presence of fertilizers.

**MATERIALS AND METHODS**
The materials included digging tools (shovel and fork), sterile wipes, 3 planting pots, soil/dirt, legume seeds (Crotalaria juncea legume), commercial inoculant (with the active rhizobia Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum phaseoli, and R.leguminosarum biovar viceae), tupperware with water, ruler (in cm), labels, paper towels, digital microscope (TOMLOV Digital Microscope), laptop, and a phone (with camera), microcentrifuge tubes, scalpel, bleach, plastic pestel.

Soil from two different locations (the walkway and backyard) were added to 3 separate pots and water was added to each pot to moisten the soil. Pot 2 and 3 both received soil from the backyard; however, pot 3 (positive control) also had the addition of commercial inoculant. Next, using a fork, 15 holes were

pressed into the soil and one legume seed was added to each hole. The digging tools and gardening fork were sterilized to prevent contamination of different soils. Then the soil was smoothed over each hole to cover the seeds. A layer of commercial inoculant was added to the soil for the positive control. For the first week, the seeds were watered every day.

After the end of the first week, the pots with the plants were photographed to take note of the growth of the legumes. For the second week of watering the legumes, the legumes were watered every other day and commercial inoculant was added once to the positive control pot. After the end of the second week, the pots with the plants were photographed to take note of the growth of the legumes. The same procedure was applied for the legumes during the third week as during the second week. During the fourth week since planting, one plant from each pot was phenotyped and its shoot was measured. Photos were taken of each plant phenotyped as data. The plants were then put back in the pots to continue to grow. During the fifth week, a different plant from each pot was phenotyped for their shoots and using a microscope, the root noodles for each plant were analyzed. Finally, the rhizobia bacteria in the root nodules was isolated by cutting the nodules off the roots and releasing the bacteria by crushing the nodules with the pestle after they were sterilized using diluted bleach.Then they were grown on a nutrient agar plate and the amount of rhizobia was analyzed after three days of growth.

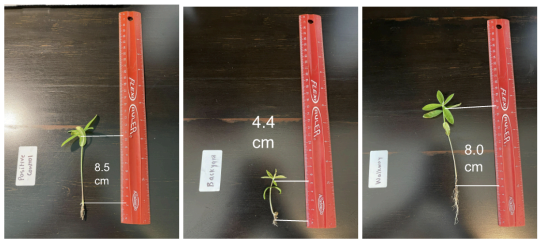
**RESULTS**


*Seedlings at 4 days post planting*

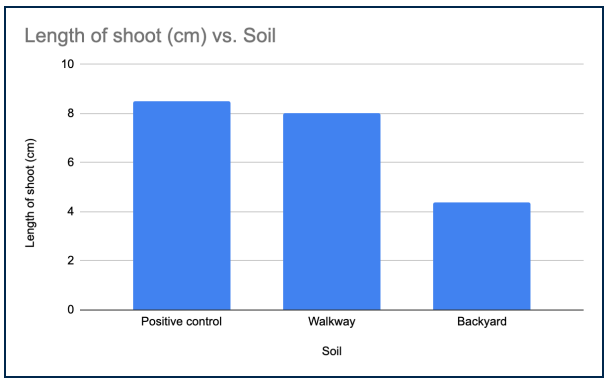

*Plants at 14 days post planting*


*Phenotyping plants four weeks after planting*

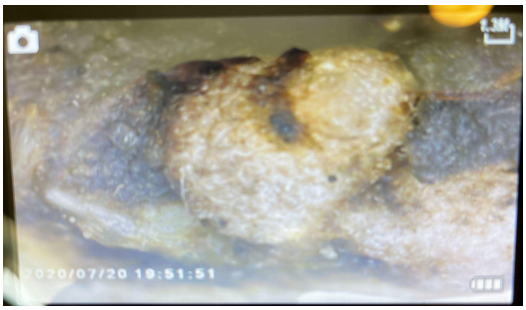

*Plants five weeks after planting*




*Phenotyping plant shoots five weeks after post planting*




Comparing the length of shoots of legume plants in each soil after the second time phenotyping



**DISCUSSION**

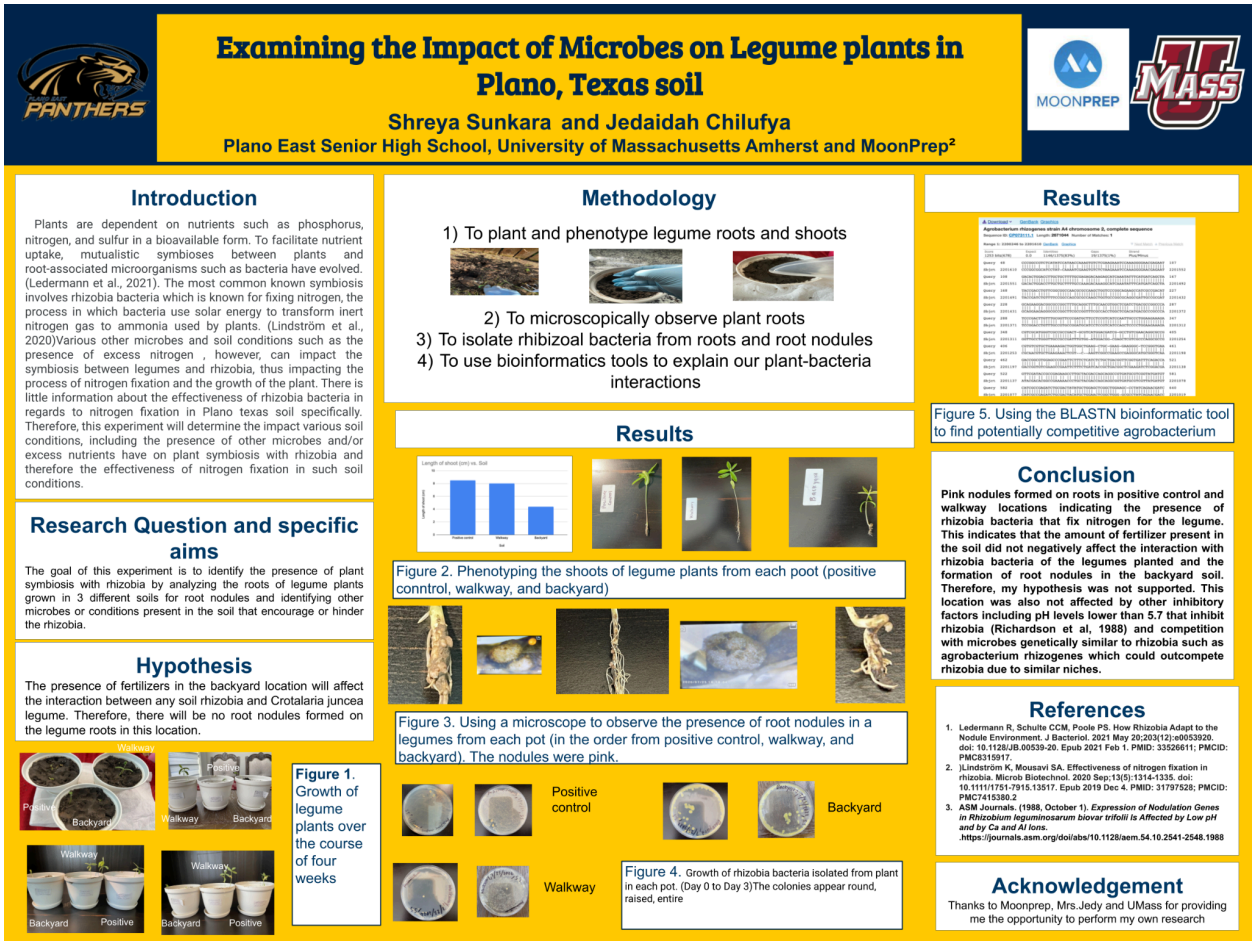
Based on the growth of the rhizobia bacteria after isolating the nodules and allowing the bacteria to grow on the nutrient agar plates for three days, it was determined that both the positive control soil and the soil from the a

walkway were able to successfully form a mutualistic relationship with rhizobia bacteria in the soil. This is due to the high amount of rhizobia growth on each plate and the higher length of the shoots of the legumes grown in the positive control and walkway soils compared to the soil from the backyard which showed very little growth in rhizobia bacteria. The presence of rhizobia in the root nodules is also supported as the root nodules appear to be a light pink color, thus showing that the rhizobia were actively performing nitrogen fixation (Pacholko, 2022).

As shown in the bar graph comparing the length of the shoots of the legumes from each soil, the length of 8.5 cm and 8.0 cm for the legume shoots in positive control soil and the walkway soil respectively were both higher than the shoot length of 4.4 cm for the legume in backyard soil. This difference in length shows how do to the lack of rhizobia and root nodules on the roots of the legume plant, the legume was not able to grow significantly due to a lack of nitrogen fixation normally performed by rhizobia that provide ammonia for plants to readily use. These results were surprising as a common factor for rhizobia not being present in soil is a significant amount of nitrogen in the soil for plants to readily use. However, there was no fertilizer, which contains usable nitrogen, applied to soil in the backyard meaning possibly no nitrogen available compared to soil in the walkway where fertilizer and therefore nitrogen was present. Therefore, the issue of nitrogen content was not a factor in why no root nodules formed in soil from the backyard.

Several factors could have inhibited the interaction of rhizobia with legume roots such as the pH of the backyard soil. Studies have shown that soils with a pH lower than 5.7 have inhibited the presence of rhizobia bacteria, thus leading to no nodule formation (Richardson et al, 1988). Competition of rhizobia with other microbes that are genetically similar could have also limited rhizobial-legume interactions. Using bioinformatics, specifically the BLASTN search tool from NCBI, the DNA sequence of common rhizobia bacteria used in commercial inoculant, Rhizobium leguminosarum biovar phaseoli, was used and a genetically-similar bacteria called Agrobacterium rhizogenes strain A4 chromosome 2 that was not part of the rhizobia genus was found. Since this bacteria was the most genetically similar to rhizobia, it was assumed that its nutritional needs were also similar to rhizobia, thus being a source of competition for the rhizobia in the backyard soil that possibly outcompeted the rhizobia. Therefore, no rhizobia would be present to form the root nodules. The lack of binding factors in backyard soil could also play a significant role in no nodule formation on the roots. Binding proteins, such as the bacterial Ca 2+ binding protein known as rhicadhesin, are involved in the attachment of bacteria, such as rhizobia, to the surface of root hair cells and the lack of such proteins could have prevented rhizobia from binding to the roots to form nodules (Smith et al, 1992).

Based on the data from the experiment, it can be assumed that the nitrogen content of the soil is not the sole factor that determines the presence or absence of rhizobia. Soil should be analyzed for microbes that have similar niches to rhizobia, for the presence of important binding proteins, and for the pH level to determine whether such soil should be used to grow legumes. From the data collected, it was determined that some soils in Plano, Texas could lack binding factors and could have microbes that are competitive with bacteria, thus preventing nodule formation despite a lack of usable nitrogen.

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Shruti Sunkara

# The Effects of Microbes and Added Chemicals like Pesticides and Fertilizers in Different Soils on the Growth of Legume Seeds in Plano, TX

**ABSTRACT**
Interactions between microorganisms and plants are crucial especially like the rhizobial interaction with plant roots as this allows plants to absorb the usable form of nitrogen, ammonia, and for the bacteria to absorb carbon representing a mutualistic relationship. These interactions aid in plant growth allowing for greater yields in crops. There was little to no information found regarding the microbes in the soil in Plano, Texas which is important to identify as this can help increase the yield in crops in legume plants. Thus, soil from the walkyard, soil from the backyard, and another pot of soil from the walkyard with positive inoculant were gathered. Crotalaria juncea seeds were placed in these pots and their height, the growth of their roots, and bacteria was isolated throughout the next six weeks after planting. It was found that the plants from the walkyard produced the plants with the most nodules, a sign of nitrogen fixation, while the plants in the other pots produced no nodules. This can be due to the fact that the plants in the backyard had already contained fertilizer as there are multiple flowers growing in this area and for the positive control there wasn't sufficient for the plants to grow fully as they sprouted much later compared to other plants.

**INTRODUCTION**
In this experiment, the interactions between legumes and bacteria were observed. Specifically, the interactions between crotalaria juncea and microbes in the soil it grows in like rhizobia. Rhizobial bacteria aid in fixing nitrogen as these microorganisms produce white nodules on the plant's roots once the early stage of this interaction has passed. For this to occur the roots must send a signal through flavonoids to the bacteria and in return the bacteria will send a signal through the nod factors. This produces white nodules on the roots in which rhizobial bacteria can grow resulting in pink rodules where nitrogen can be fixed to a usable form for plants called ammonia needed for plant growth and the bacteria gain carbon. (Annet Westhoek et al, 2021) Unfortunately, external chemicals added like pesticides and fertilizers can halt these interactions preventing nodules on roots from forming as nutrients like ammonia are already present in the soil. This can cause several detrimental environmental effects including runoff and groundwater contamination. (Yarmilla Reinprecht et al, 2020) Although there are other methods of promoting nitrogen fixation including crop rotation that don't involve adding chemicals to the soil, they haven't been as significant compared to these external additions which is why using fertilizers and pesticides is so common amongst crop producers. (Jennifer E. Fox et al, 2007) There are also microorganisms that vary from region to region and it is unknown whether or not rhizobial bacteria or other beneficial microbes are present in the soil in Plano, Texas especially as there are specific types of bacteria that can interact with these legumes although there are few plants which can interact with multiple bacteria. (Dong Wang et al, 2012).
This experiment aimed to see if there was rhizobial bacteria present in the soils of Plano, Texas and what was the effect of added chemicals including fertilizers and pesticides on the interactions between the bacteria and legumes. The soils that were tested were from two locations: the walkyard and the backyard. In the walkyard there are no plants other than grass, so fertilizer is hardly used and there are little to no insects present. In the backyard, there are several

types of flower growing that benefit from added fertilizer and also several insects including ants that are removed by pesticides. To answer these questions the same legume seeds of crotalaria juncea were added to a pot containing soil from the walkway, soil from the backyard, and a positive control with soil from the walkway and positive inoculant to aid in growth by giving nutrients and materials needed for plant growth. From this the heights of the shoots were determined, the roots of the plants were phenotype, and bacteria was isolated from the roots for about 6 weeks after planting. These results were compared to find the impact of different microorganisms in different soils in Plano, Texas on the overall growth of the legume, crotalaria juncea.

**MATERIALS AND METHODS**

The materials used in this experiment included water, fork, tray, 3 pots, a shovel, gloves, hand sanitizing wipes, legume seeds, commercial inoculant, soil, small tupperware, a black folder, a ruler, a sharpie, plastic pestles, microcentrifuge tubes, household bleach, agar media plates, a scalpel and labels.

I labeled each pot with the location of the soil. Then, I dug up soil from each location using the shovel and placed it in the respective pot ensuring to change gloves between each pot (positive control having the same soil for location 1). I brought the soil back to the sterilized workspace, filled the pots, and added 15 holes in each pot using a sterilized fork. I added water and waited for water to seep through. Next, I added another 15 holes in each pot using a sterilized fork. I added another 15 holes in each pot and added legume seeds in pot 1 and pot 2 again using different gloves. I covered the seeds with soil. I added a pinch of commercial inoculant for the positive control in each hole, added the seed, and covered the hole. Finally, I added water to the three pots and water the plants every other day.

To phenotype, I took one plant from each of the pots at three and four weeks post-planting. I added extra water into the pots to loosen the soil around the roots. I then used sterilized gloves and a shovel to gently remove the plant shoot and its root from the soil. I washed off the excess soil on the roots in a tupperware filled with clean water. Then, I patted the plant dry and placed it onto a black folder with a label with the respective title of the soil. I put a ruler next to the plant, determined the height of the plant shoot from its root, and took pictures.

To phenotype the root at the four weeks post-planting I placed the roots under the microscope and took pictures of the roots and root nodules.

After using microscopy to analyze the roots and root nodules, I isolated the rhizobial bacteria and other bacteria from the roots and root nodules. I sanitized my workspace with the hand sanitizing wipes and created the diluted bleach solution which was placed in a microcentrifuge. I then added water to another microcentrifuge. Then I took a scalpel and cut off parts of the root and root nodules and added it to the microcentrifuge with bleach and inverted the tube 5 times. Next, I added the roots and root nodules to the microcentrifuge with water and inverted that tube 10 times. I then placed the nodules and roots in another microcentrifuge and crushed them with a sterilized plastic pestle. I added water and mixed the solution with the pestle. Then I labeled the agar media plates with respective soil names and added the crushed root and root nodule mix into the plates with a sanitized swab. I took a picture, covered the plate with foil, and placed it by a window. I then took pictures for the next few days until I saw bacteria colonies growing on the plates.

**RESULTS**

At the end of the four weeks there were 3 sprouts in the pot with soil from the positive control, 1 sprout in the pot with soil from the walkway, and 2 sprouts in the pot with the soil from the backyard. The heights of each of the

sprouts were also determined from the three and four week period. The only plants that contained root nodules were sprouts from the walkway. After isolating the bacteria and placing it on the agar media plates, there was growth seen on all plates signifying there was bacteria present in all of the plant roots. Specifically, the bacteria on the positive control agar media plate were filamentous, irregular, and opaque. The bacteria on the walkway agar media plate were small Colonies, round, and opaque. Lastly, the bacteria on the backyard agar plate were small, round, and opaque. Bioinformatics was used to compare DNA sequences of predicted types of bacteria in the soil to the DNA sequences of  rhizobia. This was the information found for each of the types of soil in the pots.

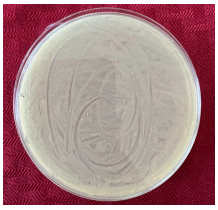
*Positive Control.* When comparing the DNA sequence of a possible type of bacteria found in the commercial inoculant, bradyrhizobium japcium, and what I believed was in the soil already, rhizobium, there was 99% identity between both sequences.

*Walkway*. When comparing the DNA sequence of a possible type of bacteria found in the soil, pseudomonas, with what I believed was already in the soil, rhizobia, there was no significant similarity found.

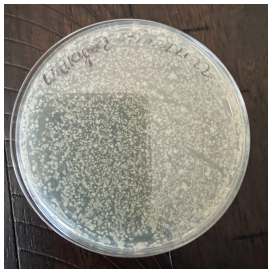
*Backyard*. When comparing the DNA sequence of a possible type of bacteria found in the soil, pseudomonas, with what I believed was already in the soil, rhizobia, there was no significant similarity found.

**TABLES, GRAPHS and PHOTOS**

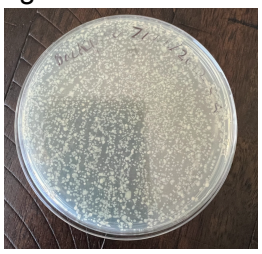
Agar Media Plate For Positive Control

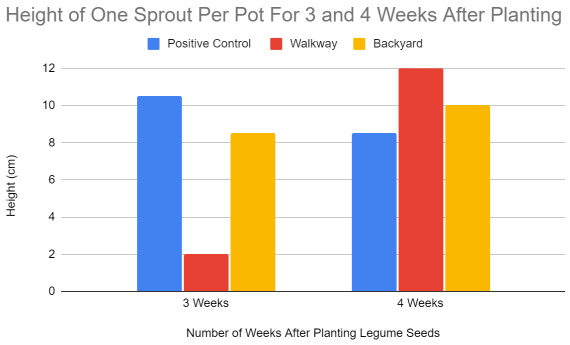


Agar Media Plate For Walkway



Agar Media Plate For Backyard

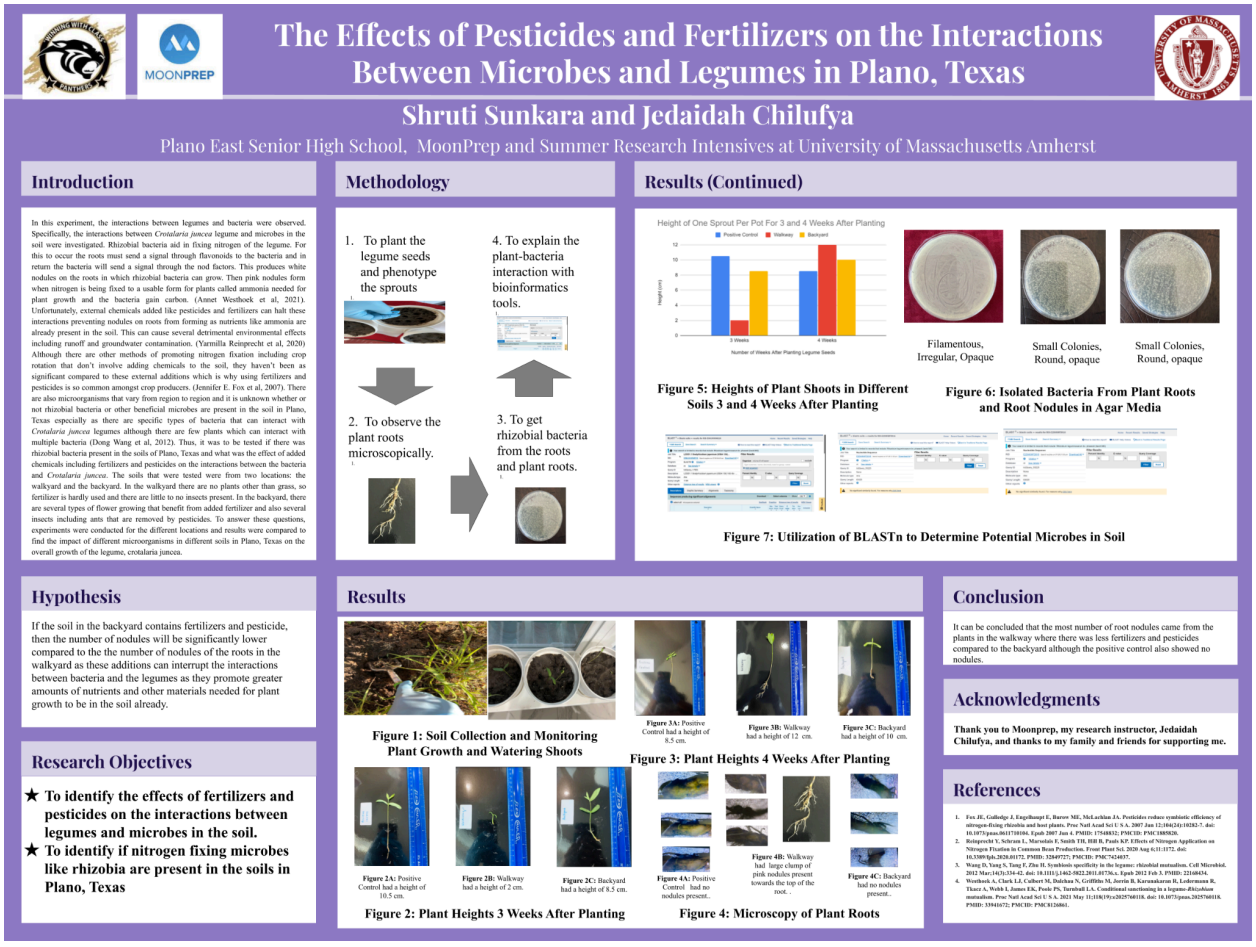





**DISCUSSION**

In the walkway there were no plants other than grass, so fertilizer was hardly used and there were little to no insects present. In the backyard, there were several types of flower growing that benefit from added fertilizer and also several insects including ants that were removed by pesticides. Thus, the creation of root nodules were prevented from forming as in the backyard as there were pesticides and fertilizers present which added external chemicals and nutrients that inhibited the early interactions between the rhizobia bacteria and the legume sprouts. Specifically,

the flavonoids were unable to be received by the bacteria and nod factors were unable to be received by the plant itself.
What was unexpected though was the positive control not having any root nodules as it contained the same soil from the walkway which had root nodules along with commercial inoculant which added nutrients into the soil. This may have been due to the fact that several of these plants wilted and weren't in the best conditions compared to the plants in the other pot as the soil became very dry. To fix this in the future, I would have added more water to this pot. This data can be used in the future as it can be used in research regarding the effects of added chemicals like pesticides and fertilizers on interactions with bacteria and legumes as nitrogen fixation cannot occur with these external chemicals. This can also be used to encourage others to utilize organic and natural means of pesticides and fertilizers that will not prevent this interaction from occurring as pesticides and fertilizers have a multitude of harmful effects ranging from runoff and water pollution to ground contamination as stated earlier.

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Siya Patel

# Examining the Interaction and Impact Between Legume and Bacteria in Piscataway, NJ


## ABSTRACT

Legumes interact with microbes on a daily basis for survival. Microbes are a necessity for legume, as they help legume remain healthy and strong. Very few experiments in Piscataway, NJ have been conducted to learn more about the interaction and impact between plants and bacteria. Therefore, multiple experiments have been conducted to study the interaction and impact between legume and beneficial microbes, specifically bacteria. Soil was collected from two different locations in New Jersey and placed into three different pots. Before planting seeds into one of the pots, commercial inoculant was added in the soil for a positive control. From there, legumes were planted in each pot. Germination of the legumes were observed and monitored on a daily basis, and given the legumes water, when needed. After this, phenotyping the legume shoots and root nodules. From there, the root nodules were closely observed by utilizing a digital microscope. Then, the rhizobial bacteria were isolated from the root nodules of the legume. From there, the rhizobia bacteria were microscopically identified. Finally, with the use of bioinformatics tools, plant-bacteria interaction was explained. The legumes that received positive control and soil from the garden had a larger quantity of root nodules compared to the soil from the backyard. In fact, the legumes from the garden soil and positive control looked healthier and stronger than the legume from backyard soil. To add on, the legume from backyard soil eventually died, while the legume from the other two pots were still healthy and strong.


## MATERIALS AND METHODS

The materials used for this experiment were as follows: legume seeds, three pots, water, soil, two trays, commercial inoculant, digging tools (shovel and the fork), large tupperware, paper towels, 30 cm ruler (12 inch ruler), dark background, ethanol wipes (at least 70% alcohol containing), LCD microscope, PC, plastic pestles, microcentrifuge tubes, scalpel, dropper, household bleach that has the active ingredient of sodium hypochlorite, swab, and agar media plates.

Soil was collected from two different locations in Piscataway, New Jersey. Legume seeds were planted in three different pots, watered, and placed near a window where sunlight was easily accessible.

Pot 1 and 2 contained soil dug from the garden in Piscataway, NJ. In Pot 3, the soil was collected from the backyard in Piscataway, NJ,. After the soil was placed in all three of the pots, these pots were labeled with the location name of where the soil was collected from. A fork was used to create about 5-6 holes in each pot to allow the legume seeds to be planted in the soil. Pot 2 served as the positive control and a pinch of commercial inoculate was placed in each hole, prior to adding the legume seeds in the holes of this pot. The holes were covered back up with soil and water was added to the soil to allow the legume plants to grow.
The germination of the legume seeds were observed on a daily basis. At four weeks post planting the plants were phenotyped.
For phenotyping, a dark and flat surface was used. The lab space was sterilized with ethanol wipes that contained at least 70% alcohol. The tallest plant in each pot was used to phenotype. The tallest plant was removed from each pot. Since the roots had soil particles, the legume plants were dipped into the large container of water, to remove the soil particles on the roots. The roots were carefully dried with paper towels. The legume plants were placed on the sterilized dark and flat surface. A label with the location name

and a 30 cm (12 inch) ruler were placed next to the legume plants. The shoot height of the legume plants were measured. Next, pictures were taken of the plant, including the label and the ruler. After pictures were taken, the legume plant was placed back in the pot by digging a hole that was about 6 inches deep, and placing the legume plant back in that hole. Next, roots were buried by the soil. After phenotyping the legume shoots, the legume roots were phenotyped under a microscope to identify any nodules on the roots. Finally, bacteria from the legume roots were isolated on the agar media plates. First, the legume shoots were phenotyped for the second time by repeating the same steps that were described above. In order to phenotype the roots, the AMcap software was downloaded on a PC, and connected the LCD microscope to the PC. The same plant that was phenotyped for the second time was used to phenotype the roots. The roots were placed under the LED light, on the stage of the microscope. The coarse adjustment knob and the fine adjustment knob were adjusted as necessary to get a clear and close up view of the roots.

In order to isolate bacteria from the roots, the lab space was sterilized with ethanol wipes that contain at least 70% alcohol. Next, the diluted bleach sterilizing solution was created by labeling the microcentrifuge tube to indicate that it contains diluted bleach. After the microcentrifuge tube was labeled, 1-2 drops of household bleach was added to the tube, by utilizing a dropper. From there, 0.75 ml of water was added in the same tube that contains 1-2 drops of household bleach. The microcentrifuge tube was then inverted multiple times to mix the household bleach and water together. The same legume plant that was phenotyped for the second time was used to cut off segments of the roots by using a scalpel. The root segments were placed inside the microcentrifuge tube that contains diluted bleach and the tube was shaken about 5 times to sterilize the roots. The root segments were taken out of the tube and placed on an ethanol wipe, while another microcentrifuge tube was filled with clean water. The root segment was placed in the microcentrifuge tube that contains only clean water. The tube was shaken for about 10 times to rinse off any bleach particles that were remaining on the root segments. The root segment was removed from the tube and placed inside another microcentrifuge tube and the roots were crushed by utilizing a plastic pestle. From there, 0.5 ml of water was added in the tube that contains the crushed segments of the root. Once the crushed suspension was made it was transferred to an agar media plate, flipped upside down, and labeled with the location name of the soil and the date.

A BLAST Search in NCBI was used to find the DNA sequence of the example bacteria in a specific scenario. The scenario was about types of microbes that could be present in the soil when there were no nodules forming on the roots. Utilizing the bioinformatic tools on NCBI, the DNA sequence was aligned with Bradyrhizobium Japoncium. The Bradyrhizobium Japoncium was one of the four bacteria present in the positive control. The similarities and differences were observed.

**RESULTS**

On day 4 there was one germinating plant per pot. After 7 days post-planting legume seeds, there were multiple germinating plants in the pot with garden soil, one more germinating plant in the positive control, and the third pot did not experience any new germinating plants, after the first seed started to sprout. By week 2.5, the pot with garden soil contained 5 healthy legume plants and 0 unhealthy or broken plants. The pot with backyard soil contained 2 healthy legume plants and 0 unhealthy or broken plants. Tiny sprouting has been observed for the third legume plant. The pot with positive control contained 2 healthy legume plants and 0 unhealthy or broken plants.  Tiny sprouting had been observed for the third legume plant.

By week 3, the pot with garden soil had 5 healthy legume plants and 0 broken or unhealthy plants. However, the backyard soil and positive control contained 3 healthy legumes and 0 broken or unhealthy legume. After phenotyping the legume roots, the shoot height from the garden soil and backyard soil was 12.5 cm. The shoot height from positive control was 14.5 cm.

The results for phenotyping legume shoots for the second time showed that the garden soil and positive control grew by a whole extra centimeter, but the legume from backyard soil only grew by another 0.5 centimeters. However, one of the plants from the backyard soil died, therefore it was removed from the pot. But the other 2 were healthy. Two of the three plants in positive control were healthy and the other one plant looked weak. The results of the microscopy of roots for the positive control and garden soil were very similar. Both of them had a larger quantity of round, white root nodules on the roots, very curly root hair, but the roots from the backyard soil had very few root nodules. The results of isolating bacteria were very straightforward. The type of colonies that were formed on the agar media plates were large colonies, opaque, entire, round.
Finally, there were no significant similarities found after aligning the DNA sequences.

**TABLES, GRAPHS and PHOTOS:**

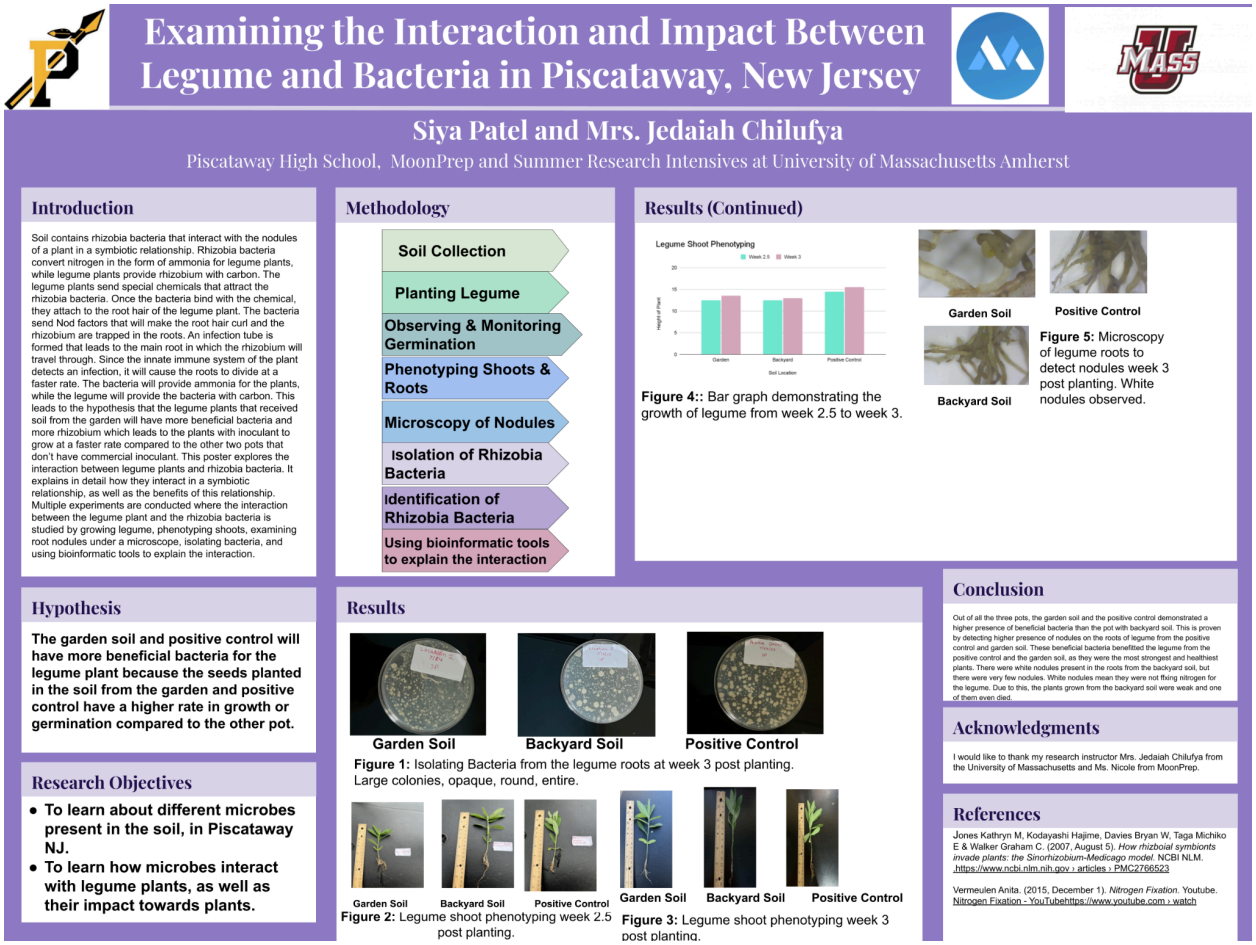
Figure 1: Soil collection from 2 different locations and legume seeds planted in the pots.

