

Analyzing the Growth of Pea Plants in an LHS-1/Vermiculite Mixture

Team Name: Central Cass Moon Mission

Team Name: #11319

Challenge Category: Highschool

Team Identification: Plant the Moon

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| Background |

Nitrogen is essential to a plant's life; without sufficient amounts of nitrogen the plant will not be able to create an essential protein that plant cells require for growth. On Earth, plants get their nitrogen through bacteria which live in the soil, or through a symbiotic relationship with bacteria living in nodules on their roots. On their own, plants cannot obtain nitrogen from the air. The bacteria that serves these functions are not found in the Lunar Regolith, which limits plant growth in this medium. Currently, to combat this lack of nitrogen on missions, large amounts of fertilizer are transported on space shuttles as it provides plants with needed nutrients such as nitrogen, phosphorus, and potassium. This is extremely costly to the program as more transported weight equals higher cost. This was the impetus of our research hypothesis.

Guided by Dr. Barney Geddes, NDSU Microbiology, our team focused on using a naturally occurring bacteria called Rhizobium to help eliminate the need for pounds of fertilizer. This bacterium helps to create a useable form of nitrogen for the plant from the air in exchange for food and shelter while residing in nodules on the plant roots. This bacterium is especially helpful in the way it converts atmospheric nitrogen to ammonia, which is what is used by the plants cells to help grow. This will eliminate the need of transporting excessive amounts heavy fertilizer, and instead replace it with much light bacterium. We focused primarily on how the Rhizobium bacterium would function in Lunar Regolith (LHS-1).

| Experiment Design |

Our experiment was conducted at NDSU ACE Greenhouses, under the direction of Dr. Barney Geddes. Before any of the setup for the experiment was done, everything was sterilized for use. This was to limit the possibility of cross contamination of natural occurring bacteria found in the room. Pots were constructed using two Leonard jars, drilling a hole in the bottom of one jar, and placing that jar on top of the other. Each setup contained a cotton ball batting as a wicking agent to bring up the water as the plant grew. This allowed researchers to not be present every day to water the plants and prevented the chances of overwatering.

In total 9 pots where planted, each contained a mixture of Lunar Regolith (LHS-1) (80%) and vermiculite (20%) that weighed a total of 368.58 grams, along with 250mL of N-free rooting

solution (Fig. 1) to give the seeds the need nutrients to start growing. The LHS-1/Vermiculite mixture was placed in the top jar, two germinated pea seeds were added ½ inch deep, then gently covered with the remaining soil. The N-free rooting solution was then added to the bottom jar as seen in (Fig.2).

Pertaining to the seeds, a sterilizing method was used containing an ethanol solution followed by a bleach solution to eradicate any unwanted bacteria that may be present. The procedure was as follows: First the pea seeds are soaked in a 95% ethanol solution for 30 seconds. Immediately after they were placed in a 2% bleach concentration solution for 5 minutes. Seeds then were rinsed 10 times with sterilized water to remove the solutions to prevent them from harming the seeds. While in the solutions, the seeds were constantly being gently mixed by turning the container in 360 degree turns. The sterilized seeds were then germinated in 0.8% water agar plates two days before planting to ensure the viability of the seeds for the best change at successful growth and to avoid the possibility of replanting.

For the planting of each pot, researchers wore gloves that were sterilized using alcohol each time they touched an outside source, and N95 masks to protect their lungs against finer particles of the Lunar Regolith. In groups, the team evenly mixed LHS-1 and Vermiculite, and followed the instructions that were provided by Dr. Barney Geddes, and what is listed above. Our design had both a negative and a positive group along with a control group. The control group received the N-free rooting solution but got no other treatment. The negative control was the group that received the Nitrogen Fertilizer (10 mM KNO_3). The positive control was the group that was received the colony of Rhizobia Bacterium. See (Fig.3) for a breakdown of conditions on starting data. Each pot was assigned its label according to its group and number, this is how data for each pot was kept individually. Each structure got 10mL of their required treatment.

The creation of the pots was a redesign of the pot choices from last year. The group from last year experienced difficulty with keep the plants well-watered. This time, watering was done weekly with the help of how the Leonard Jars were constructed. The choice to mix vermiculite with the Lunar Regolith was the way the Lunar Regolith behaved. It resembled sand, but it was much higher in density. The roots of plants would have issues trying to grow through

the finer, higher density lunar regolith, the purpose of adding the vermiculite to the LHS-1 was to combat this. The vermiculite was less fine, and when mixed into the LHS-1, would create miniscule pockets of air, allowing an easier path for the roots.