**Figure 2:** Addition of commercial inoculant to the positive control.

**Figure 3:** Growth and germination of the legume plant in all three pots (pre-class week 2).

## DISCUSSION

Out of all the three pots, the garden soil and the positive control demonstrated a higher presence of beneficial bacteria than the pot with backyard soil. This was proven by detecting higher presence of nodules on the roots of legume from the positive control and garden soil. These beneficial bacteria benefitted the legume from the positive control and the garden soil, as they were the most strong and healthiest plants. There were nodules present in the roots from the backyard soil, but there were very few nodules. Due to this, the plants grown from the backyard soil were weak and one of them died. These results were expected from the experiments and the results support the hypothesis. In the future, this research paper will be useful as it explains the interaction between legume and rhizobium, as well as proves that other than rhizobium there are other beneficial bacteria that live in the soil to provide the legume with their necessities.

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Tanush Deka

# High Amounts of Rhizobia Bacteria Found in Gardening Soil in Warrington, PA

**ABSTRACT**
This research project aims to determine whether garden soil contains higher concentration of Rhizobia bacteria and natural soil. Rhizobia bacteria was presumed to be more abundant in gardening soil. Soil was taken from 2 distinct locations and divided between three pots. For Pot 1, soil was collected from the backyard (garden soil), In Pot 2, soil was collected from the front yard (next to the sidewalk). Pot 3 served as the control pot with soil collected from the backyard. In Pot 1 and Pot 2, legume seeds (15 in each pot were planted). The soil in Pot 3 was inoculated with commercial inoculant (which contains Rhizobia) and then 15 legume seeds were planted. All three pots were exposed to the same amount of light, water, temperature and humidity. After the legume seeds grew, the height of the plants was measured daily for 3 weeks. The results demonstrated that the plants in Pot 1 (backyard soil) were taller and had a larger mass than the plants in Pot 2 and Pot 3.

**INTRODUCTION**
Nitrogen is one of the most essential nutrients for plant growth. Nitrogen is a major component of amino acids (proteins), essential for structural strength of plants (Gage, 2004). It is also an important constituent of chlorophyll (essential for photosynthesis). Nitrogen is abundantly present in the air we breathe, but plants cannot use nitrogen in the atmosphere, the nitrogen must be converted to other forms like ammonium and nitrates. This conversion is also known as nitrogen fixing, and the process is facilitated by different species of bacteria (Gage, 2004). Rhizobia is one such bacteria, however, free living bacteria cannot fix nitrogen. For the process of nitrogen fixing, Rhizobia develops a symbiotic relationship with legumes, the result of which is the formation of nodules on the root of the plant (Wang, Liu & Zhu, 2018). Within this nodule, the bacteria convert atmospheric nitrogen into ammonia, with the enzyme nitrogenase (comes from the legumes). The carbohydrate required for the fixation is also provided by the legume plant. Legumes, because of their nitrogen fixing capabilities, also help other crop growth by increasing soil nitrogen quantity (Johnson, 2019).

This experiment aimed to answer the following question: will there be a larger amount of rhizobia bacteria in gardening soil compared to regular soil? I hypothesized that gardening soil would contain larger concentrations of rhizobia bacteria and undergo more nitrogen-fixation after establishing a symbiotic relationship with the legume roots.

**MATERIALS AND METHODS**
*Materials*
Three identical white pots, gloves, disinfecting wipes, labels, marker, phone camera, garden tools, jar (for watering), legume seeds, commercial inoculant, tap water, and measuring tape were used for this project. Disinfecting wipes were used to clean tools and gloves were used to avoid contamination while collecting soil and to avoid cross-contamination of soil between the 3 pots.

*Procedure*
Three identical pots with labels including the site of collection, date, and initials were used. Pot 1 and Pot 3 (control) contained soil collected from the backyard. Pot 2 contained soil collected from the front yard. Each pot was watered with the same amount of water. Both Pot 1 and Pot 2 each had 15 legume seeds planted in them. Pot 3 (the control pot), received commercial inoculant (bacteria) prior to the planting of 15 legume seeds. All 3 pots were kept near a window next to each other

to ensure they are exposed to the same level of sunlight, temperature, and humidity. Isolation of the Rhizobia bacteria required removing a plant from each pot measuring their height and observing their roots under the microscope. Cotton swabs were used to put isolated samples into petri dishes to observe bacterial growth.

**RESULTS**

Each picture above shows the plant roots of the control, sidewalk, and backyard pots, respectively. The control plant reached a shoot length of 3.7 cm, while the whole plant was 6.5 cm long. The sidewalk plant's shoot had a length of 5.2 cm, and the whole plant was 6.8 cm. The backyard plant's shoot reached 4.7 cm, and the whole plant reached 8.9 cm.

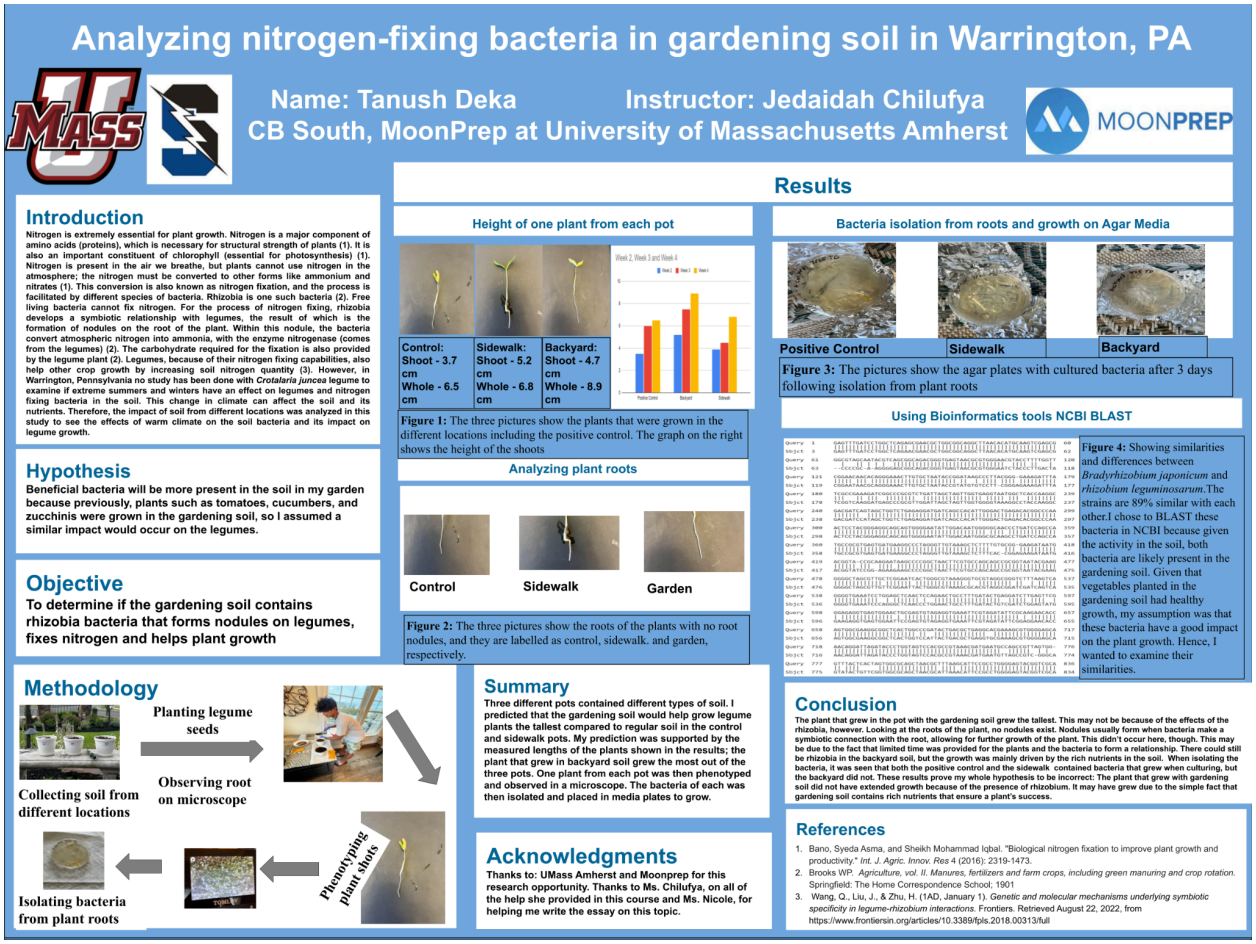
Above is Day 2 of isolating bacteria, where we can see that bacteria is beginning to grow in both the Positive Control and Sidewalk plates. However, there is no bacteria growth seen in the Backyard plate.


Left shows Positive Control Roots. Middle Shows Sidewalk Roots. Right shows Backyard Roots.

**DISCUSSION**

The seeds that were planted in gardening soil (Backyard) grew the most under the same conditions and time restraints. I originally assumed that this would occur due to rhizobia bacteria being present in the gardening soil, but this was not the case. The media plates that are shown above contain bacteria that grew over days. The backyard media plate contains no bacteria. This shows that there was no bacteria in the gardening soil. However, the positive control and the sidewalk pots did have bacteria. How did the backyard plant grow the tallest then? My inference is that since the backyard plant was placed in gardening soil, it had the best chance of growth because of the soil's enriching nutrients.

This project demonstrates how bacteria and legume roots undergo a symbiotic relationship. The legume roots form nodules and the bacteria reside within these nodules. It is here that the bacteria helps to fix atmospheric nitrogen to forms that can be used by the plant to facilitate structural growth of plants and also assist in the process of photosynthesis. Legumes, if planted in commercial crop growing fields, can also facilitate accelerated growth of other crops by increasing the amount of nitrogen in the soil.

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Tiana Mishra

# Determining the Impact of Nitrogen-Fixing Rhizobia Bacteria on the Development and Productivity of Crotalaria Juncea in Battle Creek, MI

**ABSTRACT**

The plant microbiome helps plants such as legumes absorb nitrogen from the soil. Rhizobia bacteria has a unique symbiosis with legume seeds, where it fixes nitrogen from the air into a form that can be accessed by legume seeds. Nitrogen is very important for the growth of plants. In this paper, we specifically study the impact of rhizobia bacteria on the growth of crotalaria juncea. The positive control consists of soil mixed with rhizobia bacteria which is known to accelerate legume plant growth. The other two soil samples are from two backyards in Battle Creek, Michigan. The legume seeds were planted and monitored for a length of 4 weeks. We examined the shoot length and nodule growth in each pot. Our observation was that the plants from Backyard Location 2 pot had no sparse nodules. This means the early stage messaging between the rhizobia bacteria and crotalaria juncea didn't occur. The plants in positive control interacted with the rhizobia in the inoculant and the Backyard Location 1 pot (lakeside sample) was able to interact with native Michigan rhizobia bacteria, and the plants produced were similar in quality with similar results in terms of germination, sprouting, and nodule development.

**INTRODUCTION**

Crotalaria Juncea, also known as Sunn hemp, is a legume variety widely believed to have originated in India (Irmer et al., 2015). Similar to other plants, legume plants rely on nutrients in the soil and bacterial assistance for optimal growth. There are many microbes present in the soil. Some are helpful while others are harmful. Legumes primarily depend on the use of Rhizobium bacteria for nitrogen fixation. This mutualistic symbiotic relationship between the plant and Rhizobia bacteria is utilized to assist with the plant's metabolic processes (Blanco, 2016). Rhizobia bacteria has a unique symbiosis with legume plants, where it fixes nitrogen (nitrogen) from the air into a form that can be accessed by legume plants. The rhizobia also benefit from an exchange in carbon-heavy substances like glucose and amino acids. For this interaction to occur, the plants must need nitrogen. But because the soil is full of millions of microbes, legumes and rhizobia must send signals to each other for symbiosis to occur (Nelson & Sadowsky, 2015). When the soil is nitrogen-rich due to fertilizers or many plants growing in the area, this symbiosis between legumes and rhizobia will not happen because the plants already have to access the nutrients and nitrogen in the soil (Turner, 2013). This means that the relationship to obtain nitrogen is no longer needed; therefore, no signals will be sent to start this interaction. The plant microbiome is a key determinant of plant health and productivity and has received substantial attention in recent years.

A testament to the importance of plant-microbe interactions is the mycorrhizal fungi. Molecular evidence suggests that their associations with green algae were fundamental to the evolution of land plants about 700 million years ago. Most plants, although notably not Arabidopsis thaliana and other Brassicaceae, have maintained this symbiosis, which assists root uptake of mineral nutrients such as phosphate. The plant microbiome can be considered an extension of the host genome. Abiotic conditions, such as temperature, moisture, and pH, have broad effects on the microbiome directly and indirectly through the host (Turner, 2013). We undertook this research study to better understand the

plant-microbe interactions in the soil in Battle Creek, Michigan.

The goal of this experiment is to identify the presence of plant symbiosis with rhizobia by analyzing the roots of legume plants grown in 3 different soil locations. To do this, we asked the question; how do rhizobia bacteria affect the productivity & growth of legumes, and if there is any effect? This study aims to better understand the effects of the types of soil in Battle Creek, Michigan on plant-microbe interactions. In this experiment, three different soil locations were compared to determine the best soil conditions to grow legume plants in Battle Creek, Michigan.

**MATERIALS AND METHODS**

*Soil Collection:*

Soil was collected from two different backyard locations in Battle Creek, Michigan (Figure A1). The soil for Backyard Location 1, was obtained from the lakeside. This soil was moist, dense, and dark in color (Pot 1). Plants had previously been grown in this soil. The second sample was collected from Backyard Location 2. It was dry, hard, and light in color (Pot 2). The soil from this location had a very sandy dense texture to it. This soil was from a freshly cultivated home vegetable garden where compost and fertilizers are commonly used, and vegetables like tomatoes, and squash, are typically grown. Positive control (Pot 3) was prepared by taking soil from Backyard Location 1 and adding commercial inoculant when planting the legume seedlings. The inoculant consisted of: Bradyrhizobium sp. (Vigna), Bradyrhizobium japonicum, Rhizobium leguminosarum biovar phaseoli, and R. leguminosarum biovar viceae. The purpose of the Positive Control was for comparison of nodule and plant growth to the other two pots, as known rhizobial bacteria were already present.

*Planting Seeds:*

To begin planting the Crotalaria Juncea seeds, the soil in each pot first seeped with water. To do this, 15 holes were made with the garden fork and water was poured. This process was repeated 3 times. We sterilized the garden fork when moving between pots with 70% isopropyl alcohol wipes. After this, 15 holes were made in each pot to plant the Crotalaria Juncea seeds. In Pot 1 & 2 the legume seed was placed in the hole and covered with soil. In Pot 3 a pinch of the commercial inoculant was added to each hole before the legume seed, then covered with soil. Sterile technique was followed making sure to change into new gloves and sterilize tools when moving to a different pot. Plants were watered every other day. Germination was monitored in each pot (Figure A2a,b,c). As there was not enough germination, more legume seeds were planted on day four by using the above steps.

*Phenotyping Plant Shoots & Roots:*

The plants were first phenotyped at 2-2.5 weeks post-planting (Figure A3a). To do this, one legume plant was carefully extracted from each pot. Water was used to loosen the soil and digging tools to dig the soil surrounding the chosen plant. This loosened the soil and allowed us to carefully extract the plant by hand. The soil was then washed off the plant with water. Plant shoot height was measured using a 15 cm ruler against a black background. Plants were then observed under a microscope to observe root nodules. The number of nodules, shoot height, and their microbiological characteristics were recorded at 2-2.5, 3, and 4 weeks post planting (Figure A3b,c,d,e).

*Isolation of Bacteria:*

Rhizobia bacteria was isolated at 3.5 weeks post planting and then placed into three agar media plates to examine for culture growth. We individually isolated each plant's rhizobia. To begin, 3 microcentrifuge tubes were prepared with a solution of .25 mL bleach and .75 mL of clean water (1:20), and tubes were labeled according to 3 pots. One microcentrifuge tube was prepared with 1.5 mL of clean water to wash off excess bleach. Legume plants were extracted and phenotyped using the mentioned steps above. Once located nodules in the

microscope, they were then cut off (if no nodule roots were cut off) the roots using a sterilized scalpel to then be placed in their labeled microcentrifuge tube. The tube was gently inverted up and down 10 times to sterilize the nodules and remove excess soil, and the nodules were then removed. To wash off excess bleach solution, nodules were placed in a microcentrifuge tube with water and inverted several times. Clean nodules were then placed into a new microcentrifuge tube with .25 mL of clean water, and crushed using a plastic pestle to release bacteria from inside the nodules. When the microcentrifuge tube turned 'cloudy', the crushed material was placed into general-purpose agar media plates. When this was completed, media plates were labeled and sealed using tin foil. We ensured the maintenance of optimal temperature for bacteria growth. The process was done in all three pots. Media plates were allowed to incubate and results were recorded on day 2.

**Bioinformatics (BLAST Analysis)**
During week 4, bioinformatics technology was used to better understand the legume-rhizobia symbiotic relationship. To do this, the two DNA sequences were compared using the software; BLAST. We performed a search using the online database NCBI GeneBank Link. Once the desired DNA sequences were found, we utilized open access software "BLAST" (Basic Local Alignment Search Tool) to compare the DNA sequences. DNA sequences were found for the three types of bacteria, each of which translated to different nodule phenotypes (white, pink, and no nodule growth). We further compared these to the DNA sequence of rhizobia bacteria found in the commercial inoculant using the feature in BLAST that allows for data-mining and identifying sequences producing significant alignment.

**RESULTS**
*Germination & Plant Growth:*
The germination rate was different in all three pots; however, germination did occur in each pot by day five. We noticed that most germination occurred in the Positive Control and the least in the Backyard Location 2 pot. We also found the tallest plants were identified in Positive Control and Backyard Location 1, see Table 1 for plant growth summary. All the plant shoots were red. All the Backyard Location 1 and the Positive Control plants had nodules. Backyard Location 1 plants had pink nodules, and the Positive Control plants had white and pink nodules. Backyard Location 2 had no nodules. Graphs 1, 2, and 3 showcase germination and plant growth below.

*Agar Media Plates:*
After 2 days, bacterial colonies were identified in all media plates. Backyard Location 2 media plate had the most colonies. Bacteria found in media from Positive Control and Backyard Location 1 are similar and can be compared. Backyard Location 1 colonies were; golden yellow, numerous, filamentous & regular irregular, flat opaque. Backyard Location 2 colonies were; light brown, scattered, too numerous to count, small colonies, buff pigment, irregular, irregular undulate, opaque & growth into the medium. Positive Control colonies were; light yellow, large colonies, opaque round filamentous, entirely with some growth into the medium.

*"BLAST" (Basic Local Alignment Search Tool) Analysis:*
Research was conducted to identify the type of bacteria that exist in these soil locations. The sequence of nucleotides were compared against bradyrhizobium japonicum which was found in the commercial inoculant. The roots in Backyard Location 1 had pink nodules. Rhizobium lupini is a bacteria in the soil which causes pink nodules to form. The similarity between these two bacteria is 94%. Backyard Location 2 had no nodules whatsoever, a bacteria that may cause this is streptomyces coelicolor. The sequence alignment between streptomyces coelicolor and bradyrhizobium japonicum had a similarity of 91%. Finally, the Positive Control had white nodules. Very few pink nodules

indicate the relationship was not fully established and needs a little more time. In the Positive Control,  ensifer fredii may be present in the soil which caused white nodules to form on the plant, but Rhizobium lupini may also be present as there were few pink nodules. The sequence alignment between rhizobium lupini and bradyrhizobium japonicum had a similarity of 95%. By doing a BLAST analysis we can find regions of local similarity between these nucleotide sequences.

**Table 1: Shoot Height & Number of Nodules**

| Location | Positive control | Backyard Location 1 | Backyard Location 2 |
|---|---|---|---|
| 2-2.5 weeks post planting | Day 19 7/17/22<br>Shoot height: 8.5 cm<br>Nodules: 3 | Day 14 7/12/22<br>Shoot height: 7.5 cm<br>Nodules: 2 | Day 19 7/17/22<br>Shoot height:  7.5 cm<br>Nodules: none |
| 3 weeks - 4 weeks post planting | Day 21 date  7/20/22<br>Shoot height: 9 cm<br>Nodules: clumpy 10-12 | Day 24  7/22/22<br>Shoot height: 8.5 cm<br>Nodules: clumpy 18-20 | Day26  7/24/22<br>Shoot height: 8 cm<br>Nodules: 1 big |
| 4 weeks post planting | Day 28 date  7/27/22<br>Plant 1:Shoot height: 9 cm<br>Nodules: clumpy 8-11<br>Plant 2: Shoot height: 10 cm<br>Nodules: clumpy 11-15 | Day 28 date  7/27/22<br>Shoot height: 9 cm<br>Nodules: clumpy 15-17 | Day 28 date  7/27/22<br>Shoot height: 7 cm<br>Nodules: none |

**DISCUSSION**

Legumes form the major component of all agrarian systems throughout the world. Grain legumes are the main source of protein-rich foods like soybean, groundnut, and other oilseed crops. Pasture (Fodder) legumes are important for livestock feed, many of which also produce edible fruits. Tree legumes produce other useful products such as poles and construction materials. They are particularly attractive crops from a farmers' standpoint as they require very low input systems in agriculture, making use of atmospheric nitrogen (through rhizobia) that transforms it into protein. Increased productivity through improved effectiveness of this symbiotic process is seen as a major research and development goal. This mutualism contributes to forming root or stem nodules that have rhizobia inside. Rhizobia, also called root-nodulating bacteria, are Gm-negative soil-inhabiting bacteria that contain genes required for nodulation and nitrogen fixation. These bacteria produce enzymatic mechanisms that reduce atmospheric di-nitrogen nitrogen to ammonia. They use solar energy to reduce the inert nitrogen gas to ammonia at normal temperature and pressure  (Lindström & Mousavi, 2020). The host plants supply the rhizobia with C4-dicarboxylic acids as carbon and energy source, and the rhizobia offer the plants nitrogen nutrients produced by reducing atmospheric nitrogen and incorporating it into amino acids (Wang, 1970).

We also understand that signaling molecules allow us to distinguish potentially harmful species from symbiotic partners. Secreted exopolysaccharides and the lipopolysaccharides present in the bacterial cell wall play a crucial role in triggering the expression of specific genes related to the symbiotic process. One of the main molecular determinants is referred to as the Nod Factor,

a diffusible lipochitooligosaccharide molecule produced by rhizobia. This further appears to interact with LysM receptor kinases; and many other important molecular signals (Via et al., 2016). These molecular signals exchanged between rhizobia and host legume plants have great importance in the competition with the soil microbiota and the genotype-specific perception of host plants (Cangioli et al., 2022). As we know, the natural landscapes are increasingly impacted by interference with nitrogen supplies from aquatic and airborne pollution sources. Furthermore, nitrogen enrichment can eliminate the net benefits that plants gain from nitrogen-fixing microbes such as rhizobia, potentially altering the host-mediated selection on nitrogen fixation (Wendlandt et al., 2022).

Our experiment found that native rhizobial bacteria in Michigan interact well with legume plants planted in native Michigan soil. They positively impact the growth of the plant and the production of nodules. The native Michigan soil cultivated with fertilizers did not interact with the rhizobia well (Backyard Location 2), but native Michigan soil with no fertilizers interacted very well with the present rhizobia bacteria in the soil (Backyard Location 1). We were able to compare both results to our Positive Control with the commercial inoculant. We were able to demonstrate that native Michigan rhizobial bacteria have a positive effect when interacting with legume seeds planted with no fertilizer. Answering the question of how rhizobia affected legumes planted in Michigan.

Our findings were as expected. Backyard Location 2 is filled with fertilizers and compost. Therefore, the plants in this pot didn't establish the initial interactive signals with rhizobia bacteria, and no nodules were formed. There were bacteria present in the soil which may have been Streptomyces coelicolor, a bacteria found commonly in fertilizer-treated soils, acting as an inhibitory signal. In Backyard Location 1, there has been no previous fertilizer or previous cultivation, and plants commonly grew well. The soil in this case didn't have enough pre-existing nitrogen in the soil, causing it to have a positive feedback loop, and allowing a successful relationship with a rhizobial bacteria,  rhizobium lupini. The Positive Control and Backyard Location 1 were very similar, but Backyard Location 1 plants had more root nodules. Backyard Location 1 also established a successful nitrogen fixing relationship before the positive control. This was surprising as we expected the positive control to establish a relationship first because of the use of the inoculant. However, we found that not always adding the inoculant will benefit the plant.

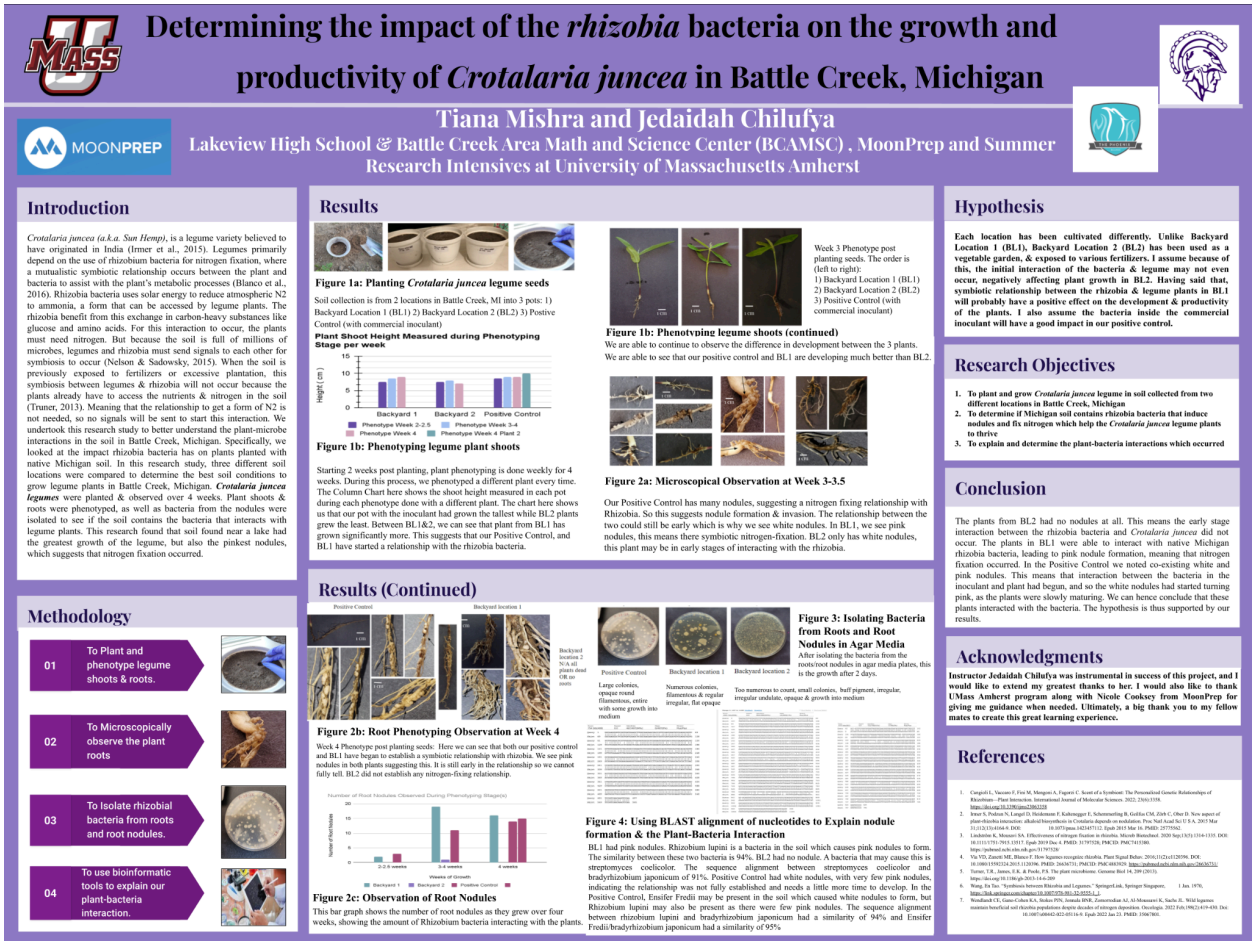
In the future, the research can be further improved by collecting soil samples from around Michigan and using a variety of legume species. This will allow us to identify the exact rhizobial species that allow certain legumes to enter symbiosis. Farmers in Battle Creek, MI, can use these findings to increase their agricultural output of legumes. They will be able to improve the productivity and growth of these plants, which will provide more food for their animals and food for human consumption.

**CONCLUSION**

After careful evaluation of experiment results, we conclude that interaction between rhizobia and plants is important for the growth and survival of plants. In this experiment, each location was cultivated differently, with respect to exposure to fertilizers, and previously existing plants. Unlike backyard location 1, backyard location 2 is not as cultivated and is heavily pre-treated with fertilizers. That is likely why the rhizobia probably may not even have sent the initial interaction signals, leading to problems with germination, lack of nodule formation, and finally early demise of the plants.  I would have to assume because of this, the rhizobia in backyard location 1 had a greater effect on the development and productivity of the plants than in Backyard Location 2. This

highlights the fact that if the fertilizer is there, the bacteria in the soil may not even try to interact with the plants. I also assume the bacteria inside the commercial inoculant would have had a good impact on our positive control pot, leading to satisfactory germination, shoot length, and nodule formation.

The hypothesis made at the beginning of the experiment was proven correct. We can demonstrate that the Rhizobial Bacteria had a positive effect on nodulation and root development of crotalaria juncea plants, but lacked the benefit of growth in terms of height for the legumes. We were also able to conclude that the soil from backyard location 1 (lakeside sample) contains more effective bacteria compared to the soil from backyard location 2 and is advantageous for plant growth. As Backyard Location 2 had fertilizers in the soil, and Backyard Location 1 did not, we can conclude the rhizobia bacteria interacts well with Michigan soil.

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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.

Aditri Balaji

### How Social Conformity Affects the Choice to Jaywalk in Boston, Massachusetts

**INTRODUCTION**

Social conformity, acting in tandem with a group of people instead of using one's individual judgment, is in many ways ingrained in human nature. Since being in a group is safer for survival situations, humans have evolved to typically feel uncomfortable when deviating from collective norms or behaviors (Levine, 2020). A 2016 research study interested in the abundance of social conformity showed that people adhered to crossing laws more often when a single stranger at the light refrained from jaywalking (Chai et al). However, the study did not test conformity with a larger group of individuals adhering to the law and assessing if others choose to wait at the crosswalk. Factors such as age, gender, and phone use can contribute to the likelihood of a pedestrian jaywalking but the biggest component is whether pedestrians feel able to break the social norms set in place by society (Shaaban et al, 2018). An additional factor that could influence one's choice to jaywalk is the relatively low repercussions that pursue after breaking this law. The legal consequence of jaywalking in Boston, Massachusetts is minimal: the Boston Municipal Court imposes a fine of $1 for the first three offenses and $2 for subsequent incidents (Mass.gov).

**RESEARCH QUESTION/HYPOTHESIS**

The main question this research experiment strives to answer is how social conformity affects someone's choice to jaywalk. Due to the emphasis society puts on social norms, my hypothesis is that when other individuals are present, pedestrians will choose not to jaywalk because of social conformity patterns.

**METHODS**

To test whether social conformity would play a role in a pedestrian's choice to adhere to or break the law that prohibits jaywalking, two different tests were set up: an experimental and a control. Experimental trials were used to measure how the presence of others following the law influenced jaywalking through conformity, and control trials were used to observe jaywalking and conformity without any outside presence. A trial began when the crosswalk sign turned to stop and ended when the walk sign appeared. A jaywalker was defined as someone who walked across the crosswalk while the sign read stop. Non-jaywalkers were people who stopped at the crosswalk and obeyed the stop sign while waiting until the sign said they could cross. For experimental trials, once the crosswalk sign changed from walk to stop, three individuals walked up to the crosswalk separately and did not make it known they were together. All three individuals were instructed to wait at the crosswalk until the sign turned to walk and then walked to the other side of the street. Two researchers observed the number of people jaywalking versus those who did not on the same crosswalk from a distance. The researchers recorded the number of people (excluding the three individuals planted for the experiment) who crossed while the sign said stop (the number of people who jaywalked) and the

number of people who waited for the sign to turn to walk to cross. The trials where no one besides the planted individuals were at the crosswalk were not viable and were not counted. For the control trials, the same two researchers observed and recorded the number of people who jaywalked and the number of people who didn't jaywalk while the three planted individuals were not participating. This was done to get a perspective on the typical amount of people jaywalking without external influence. We alternated between control and experimental trials while also alternating the orders of which type of test started first to add variation to the process and get more accurate results. This step was repeated 22 times to result in 44 total trials.

**Results**

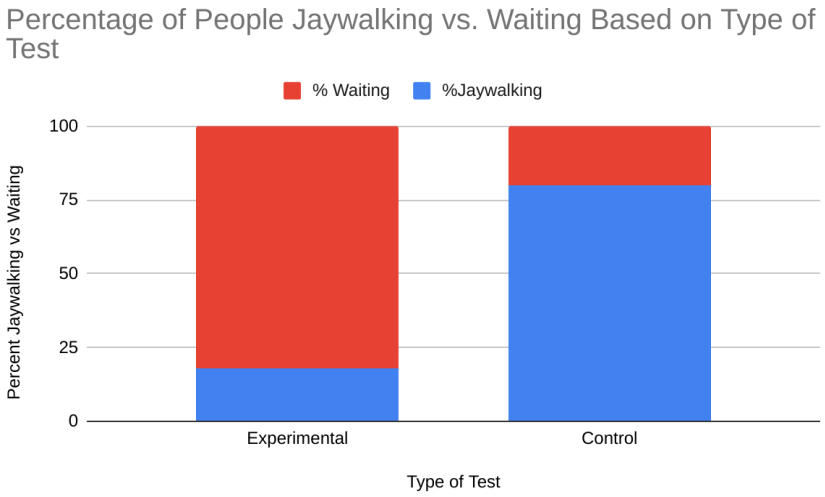


**Figure 1.** Percent of People Jaywalking verses Waiting Based on Type of Tests

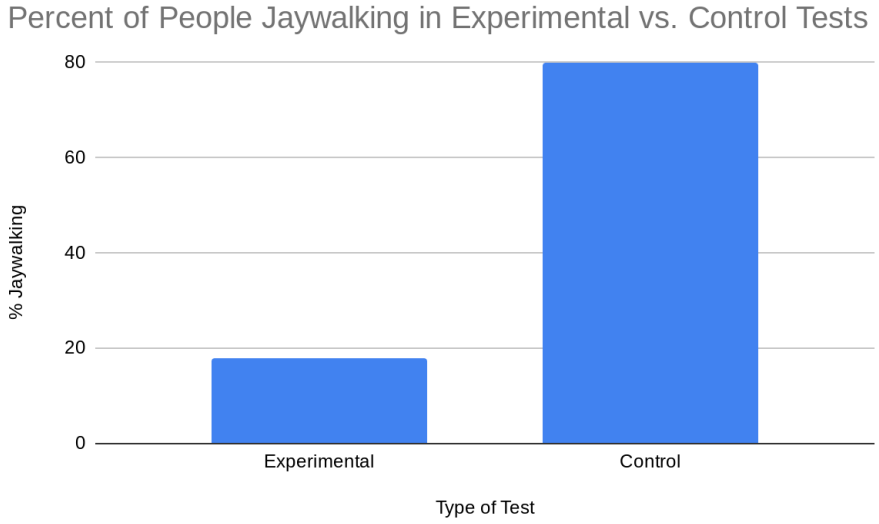


**Figure 2.** Percent of People Jaywalking in Experimental vs. Control Tests

| | Jaywalk | Waiting |
|---|---|---|
| p-value: | <0.01 | <0.01 |
| Experimental Mean: | 0.59 | 3.00 |
| Control Mean: | 3.27 | 0.55 |
| Experimental Standard Deviation: | 0.959 | 2.000 |
| Control Standard Deviation: | 2.028 | 1.262 |
| Effect Size: | 1.672 | 1.586 |

During the control test, where the three planted individuals were not present, the two researchers observed the amount of people who jaywalked vs. didn't jaywalk on a normal crosswalk. Out of the 80 total people observed during all 22 control experiments combined, 64 people jaywalked compared to the 16 who waited for the cross sign. These results show that approximately 80% of people jaywalk on a normal basis compared to the 20% of people who follow the law.

During the experimental test, the three planted individuals were present and clearly followed the law by waiting at the crosswalk when the sign was on stop and only continuing along the intersection when the sign switched to walk. In total, 79 people were observed during the test out of which 14 people jaywalked while 65 people waited with the planted individuals at the crosswalk. In this test, approximately 18% of people jaywalked compared to 82% of people who waited for the walk sign.

Statistical Package for Social Sciences, more commonly known as SPSS, is a software used to analyze quantitative data. This software was used to determine whether the results gathered were statistically significant. In order for data to be seen as statistically significant the "p-value" or the "probability value" has to be less than 0.05. As visible in the graph, the p-values for both the number of people jaywalking and the number of people waiting are below 0.01 which deems the data from the experiments statistically significant. Another indicator that the data is significant and accurate is the relatively low standard deviations. The standard deviation shows how far apart the different plots of data are in an experiment as a reflection of the reliability of the data. Since all of the standard deviations were approximately 2 or below, the data can be seen as valid and reliable.

As seen in the figures presented, 80% of people jaywalked during a control test compared to 18% of people who jaywalked during the experimental test. Figure 1 visibly shows the stark contrast in the percent of people jaywalking (blue color) and the percent of people waiting (red color) between the two different types of tests. This is further proven by figure 2 which only shows the percent of people jaywalking between the two tests. It is clear in this graph that there is a sharp increase in the percentage of people jaywalking in the control than in the experimental test. This proves that when the planted individuals were standing at the crosswalk, symbolizing a group of people

obeying the law, more people felt inclined to stick with them and conform to the group waiting rather than jaywalking. In the control test, one can see that many people jaywalk regularly in their daily lives but the need to conform to societal groups can cause people to drastically change whether they choose to jaywalk or not.

**CONCLUSION**

In correlation with what was predicted in the hypothesis, the emphasis society puts on social conformity can be seen through the pedestrians' choice to either jaywalk or wait for the crosswalk to be safe. When the three planted individuals were waiting at the intersection, there was a clear increase in the number of people waiting, compared to when no one was present. This shows that the urge to stay within societal norms is so deep that it can prompt people to change a habit engraved in their daily lives.

There were also limitations experienced during the research experimentations like weather, time of day, traffic, and experimental needs. Since it was raining on one of the days used for data collection, the weather was considered a limitation due to the probability that many people would be less inclined to wait at a crosswalk if it was raining. Additionally, more people may opt to travel by car or train when the weather is not ideal which causes fewer people to be on the streets resulting in a less accurate outcome. The next limitation experienced was the time of day since all of the data was collected on weekdays during 1-4:30 PM. This time frame is during the average person's typical work day which could make more pedestrians feel the need to jaywalk if they were running late for work or class. The third limitation was traffic since during busier times when more cars were on the roads, it is more dangerous for people to be jaywalking and crossing the road when vehicles could come through the intersection. When there are fewer cars on the road and the streets are emptier, many people may feel more safe and comfortable crossing the road when the sign is on stop.

Finally, the last limitation was the experimental needs of the research tests. For a viable result, pedestrians needed to walk up to the crosswalk while the sign was on stop and make the conscious decision to wait for it to change to walk. Each time the sign immediately switched to walk or it was already on walk, the result was not valid and a new test had to be started.

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Ankith Sureddi

# Review of Best Practices for Healthcare Professionals in the Setting of Human Trafficking

## INTRODUCTION

Human Trafficking is a global industry that is worth 150 billion dollars (Shandro et al., 2016) and is considered to be both a large public health and human rights issue. Additionally, it can affect anyone, regardless of race, gender, or other demographics (Toney-Butler et al., 2020). The International Labor Organization's estimates roughly around 40.3 million people were trafficked in 2016. In the same 2016 estimate, 72% of the total number of victims were made up of girls and women. About 5 million of these victims were also victims of global forced sexual explotation, with more than 20% of those victims being children (Toney-Butler et al., 2020). To help end the horrors of human trafficking, interventions by healthcare professionals can make all the difference. 88% of human trafficking victims will visit a healthcare professional during their captivity (Tiller and Reynolds, 2020). Through testimonies given by survivors, most human trafficking victims can receive treatment from hospitals during their time of captivity, which may be the one of the few times victims are able to receive private attention (Donahue et al., 2018). With training, healthcare professionals can better understand and screen for victims and provide help accordingly. Therefore, training that provides an increased focus on human trafficking education is recommended in hospitals and medical education institutions. Another area of research that still needs to be further studied are standardized methods that can be developed to assess patients in healthcare settings for trauma, abuse, and possible trafficking.

## BACKGROUND

This paper aims to highlight what healthcare workers in the United States can do in the face of a possible trafficking victim and how to aid the end to human trafficking as a whole. There is a large difference between human smuggling and human trafficking, though both occur throughout the globe. Human trafficking, however, involves the exploitation of a person using coercion for labor or sex. When victims are at a healthcare provider, this is where professionals can intervene for the better (Donahue et al., 2018). Due to the lack of training surrounding recognizing signs and symptoms of victimization, healthcare provider knowledge often falls short. It is becoming more clear that doctors have active and important roles in the identification and aid of human trafficking victims. Research conducted by Long and Dowdell in 2018 found misperceptions held by emergency room nurses surrounding human trafficking such as victims being prostitues, foreign born, female, or predominantly young (Pearson, 2020). An important resource that can be integrated into practice is the indepth framework for protocols related to the assistance of suspected victims of human trafficking in a healthcare setting, created by the National Human Trafficking Resource Center (Pearson, 2020).

## LITERATURE REVIEW

Saftey and protective measures for human trafficking victims exist in the United States of America. One of such protections is that a foreign person that is trafficked can utilize federally-funded benefits such as immigration assistance or access healthcare, regardless of immigration status.The immigeation assistance portion of these protective measures includes a T visa (Toney-Butler, 2022). According to the Department of U.S. Citizenship and Immigration Services, the T visa is a temporary immigration benefit that can allow for the victim to temporarily stay in the United States during the prosecution of

the trafficker for up to 4 years, with some exceptions allowed. This is critical to the safety of the victim, especially when the victim is from another country and does not have the resources to repatriate. The Justice for Victims of Trafficking Act was adopted in 2015 and provided access to government resources and funding in the aid of human trafficking victims. In addition to awareness of funding and other resources, the main resource that healthcare professionals can rely on is themselves. Some of the signs and symptoms which may raise suspicion for a possible victim of human trafficking are listed in Table 1, though not limited to only these (Tiller and Reynolds, 2020).

| Mental or behavioral symptoms | Physical symptoms |
|---|---|
| ● Anxiety<br>● Depression<br>● Excessive fear<br>● Paranoia<br>● Nervousness<br>● Avoidance of eye contact<br>● Signs of confinement<br>● Defers all decisions, belongings, and personal items to an authority figure<br>● Exhaustion<br>● Fatigue<br>● Increased suicidality and self-harm<br>● Changes in menstrual patterns in women<br>● Substance abuse<br>● Dependency<br>● Insomnia<br>● Dissociative disorders (including memory loss, loss of self, etc.)<br>● Inconsistent memories | ● Ill-appearing<br>● Malnourishment<br>● Signs of torture<br>● Signs of sexual abuse<br>● Signs of physical abuse:<br>○ Bruises<br>○ Cuts<br>○ Fractures<br>○ Previous blunt force traumas<br>○ Broken teeth<br>● Signs of restraint use |

**Table 1**. Physical and mental/behavioral symptoms of victims. If a patient presents with high likelihood to be a victim of human trafficking, healthcare professionals should always be aware of the option and protocol to signal for help if the patient or provider is in immediate danger. Additionally, providers must work with the patient to make sure that the patient's safety is not being put in danger outside of the setting of the healthcare facility.

## DISCUSSION

On a global scale, human trafficking is approximated to have placed around 20.9 million victims into forced labor (Hemmings et al., 2016). However, still more than 80% of human trafficking survivors had contact with healthcare providers and the healthcare system in general during their period of exploitation. Thus, this puts healthcare providers in an important position where they can be a potentially life-saving advocate for victims (Shandro et al., 2016). As part of a

meta-analysis of the current available body of literature, it was found that as a result of additional training, healthcare professionals felt more confident when it came to understanding human trafficking and being able to understand and identify victims (Donahue et al., 2018).

**METHODS**

A meta-analysis was performed across numerous research studies. Additionally, an online training module known as HTEmergency.com provided data about the healthcare workers who participated in the study and surveyed before and after to see how effective the courses and lessons about human trafficking were. Research and information was collected on ED Personnel that are located in two suburban hospitals. The location of these hospitals are close to a Northeast Metropolitan city and there were a total of 75 people apart from the survey that consisted of healthcare workers such as physicians, nurse practitioners, nurses, and more. According to the survey, the results found that about 89% of surveyed healthcare workers never had any prior human trafficking trainings and even before the survey was completed, about less than half of the healthcare workers in the survey knew a large amount of information about human trafficking. Thanks to the training provided, healthcare workers felt much more confident with an increase of 93% of much more comprehensive knowledge of human trafficking. 96% thought the training was useful when it came to their workspace/setting (Donahue et al., 2018).

**CONCLUSION:**

Human trafficking is a global issue and 150 billion dollar business. Human trafficking can occur anywhere and to anyone, no matter what age, gender, sexual orientation, etc.

Human trafficking can include labor or sex exploitation. Despite mental health being largely impacted during trafficking, research that is focused on the supportive needs of mental health outcomes and trafficking survivors is limited. Healthcare professionals are in a position where if they can identity the signs of human trafficking, they can intervene and possibly end the cycle of oppression for one individual. With more emphasis on human trafficking education in the medical settings, the more healthcare professionals can become advocates for human trafficking victims. Future directions may include looking into different forms of community outreach to engage the community in stopping human trafficking. An example of this would be putting up human trafficking help stickers not only in female restrooms, but also possibly male restrooms. However, the efficacy of this method is still yet to be tested, especially in regards to safety of victims, especially if their traffickers are aware of these resources, which may become increasingly relevant as the number of gender-neutral restrooms increase. Another area of research that still needs to be further studied are standardized methods that can be developed to assess patients in healthcare settings for trauma, abuse, and trafficking. Healthcare workers need better education regarding human trafficking so that way the workers can recognize the signs, step in, and intervene to prevent the escalation or prolonged victimization of individuals.

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Ariana Tanha

# Rehabilitation for Stroke Patients Using Neuroplasticity

**ABSTRACT**
This paper is about how neuroplasticity can be used to aid stroke patients. Neurorehabilitation can be very useful to someone who has had a stroke and can allow them to regain nearly all their lost functionalities. With this newfound information, medical doctors and other healthcare professionals can treat someone who has had a stroke more effectively.

**INTRODUCTION**
The brain is a key organ in the human body. It controls what people do, say, and think. Diseases and injuries that harm the brain are detrimental. One of the worst brain injuries is a stroke. Thousands of people lose their abilities to the damage caused by strokes and suffer for the rest of their lives. If doctors could find a way to use neuroplasticity to help stroke patients recover faster and more efficiently, this would benefit the population. The human brain always has the ability of neuroplasticity, causing there to be huge potential in this area. This brings forth the question: in what ways can neuroplasticity help aid stroke patients with the brain damage they have undergone?

A stroke is a medical condition in which the absence of blood flow to the brain causes circulatory disorders in the brain and can cause the brain cells to become damaged and/or die [10]. Stroke is the leading cause of long-term disability in the USA and is one of the most common health issues [9].

According to the CDC, someone has a stroke every 40 seconds in the USA [9]. Going even further, someone in the USA dies every three and a half minutes because of a stroke [9]. Stroke can occur from high blood pressure, diabetes, smoking, and heart disease, as the CDC says [9]. Symptoms of stroke include numbness, weakness, and visual loss [10]. Some people do not even notice that they have had a stroke. It is important to try to recognize when someone has had a stroke to recover the most efficiently. This is a very serious issue; strokes are debilitating millions of people, making it difficult for them to live a normal life without needing some sort of aid. Doctors have been looking for methods of therapy and medicines that would help the recovery path for stroke patients to be smoother and more efficient. One method that was found to be very effective was neuroplasticity.

Neuroplasticity refers to the functional and structural changes in the brain that occur during development, interaction with the environment, aging, and response to injury [8]. Another way of putting it is that “neuroplasticity is the ability of the brain to redevelop its neuron network at the healthy part of the brain, to take over the role of the damaged part” [7]. Neuroplasticity occurs in everyone. Anything that requires a person’s brain to change its thinking is considered neuroplasticity. This can range from learning a new language to playing the piano. Some examples of neuroplasticity that relate to injury are things relating to the loss of senses, hemispherectomies, and the rehabilitation of stroke patients. In the case of lost senses, other senses might be heightened. For example, if someone lost their sense of eyesight, their sense of sound or smell might be better to make up for it. In a hemispherectomy, half of the brain is removed or disconnected from the other half. People, most commonly children, go through this operation to avoid having severe seizures. With children, neuroplasticity is more effective. “It’s as if their brains haven’t

quite yet decided what they wanted to be when they grew up" [1]. That causes the other parts of their brains to adapt with the other roles easier. Lastly, there are similar ways in which stroke patients can be benefited by the phenomenon called neuroplasticity.

**METHODS AND MATERIALS**

Pubmed.gov was used as a database to find articles related to neuroplasticity, stroke patients, and rehabilitation. The website for the National Library of Medicine, specifically the National Center for Biotechnology Information, was also used along with The University of Pittsburgh's online library.

**RESULTS**

Recovery after stroke depends on neuroplasticity and other things relating to it. Neuroplasticity plays a huge role in stroke recovery because other parts of the brain could adapt to take the functions of the parts of the brain that died. Neurorehabilitation combines the aspect of neuroplasticity and the rehabilitation of stroke victims. Neurorehabilitation research aims to find interventions that promote recovery and to establish whether the presence or absence of improvement can be explained by any neuronal changes that occur in the post-stroke brain [6]. Maier tested 15 principles that should guide the design of effective neurorehabilitation protocols for post-stroke recovery. These included massed practice, spaced practice, dosage, task-specific practice, goal-oriented practice, variable practice, increasing difficulty, multisensory stimulation, rhythmic cueing, explicit feedback/knowledge of results, implicit feedback/knowledge of performance, modulate effector selection, action observation/embodied practice, motor imagery, and social interaction [6]. These were split into two groups, motor cortex activities and other networks. Motor cortex activities included massed practice, dosage, variable practice, task-specific practice, modulate effector selection, and multisensory stimulation [6]. Other networks included goal-oriented practice, increasing difficulty, action observation, motor imagery, mirror therapy, rhythmic cueing, implicit feedback/knowledge of results, and social interaction [6]. The research article looked at how neuroplasticity affects each of these groups and how each principle can aid stroke victims. The conclusion of the research is that an effective rehabilitation approach should thus incorporate principles of both types to counteract neuronal degradation and promote improvement.

It is extremely important to determine when the stroke occurred. There is a particular window of enhanced neuroplasticity after stroke in which recovery and rehabilitation might be more very effective [2]. It is very uncommon to specifically see trials of rehabilitation within the first two weeks after a stroke, as many people believe that the stroke victims should rest [2]. This has a negative effect rather than a positive one. It is shown that forced bed rest after stroke may harm the victim instead of helping them heal. According to Coleman, recovery starting within the first 24 hours after stroke may harm the patient recovery started within the first two weeks is very beneficial [2]. To summarize, starting recovery between one to fourteen days is very effective for stroke victims. This is probably because within the first 24 hours, the brain is still recovering from the initial stroke and after two weeks, the brain has already calmed down and accepted the new way of life.

Other than time, there are no other limits on neuroplasticity and its aid to stroke victims. Having a more intense stroke will make it harder to completely go back to living how it was. As the brain suffers more, it may get more difficult to restore it to its previous glory. The abilities lost during the stroke have no effect on neuroplasticity; it works no matter what abilities are lost. If speech is lost, that can be relearned by a different part of the brain. If movement in the left leg was lost, that can be relearned by a different part of the brain. However, if something like the memory

cortex is damaged, then it is unsure what would happen. The hippocampus is a very sensitive area of the brain. Any damage to it may cause someone to lose their memories or be unable to create new ones. If some part of the brain is still functioning, the brain can adapt itself to make it easier to live life as a normal human being.

Living with stroke is extremely difficult. According to Legg, post-stroke effects could leave victims unable to do daily tasks they must do to live, such as eating and drinking, moving around their homes, going to the bathroom, carrying out personal hygiene tasks, dressing themselves, and grooming themselves [5]. This is embarrassing to stroke victims, so many of them mostly are afraid to reach out for help or assistance with these everyday tasks. Legg goes on to talk about how using neuroplasticity and occupational therapy can help stroke victims recover and improve their ability to carry out the activities of daily living [5]. After conducting an experiment on this topic, Legg found that there was very “low-quality” evidence that occupational therapy can help improve the performance of daily tasks in stroke victims [5]. Because the study had methodological flaws, it is not possible to use this data with 100% confidence.

This article goes through research that supports cortex stimulation to trigger neuroplasticity and aid stroke patients in their recovery. In this experiment, it is researched how one can overcome TBIs, or traumatic brain injuries [4]. Cortex simulation studies in stroke patients, including ones, have provided evidence that cortex stimulation can alter brain plasticity and that it would support the recovery of functional motor skills [4]. In a clinical trial, implantable epidural bipolar electrodes were placed over the motor cortex of stroke survivors [4]. In these trials, the stroke survivors received cortex simulation during physical therapy or did not receive it during physical therapy [4]. The results were that cortex simulation helped the victims recover and, more specifically, helped them regain the ability to move their hand, which they could not before because of the stroke [4]. Cortex simulation might also be another way to help stroke victims along with neuroplasticity.

An experiment was conducted in the Indonesian region on neuroplasticity and stroke patients [7]. More specifically, it was tested how physiotherapy affects brain plasticity according to the ability of balance and functional ability [7]. Sixty-four stroke patients from the Surakarta region of Indonesia were selected for this experiment [7]. After being randomly split into half, one group received neuro-restoration protocol for a week while the other received conventional physiotherapy for a week [7]. Conventional physiotherapy includes changing positions, breathing exercises, and exercise therapy in passive and active mobilization to help the stroke patient recover [7]. The neuro-restoration protocol included cognitive, associative and automation stages. The results showed that both groups had improvements in balance and functional performance, but only the intervention group had a significant improvement in neuroplasticity regeneration [7]. When the two groups were compared instead, the neurorestorative intervention group had a greater effectiveness compared to the conventional physiotherapy intervention in terms of functional performance and balance but did not achieve statistical difference in neuroplasticity regeneration [7]. The result of the experiment was that neuroplasticity had a positive impact on balance and functional ability of stroke patients in only a seven-day period [7]. The conclusion of this experiment is that services such as physiotherapy are important for stroke rehabilitation and can aid neuroplasticity.

One study that was interesting was a study that looked at neuroplasticity in stroke patients and depression. More evidence is being presented that depression is closely related to brain structure and functional changes [11]. In patients with depression,

brain structure changes are closely associated with certain parts of the nervous system, like the frontal lobe, cingulate gyrus, and hippocampus [11]. The hippocampus is extremely important, as it controls stress and mood regulation. An autopsy study found that patients with depression showed “impaired plasticity of actual hippocampal neurons” [11]. This means that if this part of the brain did get injured, it would be harder to recover from the injury.

**DISCUSSION**

How can neuroplasticity help aid stroke patients with the brain damage they have undergone? The rehabilitation for stroke patients using neuroplasticity can be very beneficial. Neuroplasticity is the ability of the brain to rework itself to adapt to new things or injuries in the brain. A stroke is where the brain loses oxygen and some of the brain tissues die. Neurorehabilitation has been proven to help increase the chance of the brain being healed after stroke if one activity from each group is used. It is important time wise to be able to recognize when a person has had a stroke. Once determined, there must be a 24 hour wait time before neuroplasticity can work. After two weeks of waiting, though, this is too long, and it will no longer be as effective to use neuroplasticity. Other than the time limit, other factors do not affect neuroplasticity. If part of the brain can function as normal, the rest of the brain can be adapted into it to survive and restore the human's previous abilities. Stroke patients suffer every day to do common daily tasks. If neuroplasticity and occupational therapy were used properly, this would decrease the chances that stroke victims are embarrassed and too handicapped to do basic tasks.

According to Kobeissy, cortex simulation can also help aid the recovery of stroke patients and cause neuroplasticity to work more effectively. Based on an experiment conducted in Indonesia, services such as physiotherapy are important for stroke rehabilitation and can aid neuroplasticity. One study also correlates depression and neuroplasticity. It is important to make sure that the brain does not get hurt or anything that could cause someone to have depression. Throughout all this research, neuroplasticity can aid stroke patients in many ways. This is important to consider because it has the possibility to help so many stroke victims recover from extremely debilitating injuries. The brain’s ability to heal and adapt in the way that it does is really a miracle and is super interesting to see.

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Ethan Angeles

**Teen Weightlifting: Avoiding Injury and Risk**

**INTRODUCTION**

In recent years, weightlifting has become popular among the teenages. As recently as 2017, 51% of teens, according to a questionnaire conducted by the Center for Disease Control and Prevention, participate in weight training exercises three (3) or more days a week (Wang, 2021). Because of this rise in interest among teenagers, it is important to know the training risks and how to avoid injuries. A common myth is that weight training and high-impact sports can stunt your growth and damage growth plates. However, this myth may be true when improper form and weight are used while performing a specific movement. A handful of risks may happen while lifting weights, but the benefits can outweigh the risks when taking the right precautions and time (Perez, 2022). This paper will discuss the beneficial habits that teenagers should have to reduce short-term or even long-term injuries. It will also discuss the differences between bodybuilding, weightlifting, and powerlifting.

*Bodybuilding*

According to Parry, Bodybuilding is the use of hypertrophy training to increase muscle mass and size for aesthetics rather than the purpose of being able to lift a large sum of weight (2021). People might compete in bodybuilding competitions, where they are scored on their muscle control to correctly perform poses. In many competitions, there are many mandatory poses in which bodybuilders must perform, such as the front double bicep in which competitors lift their arms and bring them down to flex the biceps. Another is the lat spread in which competitors reach back, squeeze their waist, and spread their shoulders out to create a V-shape with their torso.

*Weightlifting*

Weightlifting is a competitive sport with two main lifts: the snatch, and the clean and jerk. The snatch is a movement in which the competitor lifts the barbell off the ground to above their head. The clean and jerk are similar to the snatch in which the goal is to lift the barbell above their head. However, the difference is between these two movements where the clean and jerk is a movement when the competitor lifts the barbell up but then twists their elbows forward, allowing the barbell to rest on their upper chest. They then either thrust the bar up and land in a squat position or a lunge.

*Powerlifting*

Powerlifting is much less technical compared to weightlifting. Weightlifting has more complex movements while powerlifting has more simple movements in which the bar path goes in one or two directions. The main lifts of powerlifting include the bench press, the squat, and the deadlift.

The deadlift is when the barbell is lifted off the ground close to the lifter's shins. The weightlifter's back is kept straight while lifting the barbell in a hinge motion.

The squat can be completed as a front squat or back squat. For a front squat, the weightlifter has a bar resting on their hands and upper chest. By bending their knees and keeping the back straight, they will lift back up using their quadricep muscle and hamstring muscles to lift the barbell back up.

The bench press requires the lifter to lay on a bench and push the barbell off of their chest.

**Why Teens Get Injured**

Many injuries within weightlifting often come from a poor form during a certain lift or using incorrect weight. An example of poor form in weightlifting could be not having a straight spine while performing a compound lift such as a deadlift or a snatch which could result in a spinal injury or lumbar injury.

Another way teens can get injured is by having an improper load on the bar. If they jerk the bar meaning, there is slack in their

arms and shoulders, they may cause an injury to their arms, such as a muscle strain and or tearing of a tendon. They may also lose balance while holding the improper load, which could cause them to fall over. These are some of the most common injuries that may happen while weightlifting.

**Avoiding Injuries**

The best ways to avoid injury or the increased risk are to stretch and warm up before performing any lift, using the correct load and equipment for the exercise, using correct form during exercises that heavily focus on multiple muscles, and allowing for enough rest time in between lifting sets.

*Stretching*

Stretching is one of the most important things to do before performing any exercise because it allows the muscles to become more flexible and complete more complex movements. By not stretching prior to lifting, it might mean the lifter cannot perform the exercise to its fullest and receive the expected results.

The best type of stretching to do before lifting weights is dynamic stretching. Dynamic stretching is movement-based in which the muscles are not being stretched in a fixed position. An example of dynamic stretching is leg swings against a wall in which you swing your leg from medially to laterally while supporting yourself on a wall. This can stretch the hip flexor muscles which are the iliacus, psoas major, rectus femoris, and sartorius. These muscles are most important during hinging weightlifting activities and compound leg exercises such as squats, lunges, split squats, and deadlifts.

Another example of dynamic stretching is arm circles in which you extend your arms out laterally and move them in a circular motion moving clockwise starting small and making the circular motions gradually larger for 20-30 seconds, then you repeat the motion, but in a counter-clockwise direction. This warms up the rotator cuff muscles, which are the subscapularis, teres minor, infraspinatus, and supraspinatus. Rotator cuff muscles are being used in nearly every upper body exercises, including the bench press, included dumbbell press, and lat pulldowns.

*Correct Weight*

Many people who lift weights frequently or semi-frequently may want to “ego-lift,” meaning they try to lift a maximum amount of weight while using poor form. Ego-lifting can be very dangerous and is one of the most common ways people who lift are injured because they aren’t aware of how much weight they can handle for certain repetition ranges. According to Smith, if the lifter can only do 1-5 reps of a certain weight, then that weight is considered to be “heavy” for their strength abilities (2019). If the amount of repetitions the weightlifter can do is between 6-12, then that is considered a “moderate” weight; this is what many bodybuilders choose to lift to increase muscle mass and shape. Lastly, anything over 12 repetitions is considered “light” weight.

It is often recommended to beginners and young teenagers who start lifting weights to often use a weight that is considered “light” to perfect their form to prevent damage and injuries to their body.

**How Teens Can Lift Safely**

When many teenagers think of starting to lift weights, they often try to lift as much weight as possible instead of thinking about their form during their exercise. The easiest way to perfect your form is to start with a very low weight or no weight at all to perfect the form and take the time needed during the concentric and eccentric parts of the exercise. Eccentric means the movement of the lift in which the targeted muscle(s) are being stretched and lengthened, such as the down movement during a squat or bench press. Concentric means the movement of the lift in which the targeted muscle(s) are being contracted and shortening such as the pulling movement during a pull-up.

**Why Teens Should Lift**

The main reason many teens get into weightlifting is to become stronger and show off their strength, but there are many more benefits of weightlifting for teenagers. One of these benefits is that many teenagers have increased confidence and lower depressive moods, as shown in a study by Brett Gordon, a postdoctoral fellow studying exercise and cancer at the University of Pennsylvania State (Gordon, 2018). He concluded that the increased feeling of confidence might come from the feeling of accomplishment of one's self that comes from exercising and the goal of increasing repetition ranges and weight on specific exercises.

Teens' interest in weightlight has increased dramatically in the past two decades. This increasing interest has now made it more necessary than ever, for young teens who are starting to weight lift, that many know the risks of these specific exercises and how to properly perform them with minimal risk of injury. Through proper patience and learning, many teens will be able to succeed in their goals within recreational activity and possibly within the sport.

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Gautham Ranganathan

# Observation of Keratosis Pilaris in a Multi-Generation Family and the Comparative Study of Turmeric-Rose Water and Loofah Therapies on Reducing Keratosis.

**ABSTRACT**
Keratosis Pilaris is a form of built-up keratin that presents as small bumps on areas of the body, such as the arms, buttocks, cheeks, and thighs. These bumps are harmless, non-contagious, and usually, resolve with age. Keratosis Pilaris is a hereditary dermatological disease passed from the parent(s).

This study investigates the incidence of keratosis in a multi-generation family. Keratosis pilaris or red bumps was first observed on the upper forearms at puberty onset, approximately, age 13. As part of this research, a multi-generational family was interviewed, to find out more about the hereditary nature of this disease.

The effect of applying a mixture of turmeric and rose water on keratosis was observed as a treatment option. Additionally, the effect of using a plant-based scrubber/loofah on keratosis was observed.

A comparison of the results of the turmeric-rose water therapy and the loofah therapy was made to conclude which is a better solution for keratosis.

**INTRODUCTION**
Keratosis Pilaris, a benign hereditary dermatological disease, is seen to have a genetic component [1]. The disease presents with built-up keratin as small bumps on areas of the body that resolve with age [2].

There are several clinical trials that have been conducted for keratosis treatment and each evaluates a chemical or drug application for keratosis [3]. This research focuses on the use of plant-based products to see if they will reduce keratosis.

The goal of this research is to create a toolkit for keratosis that can be passed to the author's relatives who will soon be reaching adolescent age and may develop keratosis. This toolkit containing plant-based products like turmeric-rose water, and loofah can then be socialized and widely used for other families with keratosis. This plant-based toolkit will help in improving the morale of young adolescents with keratosis.
Rose water contains many important materials that help the skin, including vitamins B and C. Rose water is said to remove oil and dirt from the skin, be antibacterial, and not clog your pores [4]. Turmeric contains an active ingredient known as curcumin that provides benefits when used on the skin. It can help exfoliate dead skin cells and reduce acne [5].

A loofah is a natural product, as it comes from the loofah plant. It is great for your skin since it takes away dead skin, dirt, and oils, and it is safe to use [6].

Existing research has studied the effects of lasers on keratosis and the application of certain acids and mineral oils to reduce keratosis [3]. There is no research that has studied the effect of natural products as therapies to reduce keratosis pilaris.
This study evaluates and compares two therapies, one, the application of a turmeric-rose water mix, and the other, the application of a loofah, to reduce keratosis. The two therapies were applied at the same time, turmeric-rose water mix on the left arm and loofah on the right. Survey respondents voted on the before and after pictures of the two treatments.

*Hypothesis*
p(turmeric-rose) - The proportion of respondents that selected the post-treatment left arm picture to have smoother skin, indicates turmeric-rose water therapy is effective.

p(loofah) - The proportion of respondents that selected the post-treatment right arm picture to have smoother skin, indicates loofah therapy is effective.

*Null Hypothesis*
The turmeric-rose water therapy is more effective or at least equal to the loofah therapy to reduce Keratosis Pilaris.

p(turmeric-rose) >= p(loofah)

*Research Hypothesis*
The loofah therapy is better than the turmeric-rose water therapy to reduce Keratosis Pilaris.

p(turmeric-rose) < p(loofah)

**MATERIALS AND METHODS**
The presence of genetic transmission of keratosis in a multi-generational family was investigated by conducting an interview with members of the author's family to find out if keratosis is indeed hereditary. The research was conducted between May 2022 – August 2022.

*Interview with the multi-generational family*
To observe the incidence of Keratosis, a Google Forms survey was created with the following questions:

1. This is a survey to obtain family data on the genetic basis for keratosis. All the data obtained in this survey will be used without disclosing personal information.
2. If you are an adult of 18 years of age or more, please answer the questions for yourself.
3. If you are a parent of a minor child less than 18 years of age, please take the survey again, for each of your children.

*Note: Only one parent needs to complete the survey for each child.*

*Table 1. Family Information.*
Q1: Are you filling the survey for yourself or your child?
Q2: What is your (or your child's) relationship with Gautham? Please specify: (maternal aunt, paternal uncle, etc.)
Q3: What age range do you belong to?
0-12, 13-18, 18-45, 45-60, Above 60
Q4: Do you (or your child) have red bumps on the skin of your upper arms? Did you ever have red bumps on the skin of your upper arms?
Q5: If yes to Q4, then at what age did you first notice them on you (or your child)?
Q6: If yes to Q4, then at what age did they subside or go away?
Q7: Do you (or your child) have these red bumps on any other skin on your body? If you would like, please mention, if not you may move on.
Q8: If yes to Q4, do you (or your child) wish you could make the red bumps disappear? Please enter any other feelings you have about this skin appearance and its impact on your (or child's) life.

These questions were sent through a survey to the author's maternal and paternal family, and the results were collected.

*Creating the turmeric-rose water mix*
A dozen red roses and 0.23 lbs. of turmeric were purchased from the local grocery store. The rose petals were separated from the bunch and the turmeric was chopped into small pieces. In a large stainless-steel pot, 6 (8oz) cups or 48 oz of filtered tap water were added. Next, the rose petals and turmeric were added to the filtered water. The mixture was boiled on the stove for 20 minutes, then left to cool to room temperature. This mixture was passed through a strainer to obtain a turmeric-rose water mixture. The turmeric-rose water mixture measured 3.5 (6 oz) cups or 28 oz and was then refrigerated.

*Pre-Condition before the experiment*
A week before the experiment the author made sure to use only soap and water in the morning shower. No dermatological treatments, antibiotics, or loofah, other than a regular moisturizer were applied. This same process was continued for the entirety of the experiment time frame. This was done to ensure that full-blown keratosis is seen on the arms and not reduced by any other factor.

*Experiment with two different therapies on the author's left and right arm*
Day1 of the experiment – May 30, 2022
Day7 of the experiment – June 5, 2022

Pictures of the author's left arm and right arm before the start of the treatment were taken, on the night of May 30, 2022. Every night the turmeric-rose water mix was applied to the left arm. The loofah was dipped in warm water and gently rubbed up and down the right arm for about 10 seconds. This treatment was repeated every night on both arms for 7 days. Pictures of the left and right arms after the treatment were taken on the 8th day, June 6th, 2022. A Nikon D90 camera was used to capture the pictures.

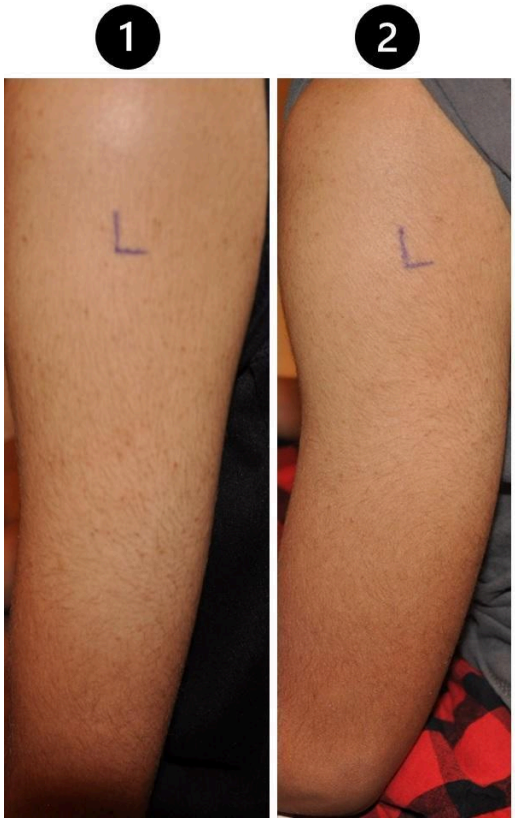
The before and after pictures were compared to see the effects of the treatment. To remove the experimenter's bias, participants were enlisted from social networking to vote on the pre-treatment and post-treatment pictures to determine the results of the application.

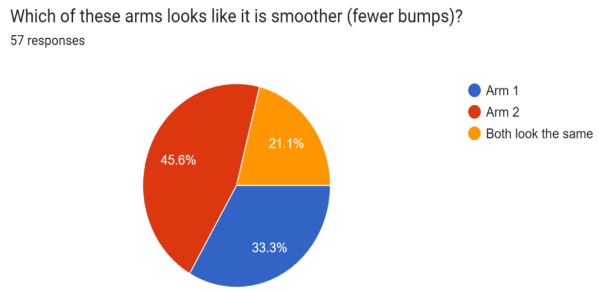
*Table 2. Survey for Results*
Q1: Picture of the left arm on Day1 before treatment and picture of the left arm on Day 8 after 7 days of treatment. Pick which is smoother
Q2: Picture of the right arm on Day1 before treatment and picture of the right arm on Day 8 after 7 days of treatment. Pick which is smoother.
Q3: Pictures of Q1 and Q2. Call therapy A and therapy B. Ask which therapy resulted in smoother skin.

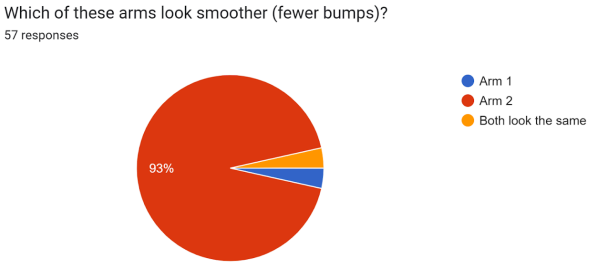
**RESULTS**
Survey results demonstrated that a child that has one parent with Keratosis Pilaris, then the child will get the condition as they age, around the teenage years. If both parents have Keratosis Pilaris, then the children exhibit this skin condition at an earlier age. If both parents did not have Keratosis Pilaris, the children did not show the condition. This observation is limited to the author's multi-generational family.

The left arm and right arm pre-treatment and post-treatment pictures were used to create a survey and sent out to family and friends. Responses were obtained from 57 participants.

*Image 1-2. Left arm before and after the turmeric and rose water treatment.*

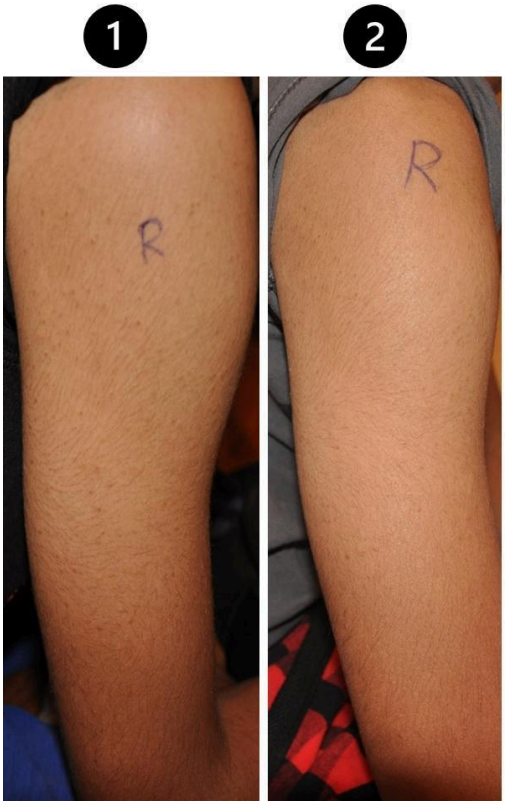





*Picture 1 – Pre-treatment;*
*Picture 2 – Post-treatment*

*Image 3-4. Right arm before and after the loofah treatment.*




*Picture 1 - Before Treatment;*
*Picture 2 - After Treatment*

*Turmeric and Rose Water Treatment*
26 out of 57 respondents agreed that Arm 2 showed fewer red bumps or Keratosis indicating treatment was effective.

12 people thought that both the arms looked the same indicating no effect of the treatment.

19 respondents thought that Arm1 showed fewer bumps indicating treatment might have worsened the condition.

*Loofah Treatment*
53 out of 57 respondents agreed that Arm2 showed fewer red bumps indicating an effective treatment. 2 people thought that both the arms looked the same. and 2 people thought that Arm1 showed fewer bumps.

*Statistical Analysis*
A two-sample proportion test was conducted using the survey results of the treatment pictures, to determine which hypothesis is correct [7].

$p_{(turmeric\text{-}rose)}$ = 26/57= 0.45614; n=57

$p_{(loofah)}$ = 53/57= 0.929825; n=57

$\hat{p}$ = (26+53)/ (57+57) = 0.692982

$Z = [p_{(turmeric\text{-}rose)} - p_{(loofah)}] / \text{sqrt}\,[\hat{p}\,(1-\hat{p})\,(1/n + 1/n)] = -5.48237$

P value is 0

$p < 0.05$, and $p < 0.01$, so the null hypothesis was rejected for both these significance levels.

That is, statistically, from the survey results, it was found that the loofah therapy is better than the turmeric-rose water therapy.

$p_{(turmeric\text{-}rose)} < p_{(loofah)}$

**DISCUSSION**

*Keratosis in the author's multi-generational family*

Every individual that exhibited keratosis pilaris had at least one parent with the same condition. When one parent had keratosis and the other did not, then the children started showing keratosis in the teenage years and beyond. When both parents had keratosis, the children started showing keratosis much earlier than teens suggesting strong evidence of the genetic basis of keratosis pilaris in the author's family.

*Which therapy is better for reducing keratosis pilaris?*

Based on the results of the statistical analysis of the before and after pictures, the loofah therapy resulted in a greater reduction of keratosis pilaris.

*Limitations:*

A genetic tree was created based only on the author's multi-generational family. It would be ideal to conduct this research on many families. The therapies were applied to only a single individual. It would be better to apply this to a cohort of patients to study the effects. Also, people from the author's social network have voted on the before and after pictures. It would be better for dermatologists to rate the effectiveness of the therapies. In addition, the pictures were taken at best effort in the same lighting, before and after. Clicking pictures in a professional lab would be more appropriate.

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Hailey Chulamorkodt

**Kinetics of Polymer Degradation with Tenebrio Molitor - Using Mealworms to Stop Plastic Waste**

**ABSTRACT**

Americans generate 32 million tons of discarded plastic waste that accumulates in landfills. Plastics can take over fifty years to decompose. Accumulating plastic waste is becoming a major problem. In a Stanford study, the Tenebrio Molitor has been shown to digest Styrofoam, a popular form of Polystyrene. The Plastics Industry Council categorized plastics into six categories based on the chemical monomer. I performed an experiment to determine if the Tenebrio Molitor could also digest all six categories of plastics. Over four weeks, Polystyrene has a mean reduction of 32.8% (SD:1.5%), Polypropylene has a mean reduction of 27.8% (SD: 2.1%), Low-Density Polyethylene has a mean reduction of 19.9% (SD: 1.8%), Polyvinyl Chloride has a mean reduction of 4.9% (SD: 0.6%), and High-Density Polyethylene has a mean reduction of 3.2% (SD: 1.1%). My experiment shows that the Tenebrio Molitor was not as effective in digesting other plastics as well as polystyrene. Although my hypothesis was not supported by my experiment, the study demonstrated that Tenebrio Molitor can be used to digest other plastics.

**INTRODUCTION**

Americans generate 35.7 million tons of plastic annually. Only 8.7% of plastic is recycled. As a result, 32 million tons of discarded plastic waste accumulates in landfills. Plastics can take over fifty years to decompose. Plastics can be augmented with additives that cause them to extend the useful life of the item, but increase the decomposition rate to 400 years. In a Stanford study, mealworms (Tenebrio Molitor) have been shown to digest Styrofoam (Polystyrene). The research shows that the Tenebrio Molitor has bacterial flora that is able to break down polystyrene into Carbon Dioxide and body mass (Jordan, 2014).

In 2014, Yang et Al published a study from Stanford University in the Environmental Science and Technology Journal about the ability for the Tenebrio Molitor to digest Polystyrene. His study showed that the mealworms, which are the larvae of the Tenebrio Molitor, were able to chew, eat, and digest Styrofoam.

In this study, the researchers studied the mealworms entirely with Polystyrene over one month. The researchers analyzed the digestive system using chromatography, nuclear magnetic resonance spectroscopy. The study revealed Polystyrene molecules that have been decomposed to depolymerized fragments in the digestive system. The study showed that over one month, there was 47.7% degradation. This biodegradation of Polystyrene brought the concept of rapid degradation of plastics waste.

There have been follow-up studies isolating the bacteria in the Tenebrio Molitor, measuring the kinetics of polystyrene digestion, and following the passage of microplastics up the Tenebrio Molitor Food chain. There has not been a formal study to determine if the Tenebrio Molitor is able to digest the six categories of plastics. The Plastics Industry Council has six plastics classes.

Plastics have become the category name for substances called polymers. The translation of polymer is "of many parts". Polymers are repeating chemicals that form a long chain of molecules. Polymers in nature include sugars such as starches, the DNA backbone, and cellulose.

Plastics are useful because they are cheap, strong, and useful. Plastics are easily shaped into useful devices. Plastics are used

for medical devices, computer parts, eating utensils, containers, and furniture. The plastics are categorized into the fundamental polymer. The Plastics Industry Council created the Resin Identification Code to categorize plastic on the fundamental repeat polymer link. The plastics are Polyvinyl Chloride, Polypropylene, Polystyrene, Polyethylene Terephthalate, High-Density Polyethylene, Low-Density Polyethylene.

This study was designed to determine if the Tenebrio Molitor can digest other plastics in addition to Polystyrene. I hypothesize that Tenebrio Monitor can digest Polyethylene Terephthalate, High-Density Polyethylene, Polyvinyl Chloride, Low-Density Polyethylene, Polypropylene at the same rate of Polystyrene.

## MATERIALS AND METHODS

*Materials*

There are two subsets for materials - construction and experiment. My equipment include Water Bottle, Grocery Bag, Toilet Brush, Bread Bag, Straws, Styrofoam Cups, Mealworms, Fast Food, Container, Digital Scale, Scissors, Rubber, Wallet, and Specimen Container

*Procedures*

The Tenebrio Molitor was obtained from a local Pet Store. The Tenebrio was housed in a dark, damp 40 degree habitat. The Water Bottle, Grocery Bag, Toilet Brush, Bread Bag, Straws, and Styrofoam Cup DVD were cut and crushed into approximately 8mm size fragments samples. These samples weighed 100mg. These samples were added to each specimen container. Thirty-five Tenebrio Molitor were added to each specimen container. Each specimen weighed weekly. When weighing the specimen, I removed the specimen fragments and left the mealworms. I weighed each fragment weekly for four weeks.

*Safety Issues*

The safety issues involve two aspects: (1) construction and (2) testing. From a construction standpoint, safety protocols were adhered to during the use of power tools and sharp instruments. A table saw was used to cut the 1” x 1” wood planks. A power hand drill was used to make pilot holes for the screws. A power jigsaw was used to cut openings in the plywood. Appropriate attire, such as safety goggles and leather safety gloves, were used during power tool operation. From a testing standpoint, the safety protocol was adhered to. Urine was not used. Water and water with food coloring was used for actual testing. Water is a reasonable substitute for urine but provides eliminated risks of bacterial infection and acidic damage.

## RESULTS

Over four weeks, Polystyrene has a mean reduction of 32.8% (SD:1.5%), Polypropylene has a mean reduction of 27.8% (SD: 2.1%), Low-Density Polyethylene has a mean reduction of 19.9% (SD: 1.8%), Polyvinyl Chloride has a mean reduction of 4.9% (SD: 0.6%), and High-Density Polyethylene has a mean reduction of 3.2% (SD: 1.1%).

Polystyrene and Polypropylene had the best digestion rates of 32.8% and 27.8%. This reflects a similar carbon backbone structure. My experiment shows that Low-Density Polyethylene and Polyethylene Terephthalate had moderate amounts of degradation at around 20%. Polyvinyl Chloride and High-Density Polyethylene had minimal degradation of around 2%.

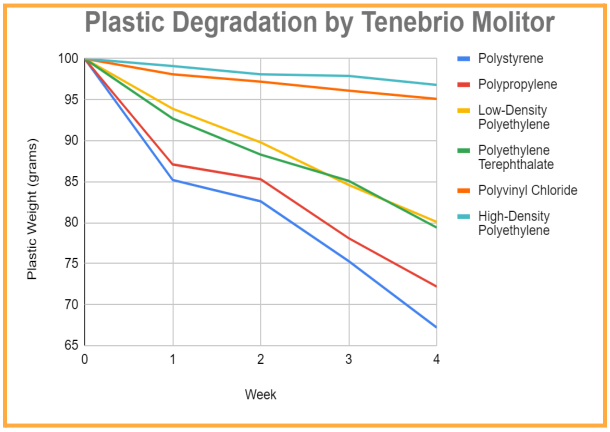


*Figure 1. Plastic Degradation by Tenebrio Molitor*

| | Polystyrene | Polypropylene | Low-Density Polyethylene | Polyethylene Terephthalate | Polyvinyl Chloride | High-Density Polyethylene |
|---|---|---|---|---|---|---|
| Week 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| Week 1 | 85.2 | 87.1 | 93.9 | 92.7 | 98.1 | 99.1 |
| Week 2 | 82.6 | 85.3 | 89.8 | 88.3 | 97.2 | 98.1 |
| Week 3 | 75.3 | 78.1 | 84.6 | 85.1 | 96.1 | 97.9 |
| Week 4 | 67.2 | 72.2 | 80.1 | 79.4 | 95.1 | 96.8 |
| | | | | | | |
| Degradation from Week 0 to Week 4 | 32.8 | 27.8 | 19.9 | 20.6 | 4.9 | 3.2 |

**DISCUSSION**

Enzymes speed up chemical reactions in the body. They bind to molecules known as substrates and alter them. The enzymes are essential for digesting food, muscles actions, nerve function, and respiration. These enzymes speed up a reaction by a process known as catalyzation. Catalyzation involves reducing the activation energy. A substrate binds to the active site of an enzyme and is converted into a product. Once the product leaves the active site, the enzyme is prepared to process another new substrate.

Enzymes work in the digestive system, DNA replication, and liver enzymes. In the digestive system, enzymes help the body break down larger complex molecules into smaller molecules. An example is the enzyme amylase that breaks down a two ring sugar to a one ring monosaccharide. The one ring monosaccharide allows the body to use them as fuel. In DNA replication, enzymes help the process of copying DNA. Each cell in your body contains DNA genetic material. These cell replications and each time a cell divides there is a process for the DNA to be copied. Enzymes facilitate this mechanism by unwinding the DNA coils and making a copy of the information. In the liver, enzymes break down large toxin molecules. The catabolism of the toxins involves the utilization of a range of enzymes.

The initial mechanism of enzymes is the lock and key model proposed in 1894. The enzyme and an active site with a specific three dimensional shape. One specific substrate fits into the active site. This allows only one substrate to be changed by the enzyme. The updated model is the induced-fit model. In the induced-fit model, the enzymes conforms around the substrate. This allows more proteins to interact with the active site. Once the substrate is positioned in the active site, then catalyzation is initiated.

Enzymes work in specific conditions. Many enzymes work best at 37°C – body temperature. At high or lower temperature the enzyme is thought to change shape and it does not work as well as or as quickly. In addition, enzymes work in a certain pH range. Some enzymes work better in an alkaline environment. Other enzymes work faster in an acidic environment. An example is gastric digestive enzymes. These enzymes function optimally in an acidic pH of 2, which is the

acidity of the stomach.

My experiment shows that the Tenebrio Molitor was not as effective in digesting other plastics compared to polystyrene. However, the Tenebrio Molitor still has demonstrated efficacy in digesting other plastics, such as polypropylene, Low-Density Polyethylene, and Polyethylene Terephthalate. The Tenebrio Molitor should be considered to be deployed to combat plastics pollution.

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Samantha Chulamorkodt, Hailey Chulamorkodt

# Harnessing the Saguaro Cactus to Treat Skin Infections - Antimicrobial Properties of the Carnegiea Gigantea Seed Extract

**ABSTRACT**
The purpose of this research is to determine if the Saguaro Cactus (Carnegiea Gigantea) extract has equivalent antibiotic efficacy to the antibiotics Ampicillin, Neomycin, and Erythromycin against Staphylococcus Epidermidis. Acute skin infections are a significant burden on the health care system. The National Hospital Ambulatory Medical Care Survey found a 65% increase in office visits, from 8.6 million visits in 1997 to 14.2 million office visits in 2005. We performed the Kirby-Bauer test to compare the antibiotic efficacy of the Saguaro Cactus extract to that of conventional antibiotics. Our results show that Carnegiea Gigantea seed extract did not work as well as conventional antibiotics. However, it demonstrated that Carnegiea Gigantea seed extract does exhibit intermediate antimicrobial properties. Additional studies could explore the Carnegiea Gigantea effect on other bacteria and Carnegiea Gigantea potentiation with other antibiotics.

**INTRODUCTION**
Acute skin infections are a significant burden on the healthcare system. The National Hospital Ambulatory Medical Care Survey found a 65% increase in office visits to 14.2 million office visits in 2005, up from 8.6 million visits in 1997 (Kaye et al, 2019). Skin and soft tissue infections (SSTI) involve the bacterial invasion of the skin and connective tissues (Stulberg et al., 2002). The SSTI severity ranges from minor to life threatening. Severe SSTI can result in surgery, sepsis, and death.

Skin infections are typically treated with antibiotics. While antibiotics are effective, the emergence of antibiotic resistant bacteria urges alternative treatments to be explored. A traditional Navajo treatment uses grounded Saguaro Cactus seeds as topical antimicrobial treatment (McCleary & Walkington, 1964). This project will explore the efficacy of this Saguaro Seed treatment. We hypothesize that the Saguaro Cactus extract has equivalent antibiotic efficacy to the antibiotics ampicillin, neomycin, and erythromycin against Staphylococcus Epidermidis.

The Saguaro Cactus (Carnegiea Gigantea) is a large cactus species native to Arizona, California and Mexico. The Saguaro fruit is used as a medicinal treatment by Native America for treatment of infections. The Saguaro fruit has been studied for treatment of high blood pressure and blood sugar levels (McCleary & Walkington, 1964).

**MATERIALS AND METHODS**
The Carnegiea Gigantea extract was prepared by crushing the seeds in a mortar and pestle. The grounds were then soaked in glycerine and distilled water solution in a 1:1 ratio. The Carnegiea Gigantea solution was soaked on different antibiotic discs overnight, which included a penicillin disc, a neomycin disc, and an erythromycin disc. An empty disc was used as the control.

The Kirby Bauer Disk Diffusion test is a protocol that determines bacterial sensitivity to an antibiotic or antimicrobial compound (Hudzicki, 2016). Clinically, the Kirby Bauer test is used to determine proper antibiotic treatment of patients with infections (Bhargav et al., 2016). In a research setting, the Kirby Bauer test tests new antimicrobial substances' efficacy. The zone of inhibition, observed as the absence of bacterial growth around disks with antibiotics, measures the ability of the antibiotic to eradicate the

bacteria.

The Kirby-Bauer test was performed by inoculating the agar plate by transferring the broth containing Staphylococcus Epidermidis. The broth was covered with a sterile plastic spreader to evenly distribute the broth over the plate. We then placed the neomycin, ampicillin, and erythromycin discs, carnegiea gigantea extract, an empty disk, and a glycine/water disk.

The agar plate was sealed and placed in an incubator at 37 degrees celsius. A total of five trials with five agar plates was prepared. After 24 hours, an antimicrobial chemical test was performed by using a millimeter caliper to measure the size of each inhibitory circle.

**RESULTS**

Ampicillin had a minimum inhibitory concentration (MIC) level mean of 21.1mm with a standard deviation of 0.7mm (Table 1; Figure 1). Neomycin had a MIC level mean of 20.1mm with a standard deviation of 0.8mm. Erythromycin had a MIC level mean of 17.6mm with a standard deviation of 1.4mm. The Carnegiea Gigantea extract had a MIC level mean of 7.2mm with a standard deviation of 0.6mm. The empty disc had a MIC level mean of 2.0mm with a standard deviation of 0.8mm (Table 1).

**TABLES, GRAPHS and PHOTOS:**

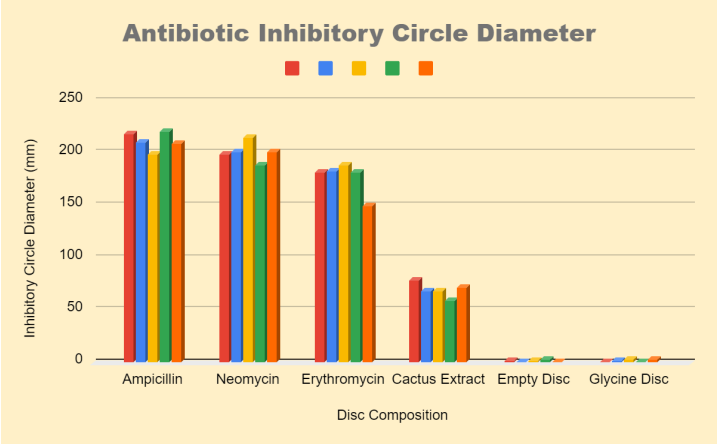


**Figure 1.** Carnegiea Gigantea extract vs Antibiotics Inhibitory Efficacy. The Larger inhibitory circle diameter reflects greater antibiotic efficacy.

| | Trial #1 | Trial #2 | Trial #3 | Trial #4 | Trial #5 | Mean | Standard Deviation |
|---|---|---|---|---|---|---|---|
| Ampicillin | 21.8 | 21 | 19.9 | 22 | 20.9 | 21.12 | 0.747 |
| Neomycin | 19.9 | 20.1 | 21.5 | 18.9 | 20.1 | 20.1 | 0.829 |
| Erythromycin | 18.1 | 18.3 | 18.8 | 18.2 | 14.9 | 17.66 | 1.401 |
| Cactus Extract | 7.9 | 6.8 | 6.8 | 5.9 | 7.2 | 6.92 | 0.649 |
| Empty Disc | 2 | 1 | 2 | 3 | 1 | 1.8 | 0.748 |
| Glycine Disc | 1 | 2 | 3 | 1 | 3 | 2 | 0.894 |

Table 1. Carnegiea Gigantea extract vs Antibiotics Inhibitory Efficacy Data. The size of the inhibitory circle compares conventional antibiotics to the Cactus Extract

## DISCUSSION

The results show that the Carnegiea Gigantea seed extract is not as effective as conventional antibiotics in treating Staphylococcus Epidermidis. However, the results show that Carnegiea Gigantea does have some antibiotic and intermediate antimicrobial properties.

Antibiotics function by blocking bacterial processes and inhibiting bacterial reproduction. Antibiotics often originate from traditional remedies used in culture for millennia. For example, the Chinese herbalist used Artemisia plants to guard against diseases. A chemical extract, Quinghaosu, was isolated for that was determined to be the anti-malarial drug, Artemisinin. Artemisinin shows improved seven day blood clearance rates of malaria than other medications.

The limitations of my research include the study of only one bacteria strain, Staphylococcus epidermidis. There are several other types of bacteria that can become pathogenic, such as Streptococcus. Additional studies could explore the Carnegiea Gigantea effect on other bacteria and explore Carnegiea Gigantea's potentiation with other antibiotics.

In conclusion, Carnegiea Gigantea extract shows moderate efficacy as an antimicrobial agent. Carnegiea Gigantea as an adjuvant therapy to conventional therapies should be explored. Conventional medicine focuses on existing inventory of pharmaceutical antibiotics. Bacteria are achieving antibiotic resistance to many of these antibiotics. Carnegiea Gigantea could lead to therapeutic modalities tools to combat these troublesome infections.

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Kevin Idicula

# Analysis of Microplastic Effects of Chlorococcum Algae and Its Implications on Marine LIfe

**ABSTRACT**
There is an urgent need to assess how the concentration of microplastics affect local ecosystems, especially in relation to the safety of the organisms living within the Long Island Sound. This research consisted of analyzing water samples from the Sunken Meadow State Park. This location is significant because it has a large population of organisms dependent on the LIS, including summer tourists and other organisms. We collected 3 samples of LIS waters. These water samples were then used to monitor the growth and development of Chlorrocumm algae. Chlorophyll concentration was evaluated over a five day growth period with a control of spring water. This study found that microplastics have no specific short term effect on chloroccocum algae.

**INTRODUCTION**
Microplastics have been introduced into our environment ever since the world has depended on the use of plastics in everyday life. The amount of consumer and commercial products that use plastics are innumerable. These microplastics can enter the environment through the degradation of larger plastics, and they pose a problem to the environment because once they enter an environment in large quantities, they can remain persistent in the environment and affect the producers and consumers in the environment.

Microplastics as defined by the Oxford English dictionary are “extremely small pieces of plastic debris in the environment resulting from the disposal and breakdown of consumer products and industrial waste”. These microplastics are entering the Long Island Sound through degrading fishing nets, industrial materials, and cleaning abrasives. Large plastics become microplastics due to the ultraviolet radiation from the sun. These microplastics can range in size from millimeters to nanoparticles.

According to the United Nations Environment Assembly (UNEP), there are an estimated 5.25 trillion plastic particles that are in the oceans right now, which is equivalent to 268,000 tons of plastic in the ocean. Research has shown that microplastics have been found in every single body of water and are doing tremendous damage to the ocean environment (Alfaro-Núñez, 2021). Another study done by the International Atomic Energy Agency shows that ever since 2008, there has been a dramatic increase of microplastic amounts in the Eastern Tropical Pacific (Jennet Orayeva, 2020) . Similarly, they do much damage to the ocean animals. According to researchers at Jiangsu Key Laboratory of Marine Bioresources Resources and Environment of China, microplastics can have toxic effects on fish and other aquatic life, including reducing food intake, delaying growth, causing oxidative damage and abnormal behaviors. Such damage is a tremendous harm to the ocean ecosystem (Qiang and Cheng, 2021).
The Long Island Sound supplies water to many residents and organisms locally. The Long Island Sound is an estuary, meaning it is a place where saltwater and freshwater mix, and hosts its own special ecosystem that is one of the most productive ecosystems on earth. The Long Island Sound is also Connecticut's largest and most important natural resource, contributing to an estimated $5.5 billion per year in the regional economy.

Millions of people live on the Long Island Sound watershed and take part in the activities that take place on the Sound like fishing, swimming, and boating. The Long Island Sound is in danger due to the substantially high concentration of microplastics in the water. According to the University of Connecticut there is an estimated 5000 microplastics per cubic meter (Reyes, 2021). Microplastics are taking a huge toll on the local organisms. This high concentration of microplastics is sure to harm the local ecosystem within the Long Island Sound. According to University of Connecticut researchers, the microplastics present in the Long Island Sound pose a threat to the local shellfish and snail population. Microplastics can also cause unknown harm to the residents of this area (Reyes, 2021).

The Long Island Sound is in danger of becoming heavily contaminated with microplastics. There's an urgent need to assess how the concentration of certain microplastics is affecting the local ecosystem for the safety of the organisms living within the Long Island Sound. The Long Island Sound is home to over 1200 species of invertebrates and 170 species of fish. These animals are part of the Sound and are important to the ecosystem. The Long Island Sound's ability to sustain this great amount of marine life is dependent on the quality of the water. According to the University of Connecticut there is an estimated 5000 microplastics per cubic meter (Reyes, 2021). This is a significant amount of microplastics in the water, and it can have detrimental effects on the marine life and ecosystem in the Long Island Sound. Polystyrene microplastics can possibly affect marine animals' ability to reproduce. An experiment done by Liyuan Qiang and Jinping Cheng of the State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, China and Hong Kong Branch of Southern Marine Science and Engineering Guangdong Lab found a correlation between the amount of polystyrene microplastics a zebra fish is exposed to alteration in the reproductive systems of the zebrafish (Qiang and Cheng, 2021). If the research goes on to show that other fish are also met with the same problems it could be a problem. Especially when the amount of microplastics in the Long Island Sound is so high. There needs to be light brought into this subject because the possible detrimental effects of microplastics is too high just to be ignored. Another reason as to why the contamination of microplastics should not be ignored is that the microplastics can be of potential harm to the human life living around it. Research done in China indicates that micro plastic contamination in crabs is worthy of attention for human health and the stability of marine ecosystems (Zhang et al., 2020)  The Long Island Sound is native to two species of animals: Slipper Snails and Sea Squirts. UConn researchers conducted a series of tests using these two organisms. These species of organisms are indiscriminate suspension feeders. Which means they do not have the ability to selectively reject particles that they ingest. Therefore, these animals are the perfect test samples because they cannot reject the level of microplastic, showing the true level of microplastics in the water. Although the research suggests that there are no harmful effects of ingesting microplastics to organisms, they specifically stated it is only at the level it is at now. Even though there are no specific harmful effects of injecting microplastic according to this source. According to researchers in China, a significant amount of microplastics are very harmful to the oceanic life and environment (Qiang and Cheng, 2021). Both research are trustworthy. Therefore, the gap arises. To what extent the concentration of microplastic is high enough to cause a negative effect on the organisms that may ingest it? If the level of microplastics exceeds the threshold of a certain amount, it causes detrimental effects to the local organisms of the Long Island Sound. Including local marine animals and the residents of human living near the Long Island Sound. It is important to conduct such research to truly understand the level of

microplastics in the Long Island Sound to prevent the level of microplastics from exceeding. Such actions will not only help protect the marine animals, but also protect the local human population.

Mircoplastics act as a poison, as both large and small creatures ingest plastic materials, believing them to be food, or microplastics, unknowingly. These plastics can lead to severe digestive problems, and even death. This pollution also has other consequences, including serious economic losses in the fishing industry. Microplastics can harm marine life, and can lead to a loss of revenue due to fewer fish being caught. Microplastics also lead to poorer quality and fewer healthy spots in the ocean, leading to fewer healthy fish being caught. This not only affects the fishing economy as a whole, but also specifically those individuals whose lives depend on the seas and the fish in them. Microplastics are small: they can be just a few micrometers long and smaller than the diameter of a piece of hair, yet still do serious damage to an organism. This makes microplastics harder to track in the environment, as sometimes you can't even see the piece of plastic. As macroplastics break down into microplastics, the more microplastics will be present, and the more harm they can cause. All types of creatures ingest microplastics, and as they move up the food chain, those microplastics may end up in the human body.

There are many other ways that microplastics make it into the environment, and as our water treatment systems are not designed to remove them, the microplastics make their way through the environment and cause harm to the organisms in the environment. According to Judith Schäli, a researcher at the World Trade Institute, the cost to marine industries in the Pacific region of Asia is estimated to be around 1.17 billion dollars per year (Matsangou, 2018). Significant change will take time and money, but if the current trend with micro plastics continues, the consequences will get worse, and it is important to know those consequences and to research microplastics to know their future impact.

The urgent need to assess how the concentration of microplastics affects local ecosystems, especially in relation to the safety of the organisms living within the Long Island Sound, led us to conduct this experiment. Our research consisted of analyzing water samples from the Sunken Meadow State Park. This location is significant because it has a large population of organisms dependent on the Long Island Sound, including summer tourists and other organisms. We collected 3 samples of Long Island Sound waters. These water samples were then used to monitor the growth and development of Chlorrocumm algae. Chlorophyll concentration was evaluated over a five day growth period with a control of spring water. We hypothesized that the amount of chlorophyll would decrease over this time period due to the concentration of microplastics. Our research procedure was advantageous in figuring out whether or not microplastics had an effect on the chlorroccum algae because our research took place in two phases. Phase one was the analyzing of microplastics, and phase two was the introduction of the micrplastics to the Chloroccum algae.

**MATERIALS AND METHODS**

Our research took part in two phases. Phase one was the collection and analysis of microplastics. Phase two was the exposure of the microplastics to Chlorococcum Algae. Phase one started with the location of a body of water from which we wanted to collect samples. We collected water samples from multiple areas of the Long Island Sound. Water samples of one liter were collected from the sound. We noted the time, date, coordinates, and weather conditions when the sample was collected. After collection, we brought the water samples to the lab and poured the contents of the liter bottle into a sieve structure. We used a squeegee bottle with water from the Long island sound to

rinse the walls of the liter bottle making sure all the particles made it into the sieving structure.

Next, with the forceps and the squeegee bottle we took and rinsed away the largest fragments such as algae, rocks, etc. Once sieve 1 was rinsed, we took it out. The particles of interest were on the sieve 2, so with the help of a spoon we took the material from the sieve 2 and put it in a glass jar. With the wash bottle of water we rinsed and collected all the remaining particles on the sieve 2 and put it in the glass jar. With the wash bottle of water we made sure all the remaining particles on sieve 2 made its way over to the glass jar. Last we closed the jar and made sure to label it with a date and place at which the sample was from.
After this filtration step of the collection and analysis of microplastics phase we moved on to the identification of the microplastics. We poured the contents of the jar which we had labeled and poured it into a beaker. Placing the beaker on a magnetic stirring plate we used the high setting and boiled off the water. After it was boiled off and only sediment was left the hot plate was turned off. We left the material to cool off for an hour. After letting the material cool off we removed the magnetic stirring bar and poured a few millimeters of water from the Long island sound to make the material more pliable. After this we scooped the material using a spoon onto a petri dish.

The final step of the collection and analysis of the microplastics phase was to identify the microplastics. We placed the petri dish on the trinocular magnifier and adjusted the light and optics until we got a sharp image on the computer. We observed the sample and used forceps with the thinnest ends to manipulate all the fragments present. After collecting data on the microplastics found we went on to the second phase of our experiment, the exposure of the microplastics to Chlorococcum Algae. First, chlorrococcum algae was grown over a 16 hour light and 8 hour dark cycle and exposed one sample to clean water as a control, and two samples to water from the sound, with microplastics in it. We then monitored the growth of both samples, analyzing any changes and differences in the two with regards to the chlorophyll content using a light spectrometer.

**RESULTS**

*Phase 1*

For the first phase of research data was collected on the location in which the microplastics came from, the perimeter of the microplastics, and the area of the microplastics. All these data points were organized into the graph shown below. The software system Image J was used to analyze microplastics with regards to the area and perimeter.

| Image # on SD card | perimeter (μm) | Area(μm²) | Location |
|---|---|---|---|
| 17 | 8.93 | | Centre Island |
| 18 | 1.89 | | Centre Island |
| 19 | 1.39 | 103.13 | Centre Island |
| 5 | 0.17 | | Centre Island |
| AVERAGE SIZE: | 3.09 | | |
| 1 | 1.16 | | Sunken Meadow |

| | | | |
|---|---|---|---|
| 2 | 8.91 | | Sunken Meadow |
| 3 | 0.16 | | Sunken Meadow |
| 4 | 0.23 | | Sunken Meadow |
| 5 | 1.02 | 55.49 | Sunken Meadow |
| 6 | 2.17 | 217.54 | Sunken Meadow |
| 7 | 0.86 | 35.87 | Sunken Meadow |
| 8 | 0.43 | 11.21 | Sunken Meadow |
| 9 | 0.55 | 16.02 | Sunken Meadow |
| 10 | 3.07 | | Sunken Meadow |
| 12 | 3.04 | | Sunken Meadow |
| 13 | 2.36 | | Sunken Meadow |
| 14 | 2.46 | | Sunken Meadow |
| 16 | 1.73 | | Sunken Meadow |
| 222 | 3.75 | | Sunken Meadow |
| 221 | 6.08 | 16.01 | Sunken Meadow |
| AVERAGE SIZE: | 2.01 | | |

*Phase 2*

Phase two consisted of the exposure of the microplastics identified in phase one to chlorrococum algae. The control group was seen to have less chlorophyll content than the group which was exposed to the microplastics. To calculate the concentration of chlorophyll absorption from the light spectrometer we used Beer's law to determine concentration of chlorophyll.

| Control Group | | | | |
|---|---|---|---|---|
| | Measured Absorbance | Coeff ($M^{-1}$ $cm^{-1}$) | Path Length ($cm^{-1}$) | Concentration of Chlorophyll (M) |
| Day 0 | 0.085 | 82931 | 1 | $1.02 \times 10^{-6}$ |
| Day 1 | 0.09 | 82931 | 1 | $1.09 \times 10^{-6}$ |
| Day 2 | 0.094 | 82931 | 1 | $1.13 \times 10^{-6}$ |
| Day 3 | 0.094 | 82931 | 1 | $1.13 \times 10^{-6}$ |
| Day 4 | 0.094 | 82931 | 1 | $1.13 \times 10^{-6}$ |

| Experimental Group | | | | |
|---|---|---|---|---|
| | Measured Absorbance | Coeff ($M^{-1}$ $cm^{-1}$) | Path length (cm) | Concentration of chlorophyll (M) |
| Day 0 | 0.114 | 82931 | 1 | $1.37 \times 10^{-6}$ |

| | | | | |
|---|---|---|---|---|
| Day 1 | 0.1388 | 82931 | 1 | $1.67x10^{-6}$ |
| Day 2 | 0.1095 | 82931 | 1 | $1.32x10^{-6}$ |
| Day 3 | 0.1352 | 82931 | 1 | $1.63x10^{-6}$ |
| Day 4 | 0.1417 | 82931 | 1 | $1.71x10^{-6}$ |

**DISCUSSION**

At the end of the experiment it was observed that the experimental group had more chlorophyll than the control group. This means that the hypothesis was rejected and the null hypothesis was accepted. Ultimately it can be concluded from the experiment which we conducted that there are no short term effects of microplastics on Chloroccocum algae, which can be backed up by the short exposure period of five days and the control group having less chlorophyll than the experimental group. This occurred possibly due to the short exposure period in which the chlorococcum algae was exposed to microplastics. Limitations to our research would be that first we only used chlorococcum algae. There are many other species of algae that could have been used and could have yielded different results. Another limitation would be the fact that the exposure period was only five days. Five days is a relatively short period of time and more data points would be needed for more conclusive results. A third limitation would be that only chlorophyll content was measured. There were many other factors that could've been measured such as nutrient ratio and oxygen production rate. Our experiment didn't measure how these factors were being affected so we don't know if microplastics had an effect on those factors.

Our study has a major implication on the local environment, The Long Island Sound region. Chlorococcum algae is native to estuaries such as the Long Island Sound. This means that due to the microplastics in the Long Island Sound there could be a harmful effect on the algae which are the producers in the environment. If the producers are affected in the environment in a negative way it can cause a biomagnification effect which would affect the entire ecosystem including the top predators. Future research would entail a longer exposure period of microplastics to the chloroccocum algae and measuring factors other than chlorophyll content such as nutrient ratio and oxygen consumption rate.

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Krish Reddy

# Examining a Possible Correlation Between Covid-19 Vaccine Accessibility and Hesitancy in High-Income Counties in the United States


## ABSTRACT

This statistical analysis will determine if there is a correlation between lower vaccine accessibility and vaccine hesitancy in the top 50 counties with the highest median household income in the United States. The CVAC index was used to measure vaccine accessibility, while a CDC survey in which people were asked how hesitant they were towards getting vaccinated was used as a measure of vaccine hesitancy. Ultimately, while higher income counties had better CVAC indexes than the national average, the percent of people hesitant to vaccinations was close to the national average[1]. There was no strict correlation found between median household income and either vaccine accessibility or vaccine hesitancy, indicating the importance of other factors in deciding the vaccination status of a county.


## INTRODUCTION

The development of COVID-19 vaccines amidst the coronavirus pandemic was the result of a coordinated international effort between governments and corporations[2]. While the timeline on which these vaccines have been developed has been unprecedented, distributing these vaccines in an efficient and equitable manner remains a challenge, both across the world and in the United States. One of the largest barriers to vaccination remains economic, with poorer counties, on average, facing more healthcare barriers than richer ones[3]. Thus, vaccination efforts in richer counties should be evaluated to measure the extent to which economic affluence plays a role in vaccine access, as well as measuring potential links between vaccine hesitancy and lower-than-expected vaccine accessibility in said counties.

## MATERIALS AND METHODS

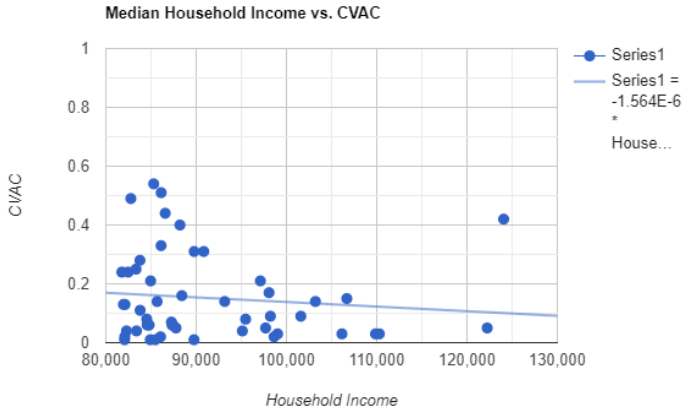
The top 50 counties with the highest median household income in the United States were chosen for this statistical analysis. The measure of vaccine accessibility used was the CVAC (COVID-19 Vaccine Coverage Index), which is measured on a scale from 0-1, with a higher score indicating it is more difficult to access vaccinations[4]. The index, developed by Surgo Ventures, utilizes five specific “themes” to capture an idea of the COVID-19 vaccine coverage in a specific county: historic undervaccination (indicated by lower vaccine coverage and historic refusal of vaccines), sociodemographic barriers (indicated by socioeconomic status and access to reliable healthcare information), resource-constrained healthcare system (indicated by low local healthcare capacity), healthcare accessibility barriers (indicated by cost and transportation barriers to healthcare), and irregular care-seeking behaviors (indicated by a lack of routine visits to a healthcare professional). The measure of vaccine hesitancy used was derived from a CDC study[5] (itself from the 2022 Household Pulse Survey[6]) estimating the number of people “hesitant”, “hesitant or unsure”, and “strongly hesitant” regarding their intention to receive the COVID-19 vaccination. These three classifications were combined for this study to form a single indicator of “% hesitant” to receive the vaccination.

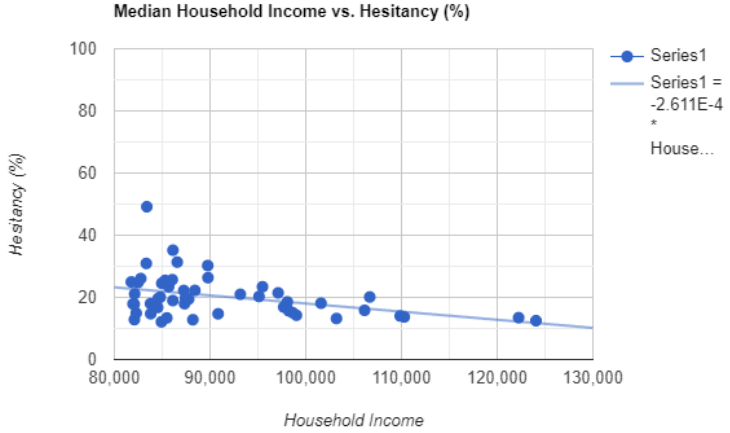
This data was inputted into a table organized in ascending order for the counties with the highest median household income. The data was then analyzed to determine if any correlation existed strictly between household income and vaccine accessibility as indicated by the CVAC index or vaccine hesitancy as indicated by the % hesitant.

**RESULTS**
There was no strict correlation found between household income and either CVAC indexes or vaccine hesitancy. 44/50 of the high income counties scored in the Very Low (0-.19) and Low (.2 to .39) level of concern on the CVAC scale, while the combined average percentage of people who were hesitant to the COVID-19 vaccine was 19%, slightly below the national average of 25%.

## TABLES, GRAPHS and PHOTOS:

| County | Median household income | CVAC Index | % Hesitant | County | Median household income | CVAC Index | % Hesitant |
|---|---|---|---|---|---|---|---|
| Santa Clara | $124,055 | .42 | 12.50% | Queen Anne's | $87,256 | .07 | 22.22% |
| Loudoun | $122,238 | .05 | 13.47% | Forsyth | $86,569 | .44 | 31.35% |
| Fairfax County | $110,292 | .03 | 13.70% | Rockwall | $86,119 | .51 | 35.18% |
| Howard | $109,865 | .03 | 14.03% | Scott | $86,112 | .33 | 18.97% |
| Los Alamos | $106,686 | .15 | 20.13% | Chester | $86,050 | .02 | 25.69% |
| Hunterdon | $106,143 | .03 | 15.83% | St. Mary's | $85,672 | .14 | 23.42% |
| Arlington | $103,208 | .14 | 13.20% | Nantucket | $85,478 | .01 | 13.38% |
| Douglas | $101,591 | .09 | 18.12% | Fort Bend | $85,297 | .54 | 25.47% |
| Somerset | $99,020 | .03 | 14.25% | Rockland | $84,951 | .21 | 24.53% |
| Morris | $98,633 | .02 | 15.08% | Norfolk | $84,916 | .01 | 12.13% |
| Montgomery | $98,221 | .09 | 15.69% | Carroll | $84,790 | .06 | 20.08% |
| Prince William | $98,071 | .17 | 18.55% | Frederick | $84,570 | .06 | 19.61% |
| Nassau | $97,690 | .05 | 16.88% | Monmouth | $84,526 | .08 | 16.75% |
| Stafford | $97,110 | .21 | 21.45% | Bergen | $83,794 | .11 | 14.73% |
| Calvert | $95,477 | .08 | 23.42% | Carver | $83,773 | .28 | 17.98% |
| Putnam | $95,117 | .04 | 20.29% | Oldham | $83,391 | .04 | 49.17% |
| Charles | $93,160 | .14 | 20.99% | Summit | $83,336 | .25 | 30.92% |
| Marin | $90,839 | .31 | 14.71% | Collin | $82,762 | .49 | 26.06% |
| Williamson | $89,779 | .31 | 26.33% | Hamilton | $82,468 | .24 | 24.79% |
| Delaware County | $89,757 | 0 | 30.24% | Fairfield | $82,283 | .04 | 14.94% |
| Fauquier | $88,409 | .16 | 22.24% | Elbert | $82,118 | .13 | 21.13% |
| San Mateo | $88,202 | .40 | 12.81% | Middlesex | $82,090 | .02 | 12.84% |
| Suffolk | $87,763 | .05 | 19.45% | York | $82,073 | .01 | 17.82% |
| Anne Arundel | $87,430 | .06 | 19.88% | Westchester | $81,946 | .13 | 17.90% |
| Sussex | $87,335 | .06 | 17.96% | Kendall | $81,765 | .24 | 24.97% |




## DISCUSSION

As a result of this study, we can conclude that pure household income, while important, plays a small part in determining the availability and perception of vaccines, which are only accurately measured through a host of demographic, historical, socioeconomic, and geographic information. Factors such as the distance to nearby major health centers play a large role in vaccination access, while education level and public confidence can heavily influence hesitancy[7]. The importance of the CVAC index must be emphasized as it is a metric that considers a variety of social indicators in a particular county, which can help public health officials locate and target particular areas of concern with regards to vaccination status. This study ultimately highlights the shortcomings of an index such as median household income, which cannot accurately consider the immense diversity and complicated intricacies of sociodemographic, economic, and geographic factors in the United States.

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Lara Nemeth

**Correlation between Diabetes and Pancreatic Cancer**

**ABSTRACT**
This study investigates the relationship between diabetes and the risk of developing pancreatic cancer. A literature review through Pubmed, The National Library of Medicine, and The British Journal of Cancer was performed to evaluate articles to determine the correlation. Type 2 Diabetes was consistently demonstrated to increase the probability of developing pancreatic cancer. While some data suggests that there is also a similar relationship between Type 1 diabetes and cancer development, the correlation remains inconclusive due to the lack of research.

**INTRODUCTION**
Type 2 diabetes is caused by insulin resistance, due to the pancreas' inability to meet the higher insulin demand as blood sugar rises. In contrast, Type 1 diabetes is caused by the lack of insulin produced by the pancreas, resulting in life-threatening blood sugar fluctuations. Type 1 diabetes can only be controlled by self-administered insulin and glucose. Approximately 37 million Americans are living with Type 2 diabetes and 1.6 million Americans live with Type 1 diabetes [1]. Considering how prevalent diabetes is in America, it is important to understand the possible long-term effects. For this reason, extensive diabetes research must be performed in order to find new targeted and effective therapies.

Since Type 1 and Type 2 diabetes are directly related to the pancreas and how it functions, there is a possibility of pancreatic cancer development in diabetic patients. Pancreatic cancer has one of the lowest survival rates because it is difficult to diagnose at an early stage [2]. Because it is resistant to many forms of radiotherapy and chemotherapy, it is a difficult disease to treat. Therefore, it is important to identify possible causes responsible for pancreatic cancer to understand disease prevention.

**MATERIALS AND METHODS**
A literature review was performed using Pubmed, The National Library of Medicine, and The British Journal of Cancer. Keywords included "diabetes" and "pancreatic cancer." Only publications that contained information on both diabetes and pancreatic cancer were included. Articles dated before 2003 were excluded from the analysis.

**RESULTS**
Five articles were identified that discussed the correlation between Type 2 diabetes and pancreatic cancer. Almost every study acknowledged that there was a definitive relationship between Type 2 diabetes and pancreatic cancer. However, it is unclear whether Type 2 diabetes increases cancer risk or vice versa. Although some studies do suggest that there is a causal relationship between Type 1 diabetes and pancreatic cancer, the research that has been conducted so far is not sufficient enough to definitively support this claim.

**DISCUSSION**
Stevens et. al reviewed 39 cases of pancreatic cancer in young-onset Type 1 diabetes [3]. It is strongly suggested that there is an increased risk of pancreatic cancer in patients with Type 1 diabetes. It was determined that for pancreatic cancer in Type 1 diabetes compared to healthy patients, the relative risk was 2.00, with a 95% confidence interval. Similarly, there seems to be a relationship between pancreatic cancer and Type 2 diabetes. However, the authors suggest that pancreatic cancer may have the potential to cause Type 2 diabetes; insulin resistance and diabetes may be induced by undiagnosed cancer or when the pancreas is exhibiting precancerous

conditions. The data demonstrated that the risk of pancreatic cancer is similarly high in both Type 1 and Type 2 diabetics.

A meta-analysis conducted by Carreras-Torres et. al focused on factors contributing to pancreatic cancer in addition to Type 2 diabetes such as obesity, hypertension, dyslipidemia, and insulin resistance [4]. Type 1 diabetes was not mentioned in this paper. The results revealed a causal relationship between pancreatic cancer and both body mass index (BMI) and genetically increased fasting insulin levels. No relationship between either Type 1 or Type 2 diabetes was found.

In contrast, Pearson-Stuttard et. al conducted a review that examined the causal relationship between Type 2 diabetes and one's risk for developing certain cancers [5]. Their findings suggest an association between Type 2 diabetes and pancreatic cancer, as well as colorectal, hepatocellular, gallbladder, breast, and endometrial cancers.

Additional research involved a meta-analysis examining whether the association between Type 2 diabetes and pancreatic cancer is causal or consequential [6]. With data collected from 9220 individuals (17 case-control and 19 cohort or nested case-control studies), the relative risk was determined to be 1.82 (95% confidence interval 1.66-1.89) with heterogeneity across the studies. The timing of pancreatic cancer diagnosis seemed to be inversely associated with the duration of diabetes: Type 2 diabetics that have been recently diagnosed within 4 years had a 50% greater risk for developing cancer in comparison to Type 2 diabetics who have been diagnosed more than 5 years ago.

Lastly, a cohort study by Zendehdel et. al was included to better understand the prevalence of cancer in Type 1 diabetics specifically. Analysis of 2,9187 test subjects revealed that there was a 20% increase in overall cancer incidence among Type 1 diabetics. It was discovered that Type 1 diabetes slightly increases risk of developing cancer overall; however, the specific cancers associated with Type 1 diabetes differ from those associated with Type 2 diabetes.

There is a strong association between Type 2 diabetes and pancreatic cancer–whether Type 2 diabetes is a precursor to pancreatic cancer or whether it is the other way around has yet to be determined. There is also some evidence of an association between Type 1 diabetes and pancreatic cancer, but further studies are still needed in order to prove this claim.

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Meghana Dasari

# Syzygium Samarangense (Rose Apple) Anthocyanin Fruit Extract Exhibits Cell Metabolic Activity against Non-Small Human HCC827 Lung Cancer Cells


## ABSTRACT

One in every 16 Americans will be diagnosed with lung cancer in their lifetime. Sixty-five percent of all cancer deaths are due to lung cancer. Despite the severity of this illness, the treatment options to combat the cancer are limited to chemotherapy, immunotherapy, or surgery. As the aforementioned treatments have dangerous side-effects, it is essential to research new plant-based treatments. Plants belonging to the Syzygium genus, a genus of plants known for antibacterial, antiviral, and anti-inflammatory activities have been used as a solid extract to test against cell lines. Syzygium samarangense, also known as Rose Apples, are known for their anticancer, antitumor, and anti-inflammatory effects, mostly imparting to their bioactive compounds (Frauches et al., 2016). Anthocyanins, water-soluble vacuolar pigments present in many fruits and vegetables, are known for their chemotherapeutic abilities, especially as they target cancer cells, while having little to no effect on healthy cells (Charepalli et al., 2016). The objective of this experiment is to determine if anthocyanins extracted from the Rose Apple demonstrate more cell metabolic activity than the Rose Apple fruit as a whole. It was hypothesized that the treatment with a concentrated form of anthocyanins should show less cell metabolic activity as the cellular reproduction of cancer cells should be slowed down compared to the treatment of the whole fruit. Using a Soxhlet apparatus and column chromatography, the two separate treatments were created and applied to HCC827 lung carcinoma cells. The cell metabolic rate was evaluated using an acid phosphatase assay. It was discovered that both treatments showed a significantly lower cell metabolic rate at 30 microliters compared to the control. However, there was no statistically significant difference between the rates of both treatments, leaving the answer to the research question asked to remain inconclusive. While the hypothesis was not supported by this experiment, future studies can be conducted in this field of inquiry as this study showed that Rose Apple methanol extracts have anticancer potential.


## INTRODUCTION

One of the most leading causes of death in the world at this moment is cancer. Lung cancer, specifically, took almost 1.8 million lives in 2018 alone. Due to the severity of this illness, the treatment options to combat different stages and types of lung cancer have improved over time, with treatments such as chemotherapy, immunotherapy, or surgery. However, due to the long-term impacts of the aforementioned treatments such as heart problems, herbal remedies are often preferred if possible. Due to their minimal side effects, it is essential to research plant-based treatment options for cancers such as lung cancer.

### *Syzygium samarangense*

In order to be used as an herbal treatment against aliments, plant species belonging to a certain genus that are known to exhibit antiviral, anticancer activity, or immunomodulatory properties are often created into solid extracts to test their potential as they exhibit similar characteristics among their genus. For example, many species in the Echinacea genus, a genus of herbaceous flowering plants, are created into extracts and used to combat colds and fevers as most of the plants in this genus exhibit similar behavior to each other (Aarland et al., 2016). As a result, plants and spices are often chosen to be tested for their properties

based on how similar species to them behave, whether they are plant species in the same genus or plant species with similar constituent compounds.
Plants belonging Syzygium genus, a genus of plants known for antibacterial, antifungal, antiviral, antidiabetic, anti-inflammatory, and anticancer activities, are often tested for their medicinal properties due to the behavior of other species in the same genus (Cock et al., 2019). As a result, other plants in this genus have been used as a solid extract to test against cell lines. For example, a previously conducted research experiment tested the anticancer potential of a clove bud dried flower bud extract against an A549 lung carcinoma cell line. The research demonstrated that clove buds, when combined with a chloroform extraction solvent, exhibited significant anticoagulant activity (Ali et al., 2019). Other plants in this genus other than clove buds have been tested for their potential as well, exhibiting similar anticancer behavior.

Syzygium samarangense, also known as Rose Apples, are known for their anticancer effects, antitumor, anti-inflammatory, anti-virus, antibacterial, and antidiabetic activity mostly impart to their bioactive compounds that include anthocyanins, carotenoids, flavonoids and gallic acid (Frauches et al., 2016).

*Anthocyanins*
Anthocyanins, water-soluble vacuolar pigments present in many fruits and vegetables, are known for their chemotherapeutic abilities, especially as they target cancer cells, while having little to no effect on healthy cells (Charepalli et al., 2016). There is also data that a direct relationship is present between the antioxidant activity and the polyphenolic content of the plant extract (Ahmed et al., 2019). Anthocyanins along [ flavones and flavonoids are considered polyphenolic, while antioxidants are beneficial in combating cancer as they work by tracking down and neutralizing harmful free radicals inside the body.

To determine the specific anthocyanins within Syzygium samarangense that are responsible for the most anthocyanin activity, a liquid chromatography–mass spectrometry (HPLC-MS) test can be done to identify them. Depending on the geographical location of where the specific apple was from, the different types and their respective percent of anthocyanins in the fruit can be different, which is why a HPLC-MS test can be helpful to fully interpret the results of the experiment (Charepalli et al., 2016). While geographical location is influential on the anthocyanins present, the common anthocyanin present in most rose apples is cyanidin-3-O-glucoside, cyanidin-3,5-O-diglucoside, and peonidin-3-O-glucoside according to (Batista et al., 2017). The anthocyanin found with the most content and with the most potential beneficial behavior is cyanidin 3-glucoside as different studies have demonstrated that this anthocyanin has the potential to demonstrate oxidative damages and the protective effects on oxidative stress, inflammation suppression, obesity prevention, attenuation of lung injury and primary hepatocytes protection (Nunes et al., 2016). Due to the anthocyanins that demonstrate bioactivity within the rose apples, there is sufficient reason to test the potential of Rose Apples against cancer cell lines.

The purpose of this experiment focused on testing the cell metabolic activity of an anthocyanin Syzygium samarangense methanolic extract to the behavior of the methanolic extract of the whole fruit against an HCC827 lung carcinoma cell line. The first extract which is the extract of the whole fruit will be created by blending the fruit and drying it so that it becomes a solid extract, which would then be combined with an extraction solvent of methanol through the use of a Soxhlet apparatus. The second extract with the anthocyanins will be created through the use of a Soxhlet apparatus with an extraction solvent of methanol, and then a column chromatography test will be

conducted to separate the anthocyanins from the extract. After creating two different extracts, the extracts will be applied to the cell line through the use of an acid phosphatase assay, which is a colorimetric assay used to determine proliferate behavior in the cell. The acid phosphatase assay measures the metabolic activity of the cells through the use of P-nitrophenyl phosphate, which is converted to P-nitrophenol through acid phosphatase enzymes and cells. So, the rate of metabolism for each of the wells can be found by measuring the absorbance at 450nm and 620nm (background absorbance). Similar to some of the previously referenced research articles, this experiment will be testing the cell metabolic activity of a Rose Apples; however, there are some differences to the previously conducted research. This experiment is different from previously conducted research because in the past, there has not been research specifically testing the difference in anticancer behavior for the natural anthocyanins from Rose Apples compared to its natural fruit extract. While there may have been experiments testing the natural fruit Syzygium extracts against cell lines, there have not been any specific tests against the cell line or tests on anthocyanins from this fruit.

## MATERIALS AND METHODS

*Lyophilization*

Seven Syzygium samarangenses (Rose Apples) were obtained from Miami Fruit and then blended with the addition of water. To lyophilize, remove the water, the blended Rose Apples were spread evenly over two trays that were fitted with parchment paper. These trays were placed inside the freeze dryer and left running overnight. The resulting extraction was free of water.

*Methanol Extraction*

The solid extract formed after the treatment was freeze dried was crushed into smaller pieces using a mortar and pestle. To combine the solid extract with methanol, a Soxhlet apparatus was set-up. The thimble was loaded halfway with the Rose Apple solid extract. The thimble was then nested inside the Soxhlet apparatus (the number three in Figure 1). Molar methanol was filled up to the halfway mark in the conical flask and wedged inside the apparatus (the number one in Figure 1). The apparatus was placed inside the fume hood and was left to run for three days. The resulting liquid (the number one in Figure 1 after the extraction was conducted) was preserved as treatment 1.

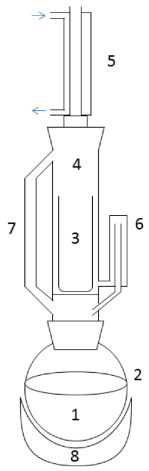


*Fig. 1.* Soxhlet apparatus with each part labeled.

*Gel-Filtration Chromatography*

A small, modified pipette was used to replicate a column chromatography process. First, a small amount of cotton was used to plug the tip. Then Amberlite XAD-7HP resin was used to pack the pipette, leaving two to three centimeters at the top. The lyophilized solid Rose Apple was packed onto the top, completely packing the entire pipette with solids. Using methanol as the solvent, the column was eluted, forming several color bands as the solid extract has an orange color contrasting with the white color of the resin. Before the elution occurred, a collection beaker was placed under the pipette. The resulting liquid was preserved as treatment 2, a Rose Apple methanolic anthocyanin extract.

*HCC827 Cell Culture*

The non-small lung adenocarcinoma cell line, HCC827, was grown to confluency using a base medium consisting of 90% RPMI-1640 Medium and 10% fetal bovine serum (FBS). The media was renewed every two to three days. The population doubling time was approximately 28 hours, as a result, cells were subcultured two times a week at a ratio of 1:4. Once grown to confluency, the cells were seeded into a 96-well-plate at a seeding density of 0.01x10^6 cells.

*Cell Treatment*

To test the metabolism of varying amounts of treatments, the two treatments were applied to the cells at different volumes, starting at 30µL to 100µL, counting by 10µL. Additionally, two experimental controls were utilized: cells with no treatment and cells with the same concentration of methanol. All treatments and controls were left for four days before evaluating cell metabolism. As shown in Figure 2, the first four rows were treated with varying amounts of treatment 1 (E1), while the last four rows were treated with varying amounts of treatment 2 (E2).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | Assay Control | Experimental Control 1: cells with no treatment | 30 µLL E1 | 40 µL E1 | 50 µL E1 | 60 µL E1 | 70 µL E1 | 80 µL E1 | 90 µL E1 | 100 µL E1 |
| B | | | | | | | | | | |
| C | | | | | | | | | | |
| D | | | | | | | | | | |
| E | | Experimental Control 2: cells with same methanol concentration in the extract | 30 µL E2 | 40 µL E2 | 50 µL E2 | 60 µL E2 | 70 µL E2 | 80 µL E2 | 90 µL E2 | 100 µL E2 |
| F | | | | | | | | | | |
| G | | | | | | | | | | |
| H | | | | | | | | | | |

*Fig. 2.* An illustration depicting the amount of each solution added to each well.

*Acid Phosphatase Assay*

After the treatments were left for four days, the acid phosphatase assay was run to evaluate cell metabolism. First, all the media was removed from the plate, and each well was filled gently with PBS and removed. This was repeated twice. Then, to each well, 100µL of 10mM p-Nitrophenyl phosphate disodium salt hexahydrate in 0.1M sodium acetate (pH 5.5) containing 0.1% Triton X-100 was added. This well plate was left in the CO2 incubator for two hours. After two hours, 50µL of 1.0M NaOH was added to each well and read on plate reader at 405nm and 620nm. The tested absorbance was at 405nm while the background absorbance was at 620nm.

**DATA AND RESULTS**

For this experiment, there were two treatments and two controls. Treatment 1 was the methanolic extract of the whole Rose Apple fruit, while treatment 2 was the methanolic extract of anthocyanins from the whole Rose Apple fruit. The first control was the cells with no treatment applied, while the second control was the cells with the 1.0M concentration of methanol applied. In Figure 3, HCC827 cells, cultured by other scientists, are shown at confluency for comparison purposes. Figure 4 shows the HCC827 cells present in the well-plate after being subcultured and seeded in.

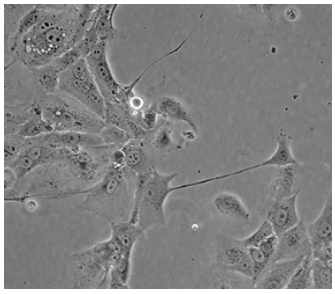

*Fig. 3.* Confluent HCC827 cells under low density microscope lens.

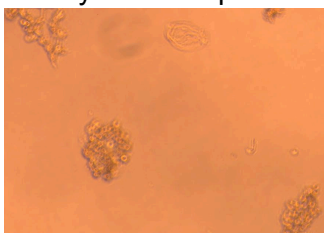

*Fig. 4.* Experimental HCC827 cells after being seeded into the well-plate.

The cells were treated according to the aforementioned procedure and evaluated using an acid phosphatase assay. The recorded absorbance for each well at 420nm was subtracted from that measured at 605nm, as at 605nm, the background

absorbance is measured. The average of each value for the same treatment was calculated and recorded in the data table. In this experiment, it was hypothesized that the absorbance, cell metabolism rates, would be less for the treatment with a more concentrated form of anthocyanins compared to the fruit as a whole. This was justified because anthocyanins are known to have bioactive compounds, and a more concentrated form of compounds known to have anticancer behavior should result in slower cellular reproduction of cancer cells compared to the extract of the whole fruit.

The data recorded in Table 1 shows the average absorbance recorded by the microplate reader. The lowest absorbance was recorded at 30 µL for both treatments, while the highest occurred at 70 µL for treatment 1, but for treatment 2, it occurred at 90 µL. The average absorbance of the cells with no treatment applied was 0.0811. The average absorbance for the methanol was not recorded properly due to measurement errors, so it was not used for comparison. Using the data recorded in Table 1, Figure 5 and 6 were created. The graphs themselves show no apparent trend.

**Table 1**
Average Absorbance Values Per Treatment

| | Average Absorbance of HCC827 Cells | |
|---|---|---|
| | **Treatment 1** | **Treatment 2** |
| 0 µL | 0.0856 | 0.0856 |
| 30 µL | 0.0669 | 0.0670 |
| 40 µL | 0.0831 | 0.0838 |
| 50 µL | 0.0811 | 0.0857 |
| 60 µL | 0.0776 | 0.0853 |
| 70 µL | 0.1000 | 0.0850 |
| 80 µL | 0.0946 | 0.0863 |
| 90 µL | 0.0910 | 0.0889 |
| 100 µL | 0.0818 | 0.0677 |

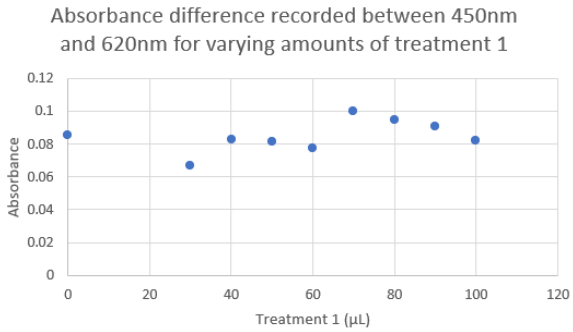


**Fig. 5.** Line graph of the cell metabolic rates for different amounts of treatment 1.

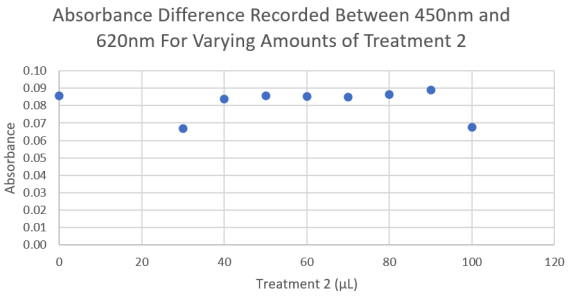


**Fig. 6.** Line graph of the cell metabolic rates for different amounts of treatment 2.

To further interpret the results of the experiment, a one-tailed unpaired t test was run between the control, HCC827 cells that were not treated, and cells applied with 30 microliters of treatment 1, as at this amount the lowest absorbance was recorded. The one tailed p-value is 0.003, which is considered extremely significant, indicating that the cell metabolism rate is lower for cells with treatment 1 compared to the control. To understand the potential of treatment 2, the same one-tailed unpaired t test was run, except this time it was between the control and the cells applied with 30 microliters of treatment 2. The p-value calculated was 0.0011, which is considered very significant, stating that the cell metabolism rate for cells with treatment 2 is lower than the control. To evaluate which of the two treatments is more effective, another one tailed t test was run between the absorbance of treatment 1 and

treatment 2. The p-value calculated is 0.4849, which is considered not significant; therefore, there is no significant difference between the cell metabolic activity of both treatments.

## DISCUSSION

*Results of Acid Phosphatase Assay*

The purpose of this experiment is to compare the cell metabolic activity of an anthocyanin Syzygium samarangense methanolic extract to the behavior of the methanolic extract of the fruit against an HCC827 lung cancer cell line. It was hypothesized that a concentrated treatment of anthocyanins (treatment 2) would have a lower cell metabolic rate due to previous research indicating that anthocyanins from this fruit exhibit anticancerous properties.

The results of this study show that compared to no treatment, both treatments show significantly less cellular metabolism; however, compared to each other, there is not a statistically significant difference. Therefore, both treatments show anticancer potential, but the main research question remains inconclusive. Multiple one tailed unpaired t-tests were run to compare the control, treatment 1, and treatment 2. With a p-value of 0.4849 for the difference between treatment 1 and treatment 2, the test claims that there is no statistically significant difference. Since only one trial was conducted, it is possible that a difference is not present due to errors that occurred in the experiment. While there was not a statistically significant difference between the treatments, this experiment demonstrates that the Syzygium samarangense fruit has beneficial cell metabolic properties, which is a new discovery.

*Errors and Limitations*

The absorbance values recorded in this experiment were heavily impacted by sources of error in this experiment. The anthocyanin treatment may not have exhibited the cell metabolic rate that was expected due to not completely separating the anthocyanins from the rest of the solution. To avoid this, a larger column should be used to run the gel-filtration, compared to the test pipette used in place. Another source of error is the microplate reader not recording the absorbance completely accurately, resulting in higher absorbance rates than what was expected.

Limitations present in this experiment also contributed to the inconclusive answer to the research question. The main limitation was time constraints. As only one trial was conducted, the data may not be an accurate representation of the results of the experiment. To validate or formulate a clearer answer, more trials may be conducted. Additionally, as a 96 well-plate was used to evaluate the cell metabolic rate, the cells were more susceptible to contamination. In the future, it would be better to explore procedures using other well-plates with a larger well volume.

*Future Research*

While the results do not show a significant difference in the absorbance rates between the cells applied with the two different treatments, it can be concluded that Rose Apples exhibit anti-cancerous behavior that can be further researched. Additionally, as the hypothesis made before conducting this experiment was not supported by the data collected, it is illustrated that anthocyanins from the Syzygium samarangense fruit do not demonstrate a similar level of anticancer behavior that was expected from previous research experiments. To understand more about the limitations of anthocyanins in regard to anticancerous behavior, it is important to conduct more research into anthocyanins of fruits from the Syzygium genus. The conclusions gathered from this experiment can be further utilized in future studies as Rose Apples demonstrate the potential to be developed as a possible herbal treatment for non-small lung cancer cells.

**ACKNOWLEDGMENTS**
The materials needed to complete this experiment were provided by Miami Fruit (Rose Apple), and Sigma-Aldrich (Amberlite XAD-7HP), Dr. Tomlinson (Soxhlet Apparatus). Additionally, Mr. Proffitt and Dr. Tomlinson provided aid in devising and conducting this experiment. The Academies of Loudoun provided materials and workspace to conduct the experiment.

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Neema Sakariya, Troy Rohn

# BDNF Levels in People with Neurodegenerative Diseases

**ABSTRACT**
The brain-derived neurotrophic factor (BDNF) gene codes for the brain-derived neurotrophic factor protein. Interestingly, mutations of the BDNF gene can lead to higher BMI. Regular versions of the coded gene are less prevalent in people who have neurodegenerative diseases, including but not limited to Alzheimer's Disease (AD). AD's common symptom of dementia is caused by death of neurons, which is linked to the decreased levels of the BDNF protein. Gene therapy is a potential treatment for Alzheimer's patients with less BDNF protein.

**INTRODUCTION**
The brain-derived neurotrophic factor gene (BDNF) encodes for the brain-derived neurotrophic factor protein—a type of protein that aids with the growth, survival, and maintenance of neurons. The protein is especially important in learning and memory development, as the protein is most present in the cerebral cortex and the hippocampus. Within the neuron, BDNF can be found at the synapses—regulating synaptic plasticity and neuronal communication. Since BDNF is a neurotrophic factor, it can be found in somatic cells, most commonly in the nucleus and endoplasmic reticulum regions. Interestingly, mutations of this gene can lead to higher BMI. In my research, I investigated the relationship between the BDNF gene and neurodegenerative diseases, specifically AD.

**MATERIALS AND METHODS**
For this study, I performed a meta-analysis by conducting a literature review through the National Library of Medicine (NCBI). Keywords that were used to search databases included"BDNF" and "Alzheimer's." Articles were excluded if they did not provide data about the BDNF protein in relation to neurodegenerative diseases and if BDNF protein was not the main focus of the article. A total of three publications were identified from NCBI, but other credible articles were also used for foundational knowledge.

**RESULTS**
The research of Mori and Ono et. al shows that the median amount of BDNF protein in people in the Normal Control (NC) group (Group 1) is significantly higher than the median amount of BDNF protein in people with mild cognitive impairment (MCI) due to AD (Group 2) [1]. The minimum amount of protein in Group 1 is also much higher than the minimum of Group 2. Similar results can be seen with the Parkinson's Disease (PD) research of Huang and Zhou et. al, with the BDNF concentration being much lower in people with PD than in people without PD [2]. In both studies, the p values are less than 0.05, confirming the statistical significance of these results.

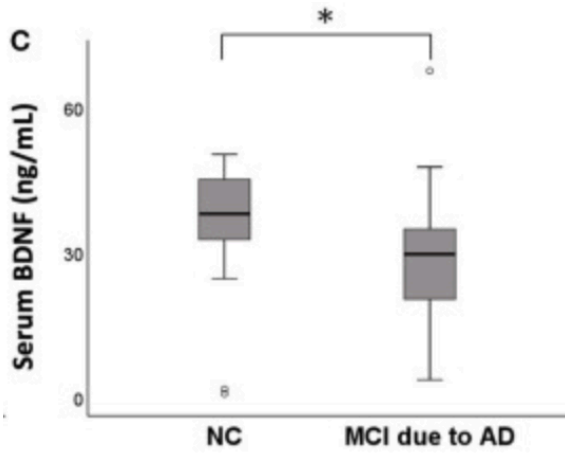


Figure 1: Box plot of BDNF Concentration (ng/mL) in normal control patients (NC) and in patients with mild cognitive impairment due to Alzheimer's Disease (MCI due to AD). *p<0.05

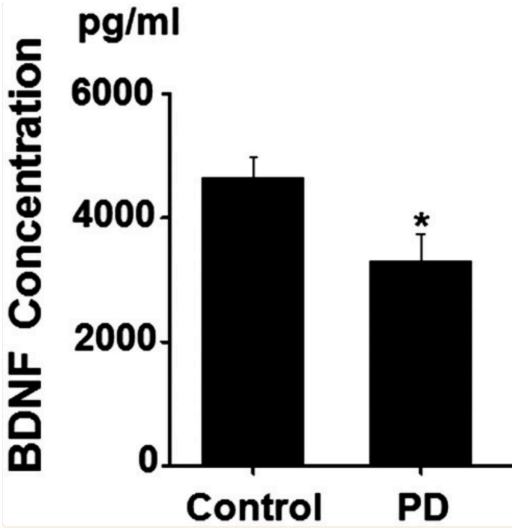

Figure 2: Serum BDNF Concentration (pg/ml) in control patients and patients with Parkinson's disease. *p<0.05

**DISCUSSION**

The results of the studies were somewhat unexpected; we were surprised by the large difference in BDNF levels between the control group and neurodegenerative disease group. However, upon further examination, the relationship between BDNF levels and neurodegenerative diseases does in fact make sense. As a maintenance protein, BDNF is found in high levels in the areas of the brain that control learning and memory. Because the protein is not present to maintain these two jobs of the brain due to decreased levels, it may cause dementia (memory loss)—a very common symptom of AD. While BDNF may not cause AD itself, it most likely is related to the dementia seen in AD patients. As we can see from the data, the results are similar for PD as well, and other publications have shown similar data. The results of these studies can be used in the future to provide treatment for neurodegenerative diseases. Indeed, research is being done on Adeno-Associated Virus (AAV2-BDNF) gene therapy, where the BDNF protein would be sent into the brain in an adenovirus since the protein does not cross the blood-brain barrier. While this study is still in Phase 1, it shows promising results that would not have been possible without the eye-opening data of the correlation of BDNF and neurodegenerative diseases.

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Pranav Bodempudi

# Analysis of Acquired Aplastic Anemia

**ABSTRACT**
Acquired aplastic anemia is a perplexing disorder as researchers remain unsure of the definite cause of the disorder. Radiation and chemotherapy treatments, exposure to toxic chemicals, use of certain drugs, autoimmune disorders, viral infection, and pregnancy are all potential causes of acquired aplastic anemia.[1] The numerous potential causes of aplastic anemia make it increasingly difficult for doctors to diagnose the disorder. The disorder targets the immune system and causes T-lymphocytes to target and destroy the most primitive cells capable of developing into hematopoietic stem cells.[1] This causes the bone marrow of the individual to be replaced with fat. The inhibition of the regular function of the bone marrow creates a deficiency of red and white blood cells and platelets (pancytopenia) and sets a precedent for future complications.[2] Blood cell transfusions can be utilized if a patient is in immediate medical danger. Treatment like this can help offset the effects of anemia for almost two months.[3] The preferred long-term treatment that is currently employed to treat this issue is a bone marrow transplant.

Epidemiology and Public Health Aspects
The term “anemia” is derived from the ability to measure red blood cells in a hematocrit. The term “aplastic” refers to the inability of marrow to form blood.[3] The first patient of the disorder was Paul Ehrlich in 1885, “anemia aplastique” and the clinical features of the disorder were discovered in the early 20th century.[1] It is undetermined what populations are susceptible to the disorder, however, research suggests the incidence varies based on geography (genetic differences) and is more common in younger populations (age).[4] The assumption can be made that a family history of bone marrow failure disorders linked to the environment, toxin, or mutations could result in increased susceptibility to acquired aplastic anemia. The complexity of the disorder also makes it difficult to track the disorder. The incidence of aplastic anemia in Europe and Israel is 1 for every 500,000 people. The incidence rate is two or three times greater in Asia. The exact incidence rates that exist in the United States are unknown although some sources say that approximately 500-1,000 new cases of aplastic anemia are diagnosed each year.[1] There have been some important occurrences of this disorder that has allowed for significant focus and advances in research on the disorder. When marrow failure was first discovered the environmental applications of the disorder were first recognized among Swedish bicycle makers and their exposure to benzenein in uncontrolled work environments. More idiosyncratically, aplastic anemia also has been associated with drugs such as chloramphenicol, which people believe to be the cause of acquired aplastic anemia.[1]

Acquired aplastic anemia is a difficult disorder to diagnose since it has symptoms that are common among numerous other disorders. Symptoms can include fatigue, weakness, dizziness, and shortness of breath which are all symptoms very common among numerous others.[2] Since the disorder is not completely understood, diagnosis of the disorder is also difficult to make due to the low number of cases that are presented each year. The standard approach to diagnosing acquired aplastic anemia is basic and the reason that many cases are overlooked. A doctor will diagnose aplastic anemia based on medical and family histories, a physical exam, and test results. Once they know the cause and severity of the condition, the doctor can create a treatment plan.[5] After there is reasonable suspicion of a hematologic disorder within the patient the doctor is able to take further action. The most common next

step is to conduct a CBC test or even Myelodysplastic syndrome (MDS). The test measures hematocrit (the portion of the blood that contains red blood cells), the amount of hemoglobin (carrier of protein in the blood), and the number and types of white blood cells. The disease number is 284.9 - acquired aplastic anemia.[5]

**DISEASE MECHANISM**

Most cases of acquired aplastic anemia are idiopathic, but researchers are led to believe that the main cause of the disorder is when the autoimmune system mistakenly targets bone marrow as a “foreign” or invading organism.[6] Although much is not known about the causes of acquired aplastic anemia, it is essential to understand the proliferation of the disease throughout the body to address its effects.

Numerous factors can initiate the process of the disorder, however, a similar pathway is followed within the body of a patient. Acquired aplastic anemia is caused by an immune mediated destruction of hematopoietic stem cells. Hematopoietic cells develop into all types of blood cells, including white blood cells, red blood cells, and platelets are regularly found within the bone marrow. The major role that these cells play within the body is the same reason why patients affected by aplastic anemia suffer life threatening complications.

The first issue that they normally encounter is an alteration of the function of the immune system. A change in such an essential system can have drastic consequences as the body begins to fight itself. Immune system cells (T-lymphocytes) target and destroy the most primitive cells capable of developing into hematopoietic stem cells within the bone marrow, leading to the bone marrow being replaced by fat. This fat is called marrow adipose tissue (MAT) which is a unique fat depot and an increase in this tissue may result from an abnormal proliferation of marrow fat cells and the displacement of the hematopoietic tissue of the marrow.[6, 7] As a result, most of the function of the bone marrow is inhibited and has widespread effects within the body as the marrow is continuously degraded.

The unnatural replacement of the bones with fat causes the fat to occupy the hematopoietic space, and directly interfere with hematopoiesis via paracrine action within the bone marrow microenvironment. The suppression of hematopoiesis within a patient leads to the patient developing a deficiency of red and white blood cells and platelets (pancytopenia) which are the reasons for the anemic factors of the disorder.[1] The decrease in red and white blood cells and platelet concentration is one of the reasons that this disorder is so dangerous to those affected. The bone marrow’s function in the body is essential for the regular production of blood cells and when a function like this is altered there are widespread impacts within the body on the essential molecules that are necessary to function.

The molecules that are involved in this disorder are the bone marrow containing hematopoietic stem cells. RBCs in adults are usually produced in the bone marrow and participate in RBC production. The RBC production process is called erythropoiesis. Platelets are small (2 to 4 microns in diameter), colorless, disk-shaped cytoplasmic cells split from cells in the bone marrow. They help constrict damaged blood vessels. They form hemostatic plugs in injured blood vessels by becoming swollen, spiky, sticky, and secretory. They provide substances that accelerate blood clotting, such as factors VIII and XIII and platelet factors.[3]

**TREATMENTS**

Acquired aplastic anemia is a disorder that is complex and requires multiple steps in order to address. The reason for this is that there is not much research that is conducted on the treatment process of the disorder due to the minimal cases and the limited amount of research and expensive treatment process for the disorder.[8]

Initially, researchers were not aware of the proper treatment plan for this disorder due to the lack of research on the limited number of cases that were examined and documented. Also due to the indefinite cause of the disorder, it was difficult for researchers to distinguish a definite solution to the disorder. This is why the first treatment resulted in the usage of a red and white blood cell transplant in an effort to accommodate for the failure of bone marrow to produce the two essential cells in the blood.[3] This was a solution to the short term effects of the disorder, but it soon became evident that this solution was not sustainable and would need a solution that would truly replicate the function or even restore the bone marrow.

Currently, the treatment process depends on the individual's age, general health, and the severity of aplastic anemia.[1] Once the treatment process is decided treatment consists of giving red blood cell transfusions to correct anemia, platelet transfusions to treat or prevent serious bleeding, and antibiotics to treat or prevent infections.[1] There are not many drugs that can simulate the replication that stem cells allow for and this is the reason why the use of certain medical drugs, also is rarely associated with aplastic anemia, as is non viral hepatitis. Both may trigger the immune system response that mistakenly destroys hematopoietic stem cells.[1]

The reason that stem cell treatment is so effective in this procedure is that stem cells are provided by bone marrow transplantation these stem cells can divide, differentiate and become red or white blood cells or platelets.[1] It has been proven that this is an effective method to regenerate the function of the cells that are normally affected by acquired aplastic anemia and is also a proven treatment for numerous other disorders that are related to the production of cells in the blood.

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Puja Tejas Bhavsar

# Analysis of the Correlation between Covid-19 and Myopia

**ABSTRACT**
Covid-19 has resulted in an increase in the utilization of technology and indoor activities. These are some external factors known to exacerbate nearsightedness among the population. Myopia, or nearsightedness, impacts billions of people around the world. It is necessary to identify whether a correlation exists between Covid-19 and myopia in order to determine whether prevention measures are necessary. Due to the inability of collecting primary data, I have utilized secondary data analysis using reliable scientific journals. After discovering data for participants affected by Covid-19, I split them up into different age groups to discern if there was a relationship between Covid-19 and myopia. The research presented in this paper supports that there is a strong correlation between Covid-19 and higher rates of Myopia in adolescents and adults.

**INTRODUCTION**
The power of a lens is determined in order to correct nearsightedness. For myopia, negative powered lenses are utilized. More diopters (the unit of measurement for lens power) are required for correction the more severe a person's nearsightedness is. The curved shape of glasses aids in appropriate eye focus on light (Nearsightedness). The Sphere stands for the prescription strength of eyeglasses, which indicates how strong the lenses must be to correct blurry vision. Cylinder indicates astigmatism, a condition in which the cornea has an irregular structure that impairs or distorts vision. The spherical equivalent (SE) is determined in order to place the sphere and cylinder on a balance: SE= Cylinder/2 + Sphere (Boyd, 2021). Myopic humans are those who have myopia, whereas nonmyopic humans have normal refraction (or emmetropes). Common myopia often has a diopter range of -5 to -6. On the other hand, high myopia typically has a diopter value of -6 or higher. High myopia increases the risk of vision problems leading to long-term vision loss or perhaps blindness. Possible vision problems include retinal damage, cataracts, and glaucoma. Pathological myopia is the term used when high myopia results in tissue damage (Nearsightedness).

Covid-19 has impacted individuals throughout the world. Whether it was through city shutdowns or closure of leisure activities, several parts of peoples' lives have been disrupted. The shift to virtual schools and workplaces are external factors that influence the vision condition of myopia that impacts billions of people Recent research predicts that half the world's population will develop some grade of myopia by 2050, with up to 1/5 at a significantly increased risk of becoming blind (Brien, 2016). This study aims to investigate whether rates of myopia increased due to external factors that arose due to the COVID-19 pandemic.
METHODOLOGY:
In this study, I conducted a secondary data analysis using information gathered from a range of sources, the majority of which are papers published in reliable scientific journals as well as the use of reliable websites. After discovering data for participants affected by Covid-19, I split them up into different age groups to discern if there was a relationship between Covid-19 and myopia.

**RESULTS**
Section 1: Covid-19 and Myopia in Adolescents
Adolescents are a major part of the population that were impacted by Covid-19. A cross-sectional study observed students from

2nd grade to 3rd grade in 12 different schools in Guangzhou, China. To discern whether effects on myopia were solely correlated to Covid-19, comparisons were made between the exposure and non exposure groups. In November and December 2019 and November and December 2020, the exposure group underwent thorough eye exams. Exams were performed on the non-exposed group in November and December of 2018 and November and December of 2019 (Hu, 2021).

Figure 1 shows the Percentage of Students with Myopia with or without Covid-19 Exposure

| Refraction-related parameter | No. (%) Nonexposure group (n = 1060) | Exposure group (n = 1054) | Difference (95% CI) | *P* value |
|---|---|---|---|---|
| SER, D, mean (SD) | 0.55 (1.16) | 0.20 (1.15) | −0.35 (−0.45 to −0.25) | <.001 |
| AL, mm, mean (SD) | 23.25 (0.78) | 23.23 (0.79) | −0.02 (−0.08 to 0.05) | .64 |
| Prevalence | | | | |
| Myopia | 141 (13.3) | 219 (20.8) | 7.5 (4.3 to 10.7) | <.001 |
| Emmetropia | 880 (83.0) | 809 (76.7) | −6.3 (−9.7 to −2.9) | |
| Hyperopia | 39 (3.7) | 26 (2.5) | −1.2 (−2.7 to 0.3) | |
| Proportion | | | | |
| Individuals without myopia, SE >−0.50 and ≤+0.50 D, No./total No. (%) | 286/919 (31.1) | 409/835 (49.0) | 17.9 (13.3 to 22.4) | <.001 |
| Total population, SE <−1.80 D | 38 (3.58) | 57 (5.41) | 1.8 (0.1 to 3.6) | .04 |

(Hu, 2021)

The results of the cross-sectional study among students in China demonstrate that higher rates of  myopia were found in the exposure group (affected by Covid-19) compared to the non exposure group (unaffected by Covid-19). Whereas 13.3% of the non-exposure group had myopia over one year, 20.8% of the exposure group had myopia (Hu, 2021). The greater percentage of myopic students during Covid-19 demonstrates that there is a correlation between the two variables for adolescents. Demonstrated by the statistical analysis, the P value for grade 3 students with the prevalence myopia with or without Covid-19 exposure is < .001. A P value of < .01 is statistically significant (Hu, 2021), which shows that the relationship between the two variables in children is significant. This study demonstrates there is a strong correlation between Covid-19 and myopia in adolescents.

**Section 2: Covid-19 and Myopia in Adults:** The Optometry Clinic of Ramkhamhaeng University in Thailand collected data on myopia for adults who visited the clinic in 2019 or 2020 and recruited them for a follow up in 2021 or early 2022. A survey was carried out using questionnaires to evaluate the lifestyle adjustments made during the COVID-19 pandemic.

Figure 1 shows the Spherical Equivalent for Adults towards the Beginning of Covid-19 and the End

| Variable | Baseline | | | | Follow Up | | | |
|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Mean | SD | Maximum | Minimum | Mean | SD |
| UCDVA (LogMAR) | | | | | | | | |
| Left eye | 1.30 | 0.02 | 0.65 | 0.40 | 1.60 | 0.00 | 0.84 | 0.50 |
| Right eye | 1.30 | 0.00 | 0.60 | 0.38 | 1.60 | 0.00 | 0.82 | 0.49 |
| Total | 1.30 | 0.00 | 0.62 | 0.39 | 1.60 | 0.00 | 0.83 | 0.50 |
| Spherical equivalent, D | | | | | | | | |
| Left eye | −0.50 | −4.25 | −1.90 | 1.04 | −0.50 | −5.13 | −2.53 | 1.36 |
| Right eye | −0.50 | −3.75 | −1.89 | 1.03 | −0.63 | −4.63 | −2.44 | 1.25 |
| Total | −0.50 | −4.25 | −1.90 | 1.03 | −0.50 | −5.13 | −2.49 | 1.30 |
| Accommodative lag (BCC), D | 0.50 | −0.25 | 0.26 | 0.18 | 0.75 | −0.75 | 0.11 | 0.34 |

The longitudinal study illustrates that myopia has increased among adult participants over the past 3 years. The Spherical Equivalent total mean was -1.90 at the beginning of Covid-19, whereas it became -2.49 towards the end of Covid-19. Along with the increase in myopia, “Most of the participants (89.2%) self-reported that their daily use of digital devices increased by approximately 7.6 ±3.2 hours” (Kohmarn, 2022).

The significant difference in power indicates there is a strong correlation between Covid-19 and myopia in adults. However, a limitation of the study is that only 37 participants were involved (Kohmarn, 2022) and as a result, the likelihood of errors is higher.

**Section 3: Covid-19 and Myopia in Elderly:** A study by the National Library of Medicine found that higher rates of myopia correlated with Covid-19 in the elderly. However, the correlation may be caused as a result of the contraction of Covid-19 as implications include vision loss in elderly. “About 1% of

patients with COVID-19 manifest ocular signs of conjunctivitis, including conjunctival hyperemia, chemosis, epiphora, or increased secretions. Additionally, conjunctivitis has been reported in 0.7% of patients with mild COVID and 3% of patients with severe COVID-19" (Franco, 2020). These lower percentages, however, demonstrate that there is not a strong correlation between Covid-19 and myopia.

**DISCUSSION**

Through the sources that have been consolidated in the paper, a positive, strong correlation between Covid-19 and myopia is shown in adolescents and adults. The information presented in this paper supports this association, but I am aware that scientific papers are typically published when a breakthrough occurs. Studies that would not have produced meaningful results may not have ever been published, therefore they would not be taken into account in this assessment.

Limitations of these studies, however, are mainly due to the reason that Covid-19 is relatively recent and not much data has been collected. For instance, although thousands of participants were part of the adolescents study, they were only between the ages 6 to 8 (Hu, 2021). Similarly, for the adults study, only 37 participants were surveyed (Kohmarn, 2022). For the elderly study, general vision loss problems were reported rather than solely myopia. These may undermine the validity of the strong correlation; however, a correlation is still observed.

The results were expected because many external factors that are the source of myopia, such as technology and indoor activities, were utilized predominantly during Covid-19. Another result that was expected was the weak correlation between Covid-19 and myopia for the elderly. Because myopia usually stabilizes by adulthood, there would not be significant changes in the later years. However, one possible cause of the slight increase may be due to inability to receive glasses or contacts, which may have exacerbated the spherical equivalent. Further research on the speculated reasons of a correlation between Covid-19 and myopia can provide a solution to mitigate the increase in myopia in the world. This data can be used to identify Covid-19 has an association with myopia and then the specific activities that have been altered can be studied if they have a large impact on the higher rates of myopia. Future study efforts should focus on understanding the ramifications of myopia, as its prevalence is rapidly rising.

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Ridhi Koner

# Rate of Photosynthesis with Varying Concentrations of Sodium Bicarbonate

**ABSTRACT**
The objective of this research experiment was to determine how photosynthetic rates fluctuated as the amount of carbon dioxide source (sodium bicarbonate (NaHCO3)) changed. The concentration of sodium bicarbonate was examined as photosynthesis necessitates a carbon dioxide source in order to function. Four beakers were used in this experiment, each with a differing concentration of sodium bicarbonate. As a control, a beaker with no sodium bicarbonate was used. With monitoring the timings at which each leaf disk emerged, the lab group was able to accurately discern the relationship between the two parameters. Photosynthesis was found to be faster in beakers with greater carbon dioxide concentrations than those with lower concentrations.

**INTRODUCTION**
The rate of photosynthesis was measured in this experiment by the buoyancy of the leaf disks. The leaf disks (spongy mesophyll layer) become more dense than water as gasses are drawn out of the gaps, forcing them to sink. The leaf disks will undergo photosynthesis if they are immersed in a fluid containing an alternate source of carbon dioxide, such as sodium bicarbonate (Turtenwald, 2018). Photosynthesis produces more oxygen, which promotes the leaves to float to the top. Tests were carried out to observe the rate of photosynthesis at different concentrations of sodium bicarbonate, with observations made at concentrations of 0.5%, 1%, and 3%. The lab tests were performed successfully using previous knowledge of photosynthesis and its cycle learned from the AP biology course, and the conclusions and discussions are documented throughout this board.

*Hypothesis*:
Photosynthesis rates will be higher in the beakers with a higher sodium bicarbonate content. This will happen because carbon dioxide is a necessary component of photosynthesis and so must be produced at a high rate. To test this, leaf disks were placed in water with varying concentrations of a carbon dioxide source (sodium bicarbonate).

**METHODS AND MATERIALS**
13.5 grams baking soda (sodium bicarbonate), liquid soap solution, 3 oral syringes, leaves of spinach, hole puncher, 3 beakers, timer, heat lamp, 3 heat sinks, ring stand, weighing scale, weigh boat, safety goggles

**PROCEDURE**
Students wore safety goggles after gathering and arranging all of the materials. 300 mL distilled water was divided evenly amongst three beakers. The weight boat was cleaned and dried well before being placed on a scale and torn. 1.5 grams of sodium bicarbonate was measured and poured into one of the three beakers, along with a drop of soap solution, and the beaker was stirred until the sodium bicarbonate was completely dissolved. This was then done with 3 grams and 9 grams of NaHCO3 to create three sodium carbonate solutions of 0.5 percent, 1 percent, and 3 percent. Then 30 leaf disks were punched out of the spinach, meeting the needs not to damage the veins. A clean syringe was used to extract 5 mL of the 0.5 percent solution, and 10 leaf disks were inserted inside. Holding a finger to the syringe entrance and pulling back the plunger for 10 seconds while simultaneously shaking the syringe to keep the leaf disks from sticking to the sides, as much excess air as possible was pushed out, and a vacuum was created by holding a finger to the syringe entrance and pulling back the plunger for 10 seconds while simultaneously shaking the syringe to

keep the leaf disks from sticking to the sides. This syringe was placed in a separate container. This step was then repeated in two additional clean syringes with the 1 percent and 3 percent solutions. The heat lamp was connected to the ring stand and turned on, and water was put into the three heat sinks in equal amounts. The contents of the syringes were poured into their respective beakers, which were then placed under the heat lamp with the heat sinks on top. The timer was then started, and the number of disks that rose to the surface was recorded every minute that passed. The experiment was completed after 15 minutes had passed or all of the disks had floated to the top. The materials were appropriately disposed of, and the surface, beaker, and syringes were cleaned.

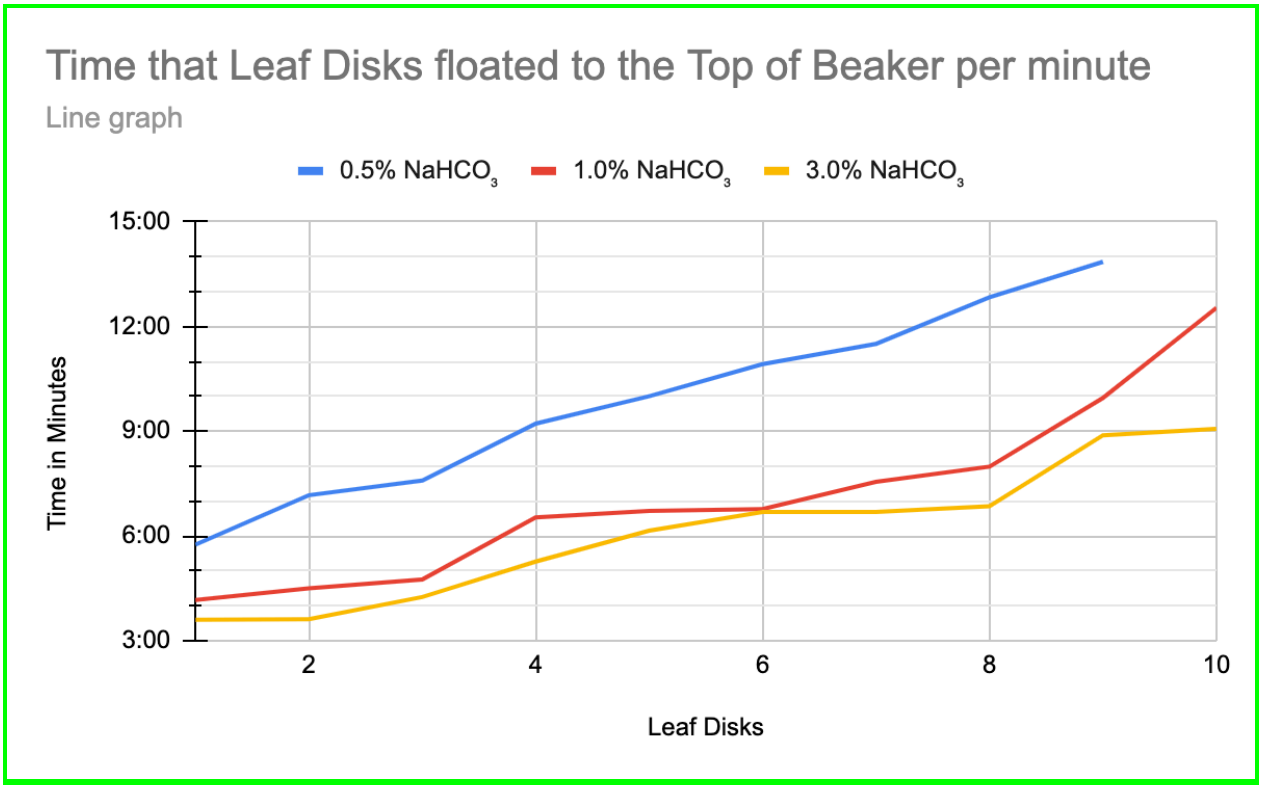
## RESULTS

The solution containing.5% sodium bicarbonate completed photosynthesis and ascended to the top of the beaker at a slower rate than the solutions containing 1 percent and 3 percent sodium bicarbonate (see graph 1).

**Table 1. Time at which Leaf Disks Floated to Top of Beaker**

| Leaf Disk | 0.0% $NaHCO_3$ | 0.5% $NaHCO_3$ | 1.0% $NaHCO_3$ | 3.0% $NaHCO_3$ |
|---|---|---|---|---|
| **1** | - | 5:45 | 4:10 | 3:36 |
| **2** | - | 7:10 | 4:30 | 3:37 |
| **3** | - | 7:35 | 4:45 | 4:15 |
| **4** | - | 9:13 | 6:32 | 5:16 |
| **5** | - | 10:00 | 6:43 | 6:09 |
| **6** | - | 10:55 | 6:46 | 6:41 |
| **7** | - | 11:30 | 7:33 | 6:41 |
| **8** | - | 12:50 | 7:59 | 6:51 |
| **9** | - | 13:51 | 9:57 | 8:53 |
| **10** | - | N/A | 12:32 | 9:04 |

**Graph 1.**




The leaf disks in this experiment were divided into four beakers, each with a different concentration of sodium bicarbonate. Beaker 1 had a concentration of 0.0 percent, Beaker 2 had a concentration of 0.5 percent, Beaker 3 had a concentration of 1.0 percent, and Beaker 4 had a concentration of 3.0 percent. The time at which the leaf disks rose to the surface of the beaker was meticulously recorded. The different concentrations produced different results in the experiment. Because there was no other source of carbon dioxide in the water for the leaf disks to photosynthesize in Beaker 1, none of the leaf disks floated to the surface. The first leaf disk floated at 5:45, and the last leaf disk floated at 13:51 in Beaker 2. The first leaf disk floated at 4:10 and the last leaf disk floated at 12:32 in Beaker 3. The results show a distinct difference: the leaf disks in Beaker 3 floated to the surface at a faster rate than those in Beaker 2. The first leaf disk floated to the surface in Beaker 4 at 3:36, and the last leaf disk floated to the surface at 9:04. The leaf disks rose at the fastest rate in the last beaker.

**DISCUSSION**

$CO_2 + H_2O$ + light energy → $O_2 + C_6H_{12}O_6$ is the photosynthesis equation. When the leaf is vacuumed, the $CO_2$ gas in the mesophyll is evacuated and replaced with the solution, causing the leaf disks to sink. All of the photosynthetic reactants are present in the experiments. As a result, when oxygen is created, it becomes trapped in the mesophyll, causing the leaf to float.

Water and light energy are kept constant and in excess in the experiment. The students altered the concentration of dissolved $CO_2$ by adding varying amounts of sodium carbonate into the same amount of water, producing an independent variable. Although the amount of leaves that float is the only thing that can be observed with the naked eye, the experiment

is actually investigating the pace at which oxygen gas is created. The faster the leaf density changes and floats, the more oxygen molecules are created and go into the mesophyll in a shorter amount of time. This experiment yielded positive results, supporting the theory. As the concentration of $CO_2$ in the cell increased, the rate at which reactants were consumed increased as more $CO_2$ molecules entered the cell, resulting in a rise in the rate at which $O_2$ was created. As a result, the leaf disks immersed in the 3 percent solution floated faster than those immersed in the 0.5 percent and 1 percent solutions, as expected.

There were no outliers, and all of the trendlines followed their expected trends, with the 0.5 percent climbing the slowest and the 3 percent rising the fastest. However, there were some possible experimental errors. Because the syringes and stirring sticks were not properly labeled, they may have been contaminated. Also, because some of the vacuum-ed leaf disks sat in the syringe for longer than the disks for the other solutions, some carbon dioxide may have leaked into the disks before they were placed in the beaker solution. This could be rectified by doing all of the trials at the same time and reducing the duration the disks spend in the syringe.

This study has the potential to help overcome the world's hunger problem. Plants that are subjected to more carbon dioxide undergo photosynthesis more quickly, as demonstrated by this experiment. In a controlled greenhouse, this experiment might be carried out on a much bigger scale, with enormous amounts of carbon dioxide supplied to the plants via pumps or water. Plants could be harvested and replaced with fresh ones as they mature. Because carbon dioxide is a renewable and recyclable material, this process may be continued indefinitely, producing far more food than is now produced.

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Saraswati Shee

# Exploration Of Effect of History on Literature Through Giovanni's Room Written By James Baldwin

**ABSTRACT**
This interdisciplinary paper explores the effect of history on literature through the book Giovanni's Room written by James Baldwin, published by Dial Press, N.Y., in 1956, a groundbreaking novel about love and the fear of love set among the bohemian bars and nightclubs of 1950s Paris. The historical context of theology, legislation, medicine, and literature is analyzed to understand the background when the book was written. The historical context is found greatly affecting the content of the book, fostering a broad public discourse of issues regarding same-sex desire.

**INTRODUCTION**
Giovanni's Room focuses on the events in the life of an American man, David, living in Paris and his struggles for self-knowledge when caught between conventional morality of heterosexuality toward his girlfriend, Hella, and repressed desires for homosexuality with other men in his life, particularly an Italian bartender named Giovanni whom he meets at a Parisian gay bar. This paper examines the historical context of theology, legislation, medicine, and literature to understand the effect it had on the content of the book. It does not examine the effect of historical context on the quality of the writing.

**MATERIALS AND METHODS**
After reading the book thoroughly, I prepared a Reader Response Paper (RRP) identifying the features operating within the text and the impact it has on my understanding. I researched my high school library and college-level databases like JSTOR to find scholarly analyses of the book as well as history of literature on homosexuality and bisexuality. I submitted a series of questions that explored the effect of the historical period on this book and I wrote this research paper outlining my investigation, methodology used, and key findings.

**RESULTS**
I found that the historical context of theology, legislation, medicine, and literature greatly affected the content of the book. The book in turn fostered a broader public discourse of issues regarding same-sex desire.

**DISCUSSION**
Since European immigrants came to "newly discovered" America with the mission to spread Christianity, Christian theology influenced gender roles, with regard to gender expression and sexuality. The ideas derived from the contents of the Bible were used to justify the hatred, exclusion, and oppression of homosexual people. For example, Leviticus 18:22 and 20:13 are two chapters in the Bible written to list rules meant to prevent the Israelites from repeating the actions of the Canaanites; specifically regarding the religious, sexual rituals where every kind of sexual practice was performed, including homosexual sex. The Bible reads, that "[t]hou shalt not lie with mankind as with womankind; it is an abomination," (Leviticus 18:22) and "[i]f a man also lie with mankind as he lieth with a woman, both of them have committed an abomination; they shall surely be put to death; their blood be upon them" (Leviticus 20:13), intending to prevent people from submitting to homosexual desires (Leviticus, The Holy Bible). Christian faith has weaponized these words to label homosexual relationships as not properly reflecting God's plan for families and society. However, this is not necessarily accurate with the context of the Biblical era. These phrases directly refer to the cultic sexual practices of the Canaanites, not everyday Christian life.

The societal norms established by theology significantly influenced the legislation that regulated society and enforced gender norms. One example was when President Dwight D. Eisenhower issued Executive Order 10450 on April 27, 1953, he banned homosexuals from working for the federal government. This executive order determined whether federal employees were considered security risks by expanding the grounds for dismissal and mandating investigation into their personal lives. In the executive order, sexual orientation is listed as grounds for dismissal of a federal government position, stating that people who identify with "sexual perversion" (referring to homosexuality) pose a security risk to the country, stating how the investigations are “designed to develop information as to whether the employment…in the Federal service of the person being investigated is clearly consistent with the interests of the national security" (Exec. Order No. 10450). The cryptic tone of this statement proves how the government viewed homosexuality as taboo and inappropriate to discuss. This order proved to be extremely damaging to the LGBTQ+ community, causing many homosexual individuals to lose their jobs; this being due to the systemic targeting from the federal government not allowing homosexuals to go against the societal expectations. This left homosexual people without jobs, livelihoods, pensions, and the ability to make a living and provide for their families. Additionally, the government provided no evidence for labeling homosexuality as a security risk, indicating that this was written into executive order because of societal bias and the pressure to conform to heteronormative societal standards.

Perhaps some of the most damaging and significant influences of American social norms have been reports of medical professionals on homosexuality. In 1952, the American Psychiatric Association listed homosexuality in the personality disorder category under the subcategory “sociopathic personality disturbance” as a “sexual deviation.” The sexual deviation included “homosexuality, transvestism, pedophilia, fetishism and sexual sadism (including rape, sexual assault, mutilation)” (DSM-I). This affected the gay community and society's view on homosexuality significantly because it turned homosexuality from being a sin to being an illness, invalidating their identity, their ties to God and religion, and their ties to their biology and health. A report by Sarah Baughey-Gill analyzed a book by Eric Marcus on gay equal rights, Making Gay History, highlights the underlying effects of the mental illness classification, stating how medical literature was “one of the major issues that emerged due to the APA classification… because it was supposedly based on scientific findings,” making “ it…difficult for homosexuals to dispute views which held them as deviant. Their opponents could dismiss any of their arguments based on the notion that they were “sick” (Baughey-Gill 7). People who opposed homosexuality used this medical diagnosis as a weapon to suggest that homosexual people were abnormal.

Theology, legislature, medicine, and the subsequent traditional gender expectations present in America affected its literature. This is demonstrated in the 1955 novel The Man In The Gray Flannel Suit by Sloan Wilson, which discusses 1950s conformity and culture. Specifically, the novel outlines the roles of men and provides insight into the male gender expectations of the period. Wilson says, "now it is time to raise legitimate children, and make money, and dress properly, and be kind to one's wife, and admire one's boss, and learn not to worry, and think of oneself as what?... I'm just a man in a gray flannel suit" (Wilson 98). This description of what society expected of men emphasizes making money, having a wife, wearing a gray flannel suit; all things that characterize masculine qualities and perpetuates the ideals of a traditional man conforming to the societal standard, like being the primary income of the family and providing for a wife and kids, hinting that

being in a heterosexual relationship was the only acceptable norm.

The novel Giovanni's Room by James Baldwin describes the atmosphere of a homosexual bar in the principal home to gay subculture in the 1950s: the Saint-Germain-des-Pres quarter of Paris (https://en.wikipedia.org/wiki/Giovanni's_Room). This vibrant and liberated area of France makes it a fitting setting where Giovanni and David's love story unfolds. This has been made more apparent after researching America's strict social gender expectations during the 1950s. The contrast between the United States and France regarding how accepting people were of the homosexual community is stark. This is not to say that Paris is free of homophobia entirely; however, the weight of homophobia ingrained in society is certainly much lighter and is not confining compared to America. Even so, Paris has been a "gay haven" and a bustling center for queer life in the 1950s, the St-Germain-des-Prés quarter as the center of the cultural hotspot. Baldwin himself moved to Paris in 1948 to come to terms with his own sexual ambivalence (https://en.wikipedia.org/wiki/James_Baldwin) and was able to articulate this through the inner struggle of David with his relationships. Hence, the historical context of theology, legislation, medicine, and literature greatly affected the content of the book.

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Saraswati Shee

# Insertion of SX4 #6 Dalton in Male Specific Lethal-1


## ABSTRACT

This paper uses modern molecular biology techniques, Mendelian genetics, and immuno-histochemistry to characterize novel fruit fly Drosophila melanogaster strains and determine the location of a P element insertion. DNA extraction and iPCR are used to get a DNA sample, and then Sanger Sequencing is used to get the nucleotide sequences of the DNA sample. The website FlyBase BLAST is then used to compare the P element and unknown DNA sequence to the already known Drosophila genome. Drosophila larvae are also dissected and imaged with fluorescence to determine where the expression is in the brain and the gut. The results determine that the P element insertion is in the Male Specific Lethal-1 gene of the Drosophila and expression is shown in the brain and possibly in the gut.


## INTRODUCTION

The work involving LexA, a novel binary gene expression tool, is important for the future studies of metabolism, development, and neurobiology of Drosophila melanogaster. The creation of 30+ novel LexA driver lines have greatly expanded the areas possible for study, including specific LexA drivers for stem cells, enteric cells in the gut, and other developmental related tissue types. This paper explains where a P element transposon gets randomly inserted in unknown genomic DNA in Drosophila and where this unknown DNA shows expression.

## MATERIALS AND METHODS

1. *Insertion Location and Mapping*

Following the creation of the P element insertion in the Drosophila melanogaster, DNA extraction, iPCR, and Sanger Sequencing were used to determine the location of the insertion in the genome. DNA extraction isolated the genomic DNA, as it broke down the cell and nuclear membranes protecting the DNA. 50mg of Drosophila was ground up in 180 µL Buffer ATL, using a micropestle, which separated the cell material from the DNA, allowing for a cleaner and clearer DNA sample. To make sure the DNA was fully purified, the next steps were to add 20 µL of the detergent Proteinase K to lyse and break open the nucleus of the cells and expose the DNA. Next 200 µL Buffer AL and 200 µL ethanol was added and the mixture was then centrifuged. Then, the DNA was washed, which separated the free DNA from any other debris from the cell and repeated for a total of two times. Next the elution process was performed, and 200 µL Buffer AE was pipetted directly on the DNeasy membrane. After extraction, iPCR was used to locate the P element insertion. This process began with digestion, where smaller pieces of genomic DNA with the P element and the unknown sequence were created. Then the restriction enzyme HpaII was used to cleave at a restriction site, CCGG, in the P element, creating sticky ends. Next ligation occurred where DNA ligase was added and the sticky ends self-ligated. This process resulted in a circular piece of DNA where PCR could be performed, allowing for the amplification of the target sequence using two primers. In this process, the unknown sequence was amplified by making the primers for the P element that initially faced away from each other, built towards each other after the circulation of the template, where one side of circular DNA was the known P element and the other part was the target sequence. Here, Kurt and Ulf were the primers used, Kurt for the 5’ end and Ulf for the 3’ end. The sequences were then sent to a lab where Sanger Sequencing was performed to determine the nucleotide

sequence of the target DNA sequence. This process used gel electrophoresis and a single sequencing primer, Sp1, complementary to the 3' end of one of the strands of the PCR product to determine the nucleotide sequence, and these results were sent back for analysis.

*2. Sequence Analysis and Primer Design*
The returned sequence from Sanger Sequencing included the P element with the eight base pair direct repeat following. In order to determine the target sequence, the end of the P element, TTTCATCATG, was found, which marked the beginning of the sequence that would be put into FlyBase BLAST for sequence analysis. Because HpaII was used as the restriction enzyme, the location of restriction site CCGG was found next to determine the end of the sequence to be analyzed in FlyBase BLAST. After determining the target sequence, the sequence between was put into FlyBase BLAST. The target sequence was then compared with the known Drosophila genome sequence, allowing the insertion of the P element to be located. For primer design, PCR was used to confirm the P element insertion site, by amplifying a region of the P element and the adjacent genomic sequence. To do this, a primer must be designed based on the insertion site of the P element. This primer was designed to anneal 1,500 bases away from the insertion site in the genomic DNA and it extended towards the P element. To design the primer, the website FlyBase was used. The program then created both a left and right primer, and the appropriate primer was chosen based on the P element orientation.

*3. Dissection and Staining*
Dissection began with selecting third instar larvae placing them in a well filled with PBS. Then one of the larvae was oriented so that the anterior end of the larva, where the mouth hooks are contained, faced the upper right corner of the well. At approximately two-thirds down the larva from the mouth hooks, the larva was cut using forceps. Following this, the larvae were inverted to expose the internal organs. Following inversion, the inverted larvae were submerged with 500 µL of 4% formaldehyde in a 1.5 mL Eppendorf tube for fixation.. Next, this tube was incubated on the rocker for 20 minutes at room temperature. After 20 minutes, the inverted larvae were removed from the rocker, the formaldehyde was removed, and 500 µL PBS was added. This was repeated for a total of three washes. Next, the fixed inverted larvae were transferred back into a well filled with PBS for dissection. During dissection, the brain and gut were isolated from the rest of the organ and the clean brain and gut were transferred into another well filled with PBS in preparation for mounting and imaging. To mount the dissected organs, a drop of DAPI, a fluorescent stain that binds strongly to DNA, was placed on the glass slide, and four halves of larval cuticle were placed in the corners of the drop of DAPI, acting as spacers. Next the larvae guts were placed into the DAPI and aligned using forceps to facilitate imaging. This process was repeated for the larval brains. The organs were then covered with coverslips and sealed with clear nail polish. Any air bubbles created were filled in by pipetting extra DAPI into the locations where air bubbles were present.

**RESULTS**

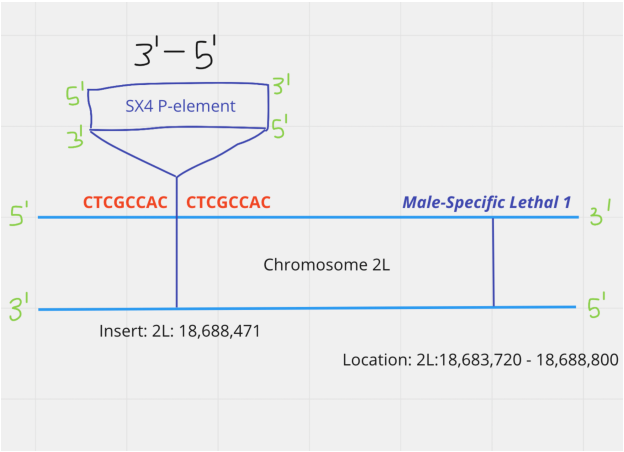
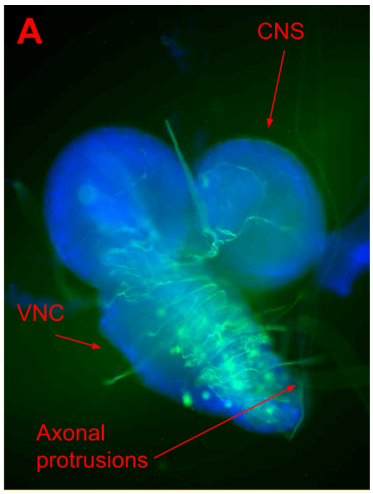


**Figure 1:** 3’ to 5’ P element with the insertion site 2L: 18,688,471. Eight base pairs direct repeat, CTCGCCAC. Located in the first exon of the Male-Specific Lethal 1 gene, which is located at 2L: 18,683,720 - 18,688,800.

For sequencing, only the 3’ end was successful and returned a DNA sequence with a match on FlyBase. Therefore, the level of certainty for the insertion is low as the insertion site could not be confirmed with the 5’ end.



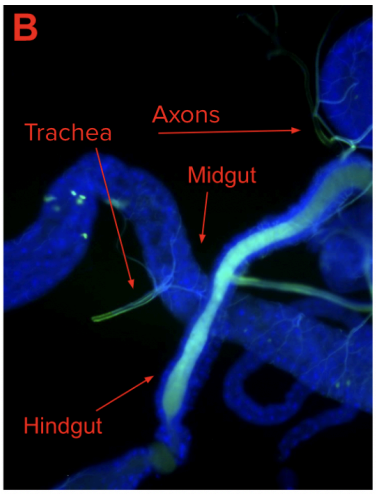




**Figure 2:** Spatiotemporal expression patterns of the gene with the P element insertion. GFP fluorescence and DAPI fluorescence images overlaid. (A) Third instar larval brain with expression of motor neurons. (B) Third instar larval hindgut folded over third instar larval midgut and gastric caeca with possible expression in the midgut. The results from (A) and (B) are reproducible, but fluorescence in the CNS and in the midgut around the gastric caeca and proventriculus need to be studied further before confirmation.

There was a background expression present in the brain. However, in the ventral nerve cord (VNC) there was some expression in the posterior end. It was out of focus, however these are believed to be expressions of motor neurons, specifically ones that aid larvae in crawling and moving around. Because of the location of the expression and the fact that Drosophila are bilateral, this expression is expected to be present on both sides of the VNC. However this image does not represent that concept as the VNC is positioned diagonally. Therefore it was very difficult to get both sides of the VNC in one focal shot. To confirm expression on both sides of the VNC, computational methods that were not available at Harvard, can allow both sides of the VNC to be seen in the same image, and confirm the expression on both sides. In the central nervous system (CNS), there seems to be some expression on the left lobe of the CNS, however this could also be an autofluorescent axon that happened to be able to show through the left lobe on imaging. Therefore it was concluded that it was unlikely that there was any expression in the CNS. The fluorescence present in the hindgut is autofluorescence from larva food. There is fluorescence in the midgut, however this was considered background fluorescence and is present in many different lines of drosophila that have been previously studied. It is unclear if there was fluorescence present near the gastric caeca, and more imaging can confirm this in the future.

*Male-Specific Lethal 1* expression is expected to be present in the larval ventral nerve cord. Expression is seen there based on the imaging of the dissected brain. This shows overlap between the expected expression pattern based on overlapping gene activity and actual enhancer activity.

**DISCUSSION**

The overlapping gene, Male-Specific Lethal 1 (MSL-1), encodes a protein that forms the structure and organization of the full male-specific-lethal dosage compensation complex. This complex upregulates the rate of transcription of most X-linked genes in male Drosophila, which results in transcription of the genes on the single X chromosome of males equal to the genes on both X chromosomes in females (Sun, 2015). Males homozygous for loss-of-function mutations in the male-specific lethal1 (msl1), male-specific lethal2 (msl2), male-specific lethal3 (msl3), maleless (mle) or males absent on the first (mof) genes die due to a failure to perform dosage compensation (Maxwell, 2000). Therefore, during dosage compensation transcription increases approximately two-fold. This occurs through histone acetylation by the male specific lethal complex, as the gene proteins have a fundamental role in the upregulation of transcription in the male X chromosome. The complex includes five MSL proteins which are required for the creation of viable males, MSL1, MSL2, MSL3, MOF, and acetyl transferase. Specifically, the MSL proteins are involved in the histone acetyltransferase for global hyperacetylation of X-linked chromatin at histone H4 at lysine16 (H4K16ac). The H4K16ac modification stops chromatin from compacting and enhances DNA accessibility and transcription (Conrad, 2012). This process happens when the MSL proteins bind, and histone acetylation occurs. As a result of the histone acetylation, the affinity of the histone for the DNA would decrease, and destabilize the chromatin structure as the interaction between adjacent nucleosomes weakened (Luger, 1997). This suggests that the chromatin structure by histone acetylation plays a large role in how Male-Specific Lethal increases the X linked gene expression in males.

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Shreya Sunkara

# Bio-waste as a Tool for Detoxification

**ABSTRACT**
The goal of this project was to create a low-cost version of activated carbon to be used for water purification as an alternative to commercially available carbon, which is very expensive for developing countries since it uses many intricate pieces of equipment. Fruit peels can be used to create raw carbon through the process of washing and drying the peels and then activating them with nitric acid. This project concluded that activated carbon prepared from chemically treated fruit peels has been proved as an effective alternative to the current expensive methods of removing dyes from wastewater. The banana peels were highly effective in removing heavy metal pollutants, especially lead. We investigated the effects of different variables such as pH, contact time, and adsorbent doses on dye removal and no significant effect was observed. From the results of the experiment, it is concluded that adsorption using low-cost, activated carbon is an effective process for the removal of heavy metal pollutants and toxic dyes from water, which serves as a potential solution to both water impurity and increasing sizes of landfills.

**INTRODUCTION**
Water pollution has become a major problem worldwide from rural countries to big cities. Every year, around 3 million people die from water-related diseases, most of which include heavy metal pollution. These heavy metals in water such as lead and manganese can cause damage to the brain and nervous system in children and disrupt the immune system through the development of autoimmunity. There have been numerous solutions created to reduce heavy metal pollutants in water, the most prominent one is the process of coagulation and flocculation, sedimentation, and filtration which is used in many communities in the USA (CDC, 2019). But many of these processes require the addition of harmful chemicals that are not fully removed. However, one method effectively removes water pollution without any further addition of pollutants and has even been posed as a solution for another world wide problem, rising landfills. This method is called commercial activated carbon.

Commercial activated carbon uses highly porous bio waste material to absorb contaminants in water like heavy metals and synthetic chemicals such as methylene blue and azo dyes which are commonly used in the textile industry for coloring clothing(Wang, 2019). While this method is highly effective in removing pollutants in water, it is also highly expensive since it uses many intricate pieces of equipment. Although commercial carbon is expensive, there is a way to replicate its methods to produce a low cost activated carbon made at home without intricate equipment and further save the environment.

Around the world, landfills continue to grow. Landfills contain items such as plastics, but they also contain high amounts of biowaste, such as fruit peels. Pathak, (2016) discussed how fruit peels contain negatively charged ions, whereas heavy metals in water have positively charged ions. Since these ions are polar opposites, the fruit peels have the ability to remove pollutants from water, therefore purifying it.

The purpose of this research was to modify fruit peels so that they can absorb common pollutants in unpurified water and compare their effectiveness. Since all fruit peels contain negatively charged ions, it was

hypothesized that all the four fruit peels used in the experiment would have the same impact on the four test waters which contain different impurities: lead, manganese, fluoride, and methylene blue. This research on the potential use of fruit peels as filters is important because it can solve two major problems in the world, water pollution and increasing landfills.

**MATERIALS**

The materials needed were 2.21 grams of sodium fluoride, 1.80 grams of lead nitrate, 1.80 grams of magnesium sulfate, 2.5 grams of methylene blue dye, 50 ml of nitric acid, sodium chloride and sodium hydroxide. Orange, pomegranate, lemon, and banana peels were also needed, enough for 600mg, 700mg, 800mg, or 900mg of each fruit according to different trials mentioned further on. Other materials needed also include PH Meters,water test strip kit for Fluoride, Lead, Manganese, Dyes detection, room temperature distilled water, test tubes and holders, gloves, safety goggles, protective apron, fruit peeler, cylindric tight container (heat safe), grinder, and Gemini-20 Portable Milligram Scale.

**METHODS**

First, the raw carbon was prepared by using the fruit peeler or knife to peel all the four fruits (lemon, orange, pomegranate, banana). The peels were washed and dried, then placed in a heat-safe container, then in a heat-safe pot over low heat for 2 hours. The fruit peels were ground up and weighed according to measurements above. This ground up peel was the raw carbon.

Next, the activated carbon was prepared by washing, drying, then soaking the raw carbon in 50 mL of nitric acid for 5 hours. Then it was re-washed and dried, and the end result was activated carbon. Next, the polluted waters were made, creating four different mixtures using sodium fluoride, lead nitrate, manganese sulfate and methylene blue, each added to 1000 mL distilled water. Next, the batch experiments using the different scenarios were performed. First, the different polluted waters were poured into the corresponding test tubes. Next, using the water test strip kit, the initial impurity level of the water was recorded. Finally, using the different scenarios mentioned below (pH, adsorbent dosage and contact time) the final impurity level for all different trials was found and recorded. The polluted waters were remade for all four trials.

The scenarios mentioned above were created as follows. For the pH variation scenario, the pH level of the water was changed from 5-8 (for a total of four different trials for pH 5, pH 6, pH 7, and pH 8) using different amounts of sodium hydroxide and sodium chloride (the pH meters were used to see if the correct pH level was reached). For the adsorbent dosage scenario, the amount of adsorbent dosage was changed (600, 700, 800, 900 mg of the modified fruit peel) for a total of four different trials (one trial for 600 mg of adsorbent, one trial for 700 mg, one trial for 800 mg, and one trial for 900 mg). The contact time was 120 minutes, the pH was 7, and 50mL impure solution was used for each trial in this scenario. Results were recorded and graphed accordingly. Finally, for the contact time scenario, the contact time was changed (120 min, 140 min, 160 min, 180 min) for four different trials. The adsorbent dosage was 600 mg, the pH was 7, and 50mL impure solution was used for each trial in this scenario. Results were recorded and graphed accordingly.

**RESULTS**

After the results were noted down, an analysis was taken on the results. The final impurity level for the four different peels in the different scenarios were compared with one another. The final impurity levels for bananas for all different scenarios for the lead, manganese, and fluoride and methylene blue were less than the final impurity levels when the other peels were used for these 3 polluted waters. Along with this pattern throughout my data, the pomegranate peels had the greatest

final impurity level for all the impurified waters with the scenarios having no effect on the results.

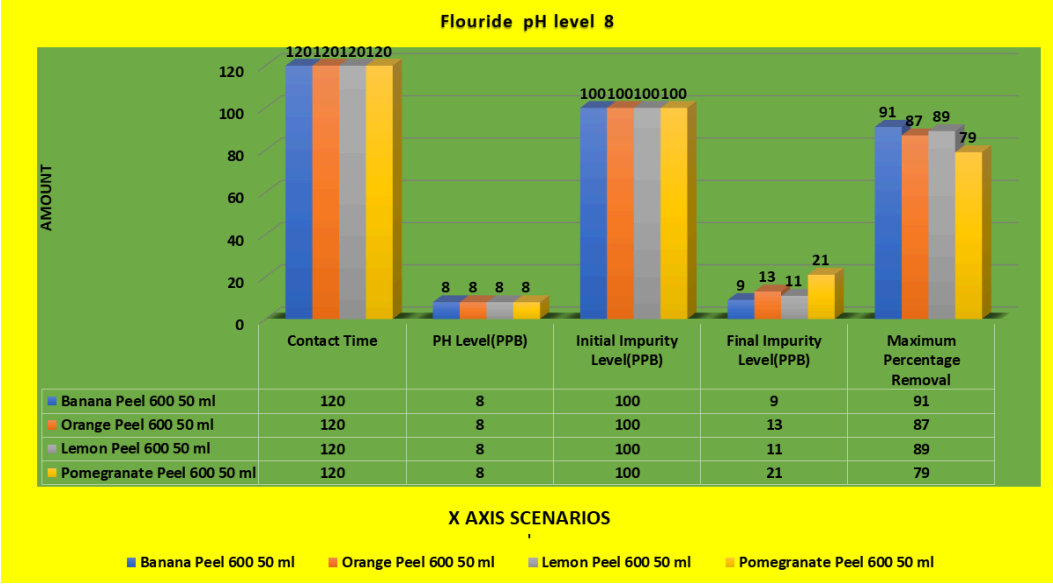
One similarity between the data was that the banana peels had the lowest impurity level for all the different impure water, despite the three scenarios of changing the contact time of the peel with the water, the amount of peels put in the water and the change in the pH level of the water. The representation of the data helps me see this because in all the graphs the bar for the banana peels for the final impurity level was lower than the rest of the peels which shows that banana peels had the lowest impurity level.

**TABLES, GRAPHS and PHOTOS**

**Experimental Design:**

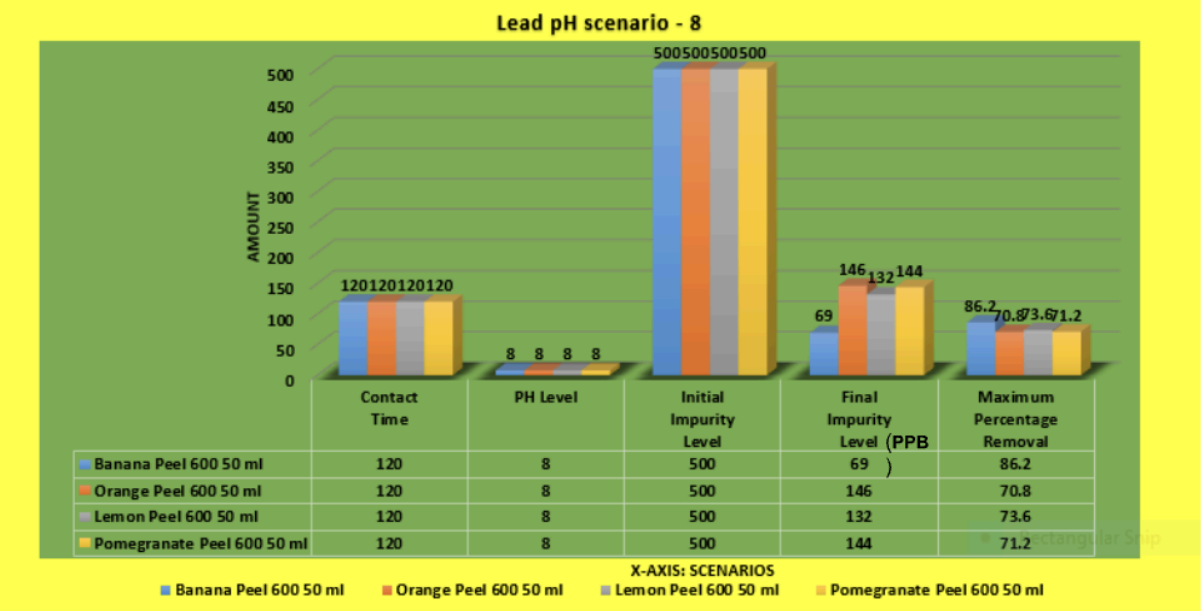
| Independent Variables | Dependent variables | Control |
|---|---|---|
| • Adsorbent dosages (amount of fruit peel used) - 600 to 900 mg<br>• pH level variations - 5 to 8<br>• Contact time variations of the peels in the water - 120 to 180 minutes | • Level of impurity of the water after peels are put in | • 50 ml of impurified water for each of the different trials |

NOTE: Two example representative graphs were chosen to showcase the data for simplification purposes since there were 24 graphs in total.




This graph shows the data for the specific scenario in which the water was polluted with fluoride at pH level of 8 with the contact time of 120 minutes. It is shown that the banana had a higher maximum percentage removal of the pollutant of 91% as well as a lower final impurity level of 9 PPB, showing how effective the banana peel was at removing the pollutant compared to the

other fruit peels. Multiple graphs were analyzed/collected showcasing the four impurities added to the water combined with the other variables (pH, Time, etc).




This graph shows the data for the specific scenario in which the water was polluted with lead at a pH of 8 and a contact time of 120 minutes. It is shown that the water with the banana peel resulted in a significantly lower final impurity level of 69 PPB compared to the waters with the other peels, showing how effective the banana was at removing the pollutant. Multiple graphs were analyzed/collected showcasing the four impurities added to the water combined with the other variables (pH, Time, etc).

**DISCUSSION**

From this data, it can be inferred that fruit peels can be used for removing impurity in water despite some having originally high acidity levels in their peels, like the orange and lemon peels. Water pollution is a very evident problem in the world, but it could have a significant improvement through the findings of this research. Banana peels have shown to have a significant effect on the final shown to have a significant effect on the final impurity level of water despite the use of different pollutants, such as lead which is a heavy metal and methylene blue, which is a toxic dye. Although banana peels have shown to be the most effective in terms of water purification, the other three fruit peels which were lemon, pomegranate and orange did show to have removed at least some of the impurity in the water. To provide support on why banana peels are more effective than pomegranate peels, banana peels have high levels of carboxylic acids, which are acids that charge ions within the peels so that they become negatively charged (Choi, 2011). This is important because these negatively charged ions bind the positively charged ions in common heavy metal pollutants. Pomegranate peels on the other hand have acids called punic acids which have hydrating and regenerating qualities especially for your skin (Shabbir et al., 2017). This could explain why they were less effective at removing impurities since they do not have the needed

properties of banana peels for absorbing pollutants in water.

For future use, the techniques for the modifications of fruit peels can also be applicable to numerous other biowaste materials since they are organic materials that have acids to help charge ions within them for the absorption of pollutants. This can lead to new avenues of research relating to the use of numerous biowaste materials for activated carbon, which serves as a low-cost way to purify water and reduce landfill waste.

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Sonal Singh

# Isolating Bacteriophages from Compost Using Gordonia Rubripertincta as a Bacterial Host

**ABSTRACT**
Bacteriophages are viruses that target and kill bacteria. However, many bacteriophages have not yet been discovered, thus encouraging researchers to identify new ones using host organisms. This study demonstrates how to isolate new bacteriophages that infect Gordonia Rubripertinca using 6 compost samples from the University of California, Santa Cruz (UCSC) campus (COSMOS Platform UC). We found that two out of the six samples on one plate showed visible plaques with central clearing, suggesting that phages had infected the host. Phage therapy can provide a potential solution for the treatment of antibiotic-resistant bacteria.

**INTRODUCTION**
Bacteriophages (or phages) are viruses that kill bacteria by infecting and replicating within bacteria. The first use of phages in a medical context is credited to d'Herelle and his hospital interns in 1919 when they instructed a 12-year-old boy suffering from severe dysentery to ingest a cocktail of phages. They were found to be greatly effective and all of his symptoms went away after just one dose (Sulakvelidze et al., 2001). Since then, researchers have used phages to remedy illnesses, now referred to as phage therapy (Lin et al., 2017). Phage therapy is often used for the treatment of diseases such as Staphylococcal lung infections, Pseudomonas aeruginosa infections in cystic fibrosis patients, urinary tract infections, eye infections, and surgical wound infections (Sulakvelidze et al., 2001). Further, doctors are increasingly using bacteriophages to treat multidrug-resistant bacterial infections.

Phages are varied in their appearance including differing sizes of the capsule or presence of a tail. They replicate in bacteria by binding and injecting their own set of genes into the cytoplasm of the cell (Steward et al., 2018). There are two main types of replication cycles termed lytic and lysogenic. In the lytic cycle, the phage DNA limits cell function and destroys the host cell DNA, which kills the cell. Then, phages are released and move on to other cells (Makky et al., 2021). In contrast, in the lysogenic cycle, the host cell isn't killed but instead used for the phage to proliferate by replicating its genome. (Kasman et al., 2021).

Virulent (lytic) phages infect and kill bacteria quickly, making phages practical for fighting bacterial diseases. Further, using a mix or cocktail of multiple phages has been shown to prevent the formation of resistant strains by targeting multiple receptors (Regeimbal et al., 2016). A recent study showed that Acinetobacter baumannii, a highly infectious antibiotic-resistant bacteria on the World Health Organization's list of most high-priority pathogens, is most effectively treated using a personalized cocktail of five phages (Regeimbal et al., 2016; Asokan et al., 2019).

The aim of the study is to investigate whether phages are effective in treating bacteria using compost from the UCSC campus.

**MATERIALS AND METHODS**
This research was conducted as part of the Cosmos program, a selective summer program conducted at four of the University of California campuses for high school students. This study was specifically conducted at the University of California Santa Cruz (UCSC).

First, compost was acquired from the UCSC campus as high levels of soil bacteria that break down organic materials are present in

these samples, creating ideal conditions for phage isolation. After gathering the compost, the culturing samples were prepared by adding 500 µl of G. rubripertincta, the host, with 4.5 ml of peptone yeast calcium (PYCa) media in a culture tube. PYCa is a general-purpose medium that has the nutrients needed to support bacterial life, including a low concentration of calcium (Segura et al., 2004). Next, 400 mg of the biological compost sample was added to the 4.5 ml of PYCa media with the host. Culture tubes were placed in a shaker at 30 °C for 48 hours and then stored at 4 °C. After 48 hours, samples were filtered with a 0.2-micron filter.

Two agar plates were prepared using a PYCa medium and 500 µl of the bacterial solution in PYCa was added to 4.5 ml of molten 0.5% agar (~60°C). Four individual PYCa mixtures were plated onto each agar plate using compost samples from different locations along with one control group containing no compost and were labeled with five distinct areas. Plates were then stored at 30 °C overnight. Then, 3 µl of the filtrate was spotted on each section of both agar plates except the control. The plates were then observed for signs of phage infection such as plaque formation with a central clearing.

**RESULTS**

A total of eight individual compost samples were evaluated on the two agar plates compared to the control group. Two of the six samples on one plate showed visible plaques with central clearing suggesting that phages had infected the host (Figure 1, right). The clear spot within the plaque is the area where the phage killed the bacteria on both plates. Sections J and M saw a major accumulation of bacteriophage, thus forming plaques with clearings in the middle. Sections S and D saw a minor accumulation of bacteriophage. In the other agar plate (Figure 1, left), all four sections had plaques with clearing, thus indicating that they had bacteriophage infection.

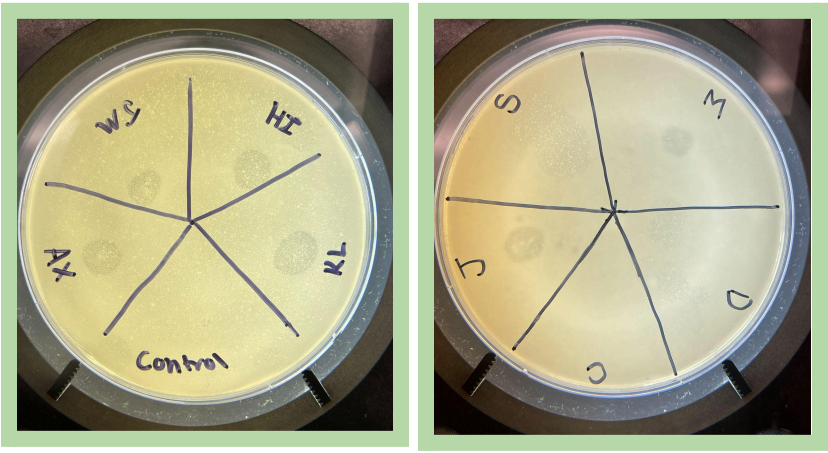


Figure 1: Plaque Formation on Two Agar Plates. (Left): Findings of bacteriophage isolation after 48 hours using four distinct compost samples compared to the control. (Right): Four additional bacteriophage samples compared to the control. Plaques with a central clearing indicate that bacteriophages attack bacteria in these samples.

## DISCUSSION

Ultimately, this study demonstrated that compost samples contained phages that were able to infect G. rubripertincta.

Our study does have some limitations. For example, only two compost samples were used. Therefore, with such a limited sample size, the precision of our results may have been affected. Nonetheless, the plates were very similar in terms of results and eight different compost samples from the UCSC campus were used. Further, plaque formation is a relatively subjective measure of phage infection. However, given that there were distinct plaques on the plates that were visible to the naked eye, this helps to mitigate the concern as the infection was very apparent. Finally, we only used one type of bacteria in this study, thus making our findings less generalizable. However, the findings we did have using one bacteria were helpful for setting up future experiments.

Through this experiment, it is clear that the bacteriophage successfully infected G. rubripertincta. It was evident that there were virulence factors in the soil meaning we had a successful isolation. Given that they were able to kill the bacteria, we think that this was a phase of the lytic cycle.

Ultimately, the application of phage therapy in modern medicine has allowed for a potential solution for the treatment of antibiotic-resistant bacteria (Lin et al., 2017). Nonetheless, phage therapy has been proven a challenge due to the limited target species of phages. Future research should focus on characterizing the strains of bacteriophages found using PCR and determining which phages might be most efficient in the patient setting (Hyman et. al, 2019). These findings can help provide a potential solution for antibiotic-resistant bacteria and potentially other diseases in the clinical setting.

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Sruthi Sudarsan

**Effects of Maternal Environmental Factors on Early Fetal Development**

The prenatal period, the period of time during which the fetal develops, shapes many aspects of a child such as behavioral, physical, and genetic characteristics. During this time, the growth and development of a fetus can be influenced by environmental factors from the mother. The process through which one's environment affects the phenotype or genotype of an individual is referred to as epigenetics. Epigenetic traits are heritable phenotypes or changes in gene expression, resulting from changes in chromosomes without any alterations to the original DNA sequence. Many of these alterations are a result of environmental factors such as nutrition, UV exposure, physical activity, alcohol, and stress. These traits can influence characteristics such as behavior, metabolism, and increase the likeliness of psychological and physical illnesses. Oftentimes, epigenetic traits are expressed after frequent exposure to a specific environmental condition, which may cause a gene to be silenced or expressed. Throughout pregnancy, the mother and fetus share resources such as nutrition and oxygen, allowing the fetus to survive. This is done through the placenta and umbilical cord, both connecting the mother and the developing fetus. As a result, environmental factors which affect the biological mother of a child during pregnancy also have an effect on the fetus. This is known as maternal epigenetics, or the effects of the maternal environment, phenotype, and/or genotype on the genetic makeup and phenotype of the offspring.

While environmental hazards are a clear threat to the developing fetus, they may not always result in harm. The impact of such dangers is determined by the combination of several factors, including the timing of exposure, the duration of exposure, and any genetic vulnerabilities that may exist. The specific time and intensity of when the growing organism is exposed to the risk might have a significant impact on the fetus and how it reacts to the environmental conditions. Critical periods occur throughout fetal development and are times of increased sensitivity.  In the first eight weeks following conception, for example, an embryo is most sensitive to teratogens. Pre-conception consultations with obstetricians and maternal-fetal medicine specialists are therefore critical to determine the best medication regimen for controlling a patient's underlying conditions. However, serious organ damage, such as damage to the brain and vision, can occur in the latter weeks of pregnancy. Apart from abstaining from drugs, alcohol, pharmaceuticals, and other substances, good medical treatment, social support, and postnatal care can all help to reduce the risks posed by environmental contaminants.

Congenital malformations, higher odds of miscarriage, preterm birth, intrauterine growth restriction, and stillbirth are variables that may contribute to negative pregnancy outcomes. Additionally, other reproductive processes, such as menstrual abnormalities and infertility, may be affected. Smoking, video display terminals, anesthetic gasses, antineoplastic medications, and exposure to lead, selenium, and inorganic mercury are among environmental variables that have been linked to poor pregnancy outcomes. Amongst these, smoking during pregnancy has been identified as the most common environmental risk associated with adverse pregnancy outcomes. Cigarette smoking during pregnancy is still a serious public health risk.

Epigenetic changes take place in numerous forms. One example of how an epigenetic change may take place is through DNA methylation, which occurs when a methyl group is added to DNA, suppressing gene transcription. DNA methylation causes permanent changes to phenotypes, which are inherited through several cell division cycles. This modifies the function of a specific gene, therefore modifying gene expression. DNA methylation has been observed to have a direct correlation to diet and various other environmental factors.

Another way epigenetic changes impact DNA is through histone modification.Histones are a class of alkaline (basic pH) proteins in the nucleus and help compress DNA into chromatin. Their positive charges allow them to bind with DNA. They're present inside eukaryotic cells' nuclei. Nucleosomes are made up of DNA and histones that are packed together to form chromatin. Two chromatids make up a chromosome. Some histones are also associated with gene expression. Histone modification factors control gene transcription and chromatin structures, therefore causing an impact on gene expression and phenotypes. Environmental variables such as food, obesity, and physical inactivity have a significant role in the development of type 2 diabetes. These variables affect gene expression through epigenetic pathways, contributing to the establishment of diabetes. NF-B-p65, PPAR-, Pdx1, PTEN, and glut4 are among the genes whose activity is changed in T2DM. Although several epigenetic modifications to diabetes-related genes, including DNA methylation, have been found, there has been no evidence of a link between global gene methylation and T2DM, glucose, insulin, or insulin resistance.

DNA is wrapped around histones, tightly wrapped DNA cannot be read by the protein, which results in the gene being turned “off”. DNA that is not wrapped around histones, leads to the gene being read as “on”. As a gene is read as either “on” or “off”, this has an effect on the phenotype of an individual. Chemical groups can change whether a gene is expressed as on or off, and if the DNA is wrapped around a histone. Additionally, Non coding RNA sequences do not encode functional proteins and coding RNA is used to synthesize proteins. Non coding RNA attaches to the coding RNA and breaks it down so that it can be used to make proteins. This ultimately contributes to gene expression, and relates to epigenetics as this process is influenced by environmental factors.

Different phenotypes can be generated by activating and suppressing the activity of certain gene sets, a process regulated by transcription factors. Transcription factors function by identifying and binding small regions of DNA termed cis-regulatory sequences in the genome. An enhancer is a cis-regulatory region that produces a reduction in gene activity when bound by transcription factors, whereas a silencer is a sequence that causes a decrease in gene activity when bound by transcription factors. These transcription factors can be influenced by environmental factors, causing a gene to be enhanced or scileneed. This could lead to changes in the phenotype of the individual or fetus.

Epigenetic inheritance refers to the passing of epigenetic traits through several organisms and generations. Changes to a parent's phenotype caused by environmental factors may be passed on to their offspring. For example, stressful circumstances parents were placed in before reproducing, may impact their children in the form of pathological conditions, represented as stress response mechanisms. In this case, the environmental trigger causing epigenetic inheritance was stress. This is present in pregnancy as well, where environmental factors the mother faces during pregnancy, can cause genetic changes in the fetus which may be present for the duration of their life. Several new studies have discovered methods by which maternal nutrition, stress,

and toxic substances influence the expression of imprinted genes during pregnancy, altering fetal and neonatal phenotype and susceptibility to disease development later in life. Fetal and neonatal phenotype are both influenced by maternal epigenetics. Maternal epigenetics refers to diet or other environmental changes in the mother which contribute to the growth, development, and epigenetic traits of the fetus. Maternal lifestyle variables including smoking (nicotine and caffeine), alcoholism, and psychosocial stress have been linked to epigenetic molecular pathways, resulting in abnormal neurological syndrome in children. During pregnancy, smoking and alcohol use are two of the most harmful behaviors that have been demonstrated to influence offspring's language, speech, hearing, and cognitive development. Smoking's negative consequences are well-known. Despite this, women's smoking rates have remained high over the previous 25 years. Despite warnings about the dangers of smoking, about 20–25 percent of adult women in the United States smoke, with rates much higher among younger women and those from poorer socioeconomic backgrounds. In women, there has been a parallel increase in cancer, heart disease, and other ailments directly linked to smoking. Women of reproductive age account for the majority of female smokers. Low birth weight, intrauterine growth retardation, placental difficulties, premature delivery, and spontaneous abortion are all linked to smoking during pregnancy. In the year 2000, there were 4381 preterm births and 3200 low birth weight babies in Connecticut alone. Smoking mothers are twice as likely to give birth to babies with low birth weight (LBW). These babies weigh 150 to 250 grams less than babies delivered to non-smoking moms. In addition to the risks of smoking for the mother, exposure to tobacco smoke in the surroundings increases the risk of Sudden Newborn Death Syndrome (SIDS), ear infections, asthma, and other respiratory problems in the infant. Not only does smoking during pregnancy harm placental function, but nicotine also penetrates the placenta and acts as a neuro teratogen. It affects the neurological system of the fetus, interfering with its development. Nicotine alters the pattern of cell proliferation and differentiation in the embryonic brain by targeting nicotinic acetylcholine receptors. Cell death and neural damage ensue as a result of this. This has been linked to the likelihood of cognitive and auditory processing abnormalities, as well as social behavior impacts.Obese women have an increased chance of having children with autism spectrum disorders. According to meta-analysis findings, late maternal age is a risk factor for autism in children.

Poor diets deficient in critical nutrients such as iodine, iron, folate, calcium, and zinc during pregnancy can lead to anemia, pre-eclampsia, hemorrhages, and maternal mortality in women. Stillbirth, low birthweight, wasting, and developmental delays in children are all also possible outcomes. Nutrition of the mother is one such environmental factor which can alter the phenotype and genotype of the fetus during development. Pregnancy involves a series of subtle, ongoing physiologic changes that influence all nutrition metabolism. The changes will likely differ from woman to woman, based on her pre-pregnancy diet, genetic predictors of fetal growth, and lifestyle habits. Studies of protein and energy metabolism show that adjusting how those nutrients are used can help to conserve fetal supplies. By the second trimester of pregnancy, changes in nitrogenous chemical metabolism have taken place. These modifications allow for positive nitrogen retention during the latter quarter of pregnancy, when fetal needs are highest. The energy needs of basal metabolism are regulated by maternal diet throughout pregnancy and fetal growth. If the maternal energy reserves are low at the time of conception, the basal metabolic rate is reduced to save energy. In addition, mothers who have larger children have higher increases in their basal metabolic rate and poorer maternal energy storage rates.

Physiologic modifications may be aided by changes in mother eating and physical activity habits during pregnancy. However, because of the wide range in dietary consumption and physical activity, demonstrating those changes is difficult. For all nutrients, there are thresholds in the ability to regulate nutrient usage to the amount provided. Fetal growth and development are harmed more than mother health when intakes fall below the threshold. This can be caused by both undernutrition and overnutrition. It is crucial that mothers follow a balanced diet.

Mothers who were malnourished often had children who were delivered severely underweight. This has caused lasting effects on the metabolism of the offspring, even well into their adult life. We see lasting effects of malnutrition during pregnancy through major historical events.

During World War II, food supplies in The Netherlands grew increasingly short as the country experienced an extremely hard winter. The Nazis cut off food and fuel supplies to western Holland, causing 4.5 million people to go hungry and starve. The Dutch Hunger Winter, often known as the Dutch Famine, occurred at this time. By the time food supplies were restored in May 1945, when the Allies conquered The Netherlands, between 18,000 and 22,000 people had perished from famine. Many more people were extremely malnourished, including pregnant or soon-to-be-pregnant women. Medical experts in the Netherlands recorded the weight and blood pressure of pregnant women, as well as the weight and size of newborns and placentas at delivery, the length of umbilical cords, and written accounts of labors and births. The Dutch Hunger Winter cohort refers to the children born to these women. Many studies have tracked the cohort's health throughout their lifetimes as a result of the famine. The purpose of this study brief is to examine how placentas reacted to the famine at various stages of pregnancy.

Overall, babies born during the famine or while still in the womb had smaller placentas, were shorter and thinner at birth, and had smaller head circumferences than babies born before the crisis. The smaller placenta changed the baby weight per gram of placenta ratio, which is used to determine placental efficiency - how well it performs its role. Depending on the stage of pregnancy during the famine, the placenta became more or less effective.

The Dutch Hunger Winter Famine, spanning from November 1944, to April 1955 was a time of near starvation for several communities. This was caused by an exceptionally harsh winter, years of brutal war, and an insufficient supply of crops as a result. During this time, some of the lowest temperatures in European history were recorded. Food rations dropped from 1,000 calories a day, to 500, and even lower. As the famine was reaching its end, towards April of 1945, individuals were limited to one piece of bread and 5 potatoes for the whole week. Several scientists conducted research on how stress during the famine impacted children and caused side effects during their adult life.

Women who survived the famine and had a child later showed lower placental size and thickness for up to 18 months after the famine (the end of the study period). Their placentas were also more oval in form than those of newborns delivered before to the famine, indicating that implantation was impeded for some time after being subjected to starvation. The scientists highlight that this oval shape is comparable to preeclamptic placentas - a condition caused by poor implantation.
This famine additionally demonstrated the effects of nutrition of the mother during fetal development. Women who were pregnant during the famine gave birth to children who were more likely to develop type 2 diabetes, schizophrenia, and heart failure. These children were also at a much greater risk for obesity, and often lived a shorter lifespan than those born outside of this time period.

Compared to their siblings that were born either before or after the famine, these offspring had a change in methylation, which was either increased or decreased in certain areas. This change in methylation most likely accounted for the variance in phenotypes between children born during the Dutch Hunger Winter Famine, and those born before and after the event. Additionally, similar effects were seen in children who were born during the seven year famine in Sweden.

Smoking has also been observed to have significant effects on fetal development when done while pregnant. Babies delivered under these circumstances were seen to be delivered much earlier than expected, likely to suffer from SIDS (sudden infant death syndrome), low birth weight, and physical differences such as underdeveloped mouth and lip. In utero development is one of the most important periods during which poor intrauterine circumstances and exposures can affect the fetus's growth and development, as well as the child's future postnatal health and behavior. In prenatal exposure to maternal cigarette smoking is still reasonably prevalent, although it is still dangerous. Prenatal smoking exposure has been linked to lower birth weight, poor developmental and psychological outcomes, and an increased risk of illnesses and behavioral problems later in life
The concept of epigenetics can be used by scientists to control the activation of certain genes or genetic markers present in various individuals. The aberrant silencing of non mutated tumor suppressor genes, which acts as an alternative to mutations for causing gene function loss, is a significant example of epigenetic modification in cancer. The discovery of mutations in genes that encode several proteins that regulate the epigenome in practically every cancer type  is a new intriguing indication of the relevance of epigenetic alterations. Understanding the consequences of these genetic alterations is critical for cancer research.This technology could be used to suppress genes such as those which code for cancer or other dangerous illnesses. A gene can be turned on or off using techniques from DNA methylation. In order to chemically flip a gene to turn it on, a methyl group should be added to the side DNA of a chain. However,iIn order to chemically flip the gene off, a demethylate compound should be introduced.  Epigenetic drugs are extremely important as advances in the medical field are continuing to be made. Eventually, it will be possible to simply “turn off” the expression of genes of several deadly or harmful illnesses such as cancer, autism, and Alzheimer's.

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# The Viability of Using Induced Pluripotent Stems Cells and Olfactory Nerve Cells as Treatments

**ABSTRACT**

Induced pluripotent stem cells (IPSCs) are artificial cells that are derived from somatic cells that have been reprogrammed and re-purposed to become pluripotent through pluripotency gene factors. While these cells do have success in being expressive and differentiating into the necessary cells needed in the body, this is only applicable to mice. A more natural but little explored cell type that can be used in stem cell research are the neural stem cells found in the olfactory bulb. Though there is very little known about the possible usage of the cells, it has been established that their regenerative abilities in the nasal cavity make it possible to point to olfactory cells as treatment as well. This article will discuss and summarize the findings from electronic databases that focus on either IPSCs or olfactory cells as well as explore in stem cell research and look towards the viability of using IPSCs and olfactory cells in treatments to reverse damage on systems that eventually render individuals with a loss of motor and emotional skills. The results highlight the efficacy of IPSCs and olfactory cells for treatments but also point towards caveats that may induce secondary diseases or the lack of function. It should be noted that much of the cases to use stem cells have been performed on rats and rather few experimental treatments have been used for humans due to ethical implications.

**INTRODUCTION**

Stem cells are specialized natural cells that have the ability to differentiate into various other cells found within the body. These can range from damaged tissue cells to muscle cells and are mainly found as two certain types: embryonic and adult stem cells. Embryonic stem cells (ESCs) come from unused embryos and come as donations. These cells are pluripotent, allowing them to differentiate into more than one type of cell[1]. The use of ESCs as potential treatments does come with several ethical issues, mainly due to the fact that it must be harvested from human embryos. While it can also be found in amniotic fluid, it does breach the safety of women and as a result raises another question that focuses on the ethics of its harvest. Adult stem cells on the other hand are multipotent cells, allowing them to differentiate into other types of cells but being limited to certain types depending on what regions they are extracted from. These cells exist in a very small quantity and due to their limited manipulation, it puts the usage of adult stem cells as treatments within the constraints of where it is being extracted from.

As stem cells are limited from the restrictions that are placed on them due to ethics and differentiation constraints, it necessitates other alternatives to be used for the possible cures for neurodegenerative diseases. Induced pluripotent stem cells (IPSCs) do open a gateway into regenerative cell treatments due to their generation to occur from healthy patients. These cells are re-purposed and reprogrammed through pluripotency gene factors[2]. The 2019 study[3] shows that due to their artificial nature, these cells must be specialized first prior to being injected into humans or they form as teratomas, tumors that are formed as result of undifferentiated and fast growth. Furthermore, the study gathers the present immunological threat recipient bodies face as a result of human leukocyte antigens (HLA) being attributed to the donor IPSC and triggering an immune response in the recipient body. Though there are several challenges present

with using IPSCs, they are a valuable resource for possible new drugs and being used through cell therapy.

The use of olfactory cells as treatments is a relatively new concept and attributes to the regenerative abilities of olfactory neurons located in the nasal cavity. These cells have a lifespan of upto six to eight months to which they are replaced by new neurons as a result of undifferentiated olfactory bulbs specializing into the weakening neuron's place. Although these cells do allow for differentiation, it is thought to be only multipotent due to their localization in the basal layer of the olfactory epithelium and vomeronasal organ. As individuals age, the regenerative capacity of the olfactory system decreases, especially if affected by neuro- degenerative diseases and other pathophysiological diseases affecting the nasal cavity[4]. But their rapid replenishment ability does make way for drug screenings and other cell therapies as well.

**MATERIALS AND METHODS**

Using electronic databases such as the the National Library of Medicine, Nature Reviews Clinical Oncology Journal, Stem Cells Journals, Biomed Central, and The Mayo Clinic were used to pool together articles that could be used for the meta-analysis. The articles containing the key words "olfactory cells", "induced pluripotent stem cells", "motor functions", and "sensory functions" were used as well as followed by having two main criterias: they must discuss the properties of IPSCs and olfactory cells and the article must be in English. Previous meta-analysis articles were also included. Articles were excluded if they were in languages other than English and if the study sample was too small to come up with a conclusive result.

**RESULTS**

The data from the meta-analysis showed the efficacy of both IPSCs and olfactory cells as regenerative treatments through mice models and in vitro studies (Table 1).

*Induced Pluripotency Stem Cells*

IPS cells have shown several successes over the course of years to allow for differentiation, although it is limited to mouse trials. Chambers et al.[5] shines light on the use of human somatic cells to develop pluripotent cells that have the ability to go into a state of differentiation by day 11. By inducing the human induced pluripotent stem cells (hIPSCs) into the neural pathway, the cells were readily able to differentiate into dopamine neurons and other motor neuron subtypes. Swistowski et al.[6] also confirms this success in a xenofree system; thus indicating that hIPSCs can be used to treat neurodegenerative diseases such as Parkinsons by producing dopamine neurons and receptors throughout the body. Rufaihah et al.[7] also confirms the transplant of hIPSCs into other mammals does indicate the promotion of tissue growth. Through immunofluorescence staining and laser Doppler data, it can be identified that when hIPSCs are injected in the ischemic hindlimb, the capillary density increases. Alipo et al.[8] does show IPSCs being used in mouse bodies to treat hyperglycemic phenotypes. Using skin fibroblasts, retroviral transcription factors were transduced into the fibroblasts to create IPSC colonies which would then be injected into the mice to correct the hyperglycemic phenotypes. The initial skin fibroblast conversion into the β-like cells were in vitro but following their differentiation, it would be injected into the mice and followed to see insulin being secreted after marking glucose in the mices' bodies.

While most studies mentioned above are integrative and direct in their approach to introducing the IPS cells into the body of the mice, Moradi et al.[3] suggests that non-integrative methods are much better at delivering the purpose of pluripotency cells. By inducing transgenes into the pluripotent cells, there is a possible risk of activating another gene or changing the purpose of the cell entirely, rendering the cell useless and the subsequent daughter generations useless as well. Because of their low reprogramming efficiency, if coupled with microRNAs or other

small molecules that assist in the reprogramming phase of the pluripotent cell, there would be a significant rise in the overall effectiveness of the IPS cells.

Integration of IPS cells into the body does pose several risks as the possible acquisition of secondary diseases as well as loss of function does pertain to heavy risks overall. Teratomas are also indicative of IPSCs proliferating, however, at an unchecked growth rate and without any differentiation. It necessitates for a differentiation gene being introduced prior to its introduction into the recipient body alongside with preventative measures to keep the HLA immunological defense mechanism from triggering.

*Olfactory Cells*

Olfactory cells are unique in the sense that they are some of the only cells that have the ability to be pluripotent (after a long believed theory that they are multipotent)[9] but can only do so in certain conditions. Unlike bone marrow cells that are multipotent, Costanzo's study[10] highlights the spatial challenges that are presented to regenerating nerve cells due to lesions to the olfactory nerves. These lesions cause axon sheath alignment to be disrupted, causing nerve links to be severed and preventing electrical impulses to get through for motor and sensory functions. Marei et al.[11] study highlights the same issue of lacking motor and sensory functions within mice despite adult human olfactory bulb neural cells being engrafted into the mice. The olfactory cells did differentiate into neurons and other neural networks, however, there was a lack of function presented in these mice.

With the Beecher study[4], it sheds light on possibly new cell transplantation therapy with olfactory ensheathing cells (OECs). By expressing directional and matrix molecule support to growing nerve cells, it indicates that these cell types are extremely important in growing nerves located within the nerve fiber layer of the olfactory bulb. Seo et al.[12] does suggest that some sensory functions can be returned through a combination of prednisolone and G biloba compared to only prednisolone through a randomized trial. Though there was not much of a statistical difference in the two therapies, the combination therapy does suggest better efficacy to treating lost olfactory sense.

Table 1. Summary of Stem Cell Meta-analysis

| Article Type | Study | Outcome |
|---|---|---|
| Induced Pluripotency Stem Cells | Moradi et al. (2019)[3] | IPSCs have better success through non-integrative methods |
| | Chambers et al. (2009)[5] | Directed differentiation allows for uniform and fast neural conversion |
| | Swistowski et al. (2010)[6] | hIPSCs differentiate in xenofree systems |
| | Rufaihah et al. (2012)[7] | hIPSC transplant showed higher capillary density |
| | Alipo et al. (2010)[8] | β-like cells were differentiated by mouse skin IPSCs |
| Olfactory Cells | Beecher et al. (2018)[4] | Olfactory ensheathing cells (OECs) can be used for cell transplantation |

| | | |
|---|---|---|
| | Costanzo (2009)[10] | The disruption of axon sheath alignment from regenerating nerve cells |
| | Marei et al. (2016)[11] | Engrafted cells failed to bring back motor and sensory functions |
| | Seo et al. (2009)[12] | G Biloba and prednisolone showed clinical efficacy in recovering sensory function |

**DISCUSSION**

A major drawback of IPS cells being used as regenerative cells is that they need to be differentiated prior to being injected into the recipient body. Although it requires the gene to be reverse transcribed into the pluripotent cell, this gives rise to the possible issue of introducing secondary diseases and redefining purposes of the IPSCs. If medications targeting the relaxation of the HLA immunological attack were prescribed to the recipient, this would allow for the proliferation and regenerative abilities of the already differentiated and localized IPSCs, however, it would also render the recipient open to other attacks from viruses and diseases that could go unchecked due to a relaxed immune system.

In cases where IPS cells are taken from an individual who may have a genomic defect, if coupled with genome editing tools such as CRISPER/Cas9, those genomic defects may be mitigated. While this does sound feasible, it opens itself to several ethicality questions and renders questions on whether the editing coupled with integrative IPSCs could possibly induce a larger and unanticipated issue in the genomic makeup of the person.

There should be a focus on the safety of transgenic cell therapy. While there is high safety in using non-integrative methods of introducing IPS cells, it would require a lot more resources and necessitate for viral vectors to deliver the genes reverting the cells back into pluripotency to be at the highest clinical grade possible. It should be noted that with subsequent daughter cells, they must match the native pluripotent cell that differentiated and cannot be mutated, lest causing damage to the area where the cells are localized.

In terms of olfactory cells, it does reveal surprising results of being able to be pluripotent unlike other cells found in the body. While this does open several gateways to using these cells, the biggest challenge it presents is its inability to recover motor and sensory functions. While most studies do focus on recovering olfactory senses, there is not much known about grafted olfactory nerves in locations other than the nasal cavity and testing the efficacy of recovered sense from those locations. It is a possibility to use OECs to help bridge the gaps between nerve cells to send electrical impulses and create a feasible link for the motor and sensory functions to work, however, it still does not answer the spatial challenge presented. For nerves to span across in an entirely new location would require glia cells and axonal outgrowth cells alongside growth factors localized only to that area.

Furthermore, due to lesions, even minimal or no damage to the olfactory bulb presents the problem of spatial issues and possible incorrect re-wiring in the nasal cavity. For the re-wiring process to fail in mice models and cause odor discrimination, it would require a deeper investigation in the re-wiring mechanisms.

The most surprising finding of this meta-analysis for olfactory cells is the lack of function. Even if nerve cells were to regenerate, there is still no possible way to

recover the function that used to be present in mice. While it may be possible to use therapy, it creates the question whether the regeneration of the nerve cells must be coupled with therapy to regain function or if electrical impulses must be artificially grafted through technologies such as Neuralink to bring function.

The use of both IPSCs and olfactory cells for treatments is viable; however, each cell type brings its own challenges to the table. The necessity of differentiating IPSCs prior to injections and keeping the immune system from fighting back requires a type of intervention that focuses on ensuring IPSC growth can be kept at check while using the immune system to terminate the growth if the IPSC growth is on track for teratoma formation. For olfactory cells, once there is a clear grasp on how to use OECs for connecting olfactory-turned-nerve cells for different body locations, it would allow us to get past the spatial connection issue and use these cells for possible cell therapy treatments.

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