Fig.1

N-free rooting solution

Chemical	Final Concentration	Final weight (g/L)
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1mM	0.07351
KCL	100uM	0.00373
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	800uM	0.09859
Fe EDTA	10uM	0.001840
H_2BO_3	35uM	0.000216
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	9uM	0.0001780
ZnCl_3	0.8uM	0.0000109
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.5uM	0.0000121
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.3uM	0.0000075
KH_2PO_4		0.5
Na_2HPO_4		0.568
	Total	1.2460945

Fig. 2 Leonard Jar Setup



Fig. 3 Pre-Growth Data

Pot	Soil	Adjustments	Crop	pH of N Solution	Total Mass of soil (grams)
1	80% LHS-1 20% Vermiculite	None: Control	Edible Peas	7pH: Neutral	368.58 grams
2	80% LHS-1 20% Vermiculite	None: Control	Edible Peas	7pH: Neutral	368.58 grams
3	80% LHS-1 20% Vermiculite	None: Control	Edible Peas	7pH: Neutral	368.58 grams
4	80% LHS-1 20% Vermiculite	Nitrogen Fertilizer (10mM KNO ₃)	Edible Peas	7pH: Neutral	368.58 grams
5	80% LHS-1 20% Vermiculite	Nitrogen Fertilizer (10mM KNO ₃)	Edible Peas	7pH: Neutral	368.58 grams
6	80% LHS-1 20% Vermiculite	Nitrogen Fertilizer (10mM KNO ₃)	Edible Peas	7pH: Neutral	368.58 grams
7	80% LHS-1 20% Vermiculite	Rhizobia Bacteria	Edible Peas	7pH: Neutral	368.58 grams
8	80% LHS-1 20% Vermiculite	Rhizobia Bacteria	Edible Peas	7pH: Neutral	368.58 grams
9	80% LHS-1 20% Vermiculite	Rhizobia Bacteria	Edible Peas	7pH: Neutral	368.58 grams

| Hypotheses |

If edible pea seeds are grown in an 80% LHS-1/20% vermiculite mixture, and naturally occurring rhizobium bacteria is added to the positive control group, (pots 7-9) faster plant growth will occur than in the negative (4-6) and control (1-3) groups.

| Independent Variables |

The independent variables present for this experiment would be the treatment each group is receiving. This includes the nitrogen fertilizer, rhizobia bacteria, and the water.

| Dependent Variable |

The dependent variables in this experiment would be plant growth. Plant mass, chlorophyll levels, and pH levels of soil were taken to compare to the control group.

| Measurements |

To measure the growth of plants, a sterilized rule edge was placed lightly into the substrate, and the plant was gently guided to stand up straight. The top tendril of the pea plant

was extended to its full height, and the measurement was recorded in centimeters. While handling plants, sterilized gloves were used as an extra precaution. This was done weekly, on Wednesdays at 4:30pm.

Using MColorpHast pH testing stripes, pH test of the mixture was conducted at the end, using a single pot from each group along with a sample of the Lunar Regolith. Using the method recommended, two pH strips were ran per group (Fig. 4). The following procedure was used: Place 20 mL of distilled water and 10 grams of soil sample in a glass bottle. Shake vigorously for 1 minute. Every 5 minutes shake again for 1 minute. Repeat this step four times. Let the standing liquid “soil solution” sit for 60 minutes, then test with the pH strips. The amount of water each plant received was also recorded, but it was not the focus of the experiment.

Regolith	Rhizobia	Fertilizer	Water
5.5	5.5	6.5	5.5
5.5.	5.5	6.5	5.5.

| Controls |

The controls in this experiment were as listed: soil mixture of LHS-1 (80%) and vermiculite (20%), the amount of daylight, the temperature (72°-73°), the number and type of edible pea seeds per jar, the Leonard jars and N-free rooting solution (250mL).

| Results |

The success of the plants was measured in the height, amount of pea pods, and if nodules formed on the root systems (Fig. 5). In a broad overview of the results, we can infer that our original hypothesis was supported; the experimental group that received the rhizobium bacterium had overall better results than the other groups. At the end of the growing period, there were four remaining plants, three from the control group and one pot from the Rhizobium treatment group (Fig.6). The rest of the plants had died the week prior. See (Chart 1) for growth data for the eight-week period. Also note that the amount of water that the plants required increased as the plants grew, as shown in (Fig.7) but this was to be expected. Upon harvesting, it was noted that cross contamination had occurred on two of the three water control group at one point during the grow period. The best inference on how this

occurred would be the fruit flies present in the green house had transferred micro-portions of the rhizobium bacterium, from the positive control to the control group. See (Fig.8) for non-contaminated roots, and (Fig.9) for contaminated roots



Figure 5: Located on the upper most parts of the roots, nodules are present. The pink hue that is visible (the nodules) indicates that nodule was receiving nitrogen. This is a successful harvest of the Rhizobia treated plant.

Final growth, weight, nodules and chlorophyll concentration of surviving plants

	Shoot Height	Shoot Weight	Nodule #	SPAD
Rhizobia 1	39cm	9.5g	55	30.5
Rhizobia 1 (Seed 2)	16.2cm	1.3g	10	31.9
Water 1	24cm	4.5g	60	28.9
Water 2	13.2cm	1.4g	None	19.2
Water 3	36cm	2.5	40	19.5



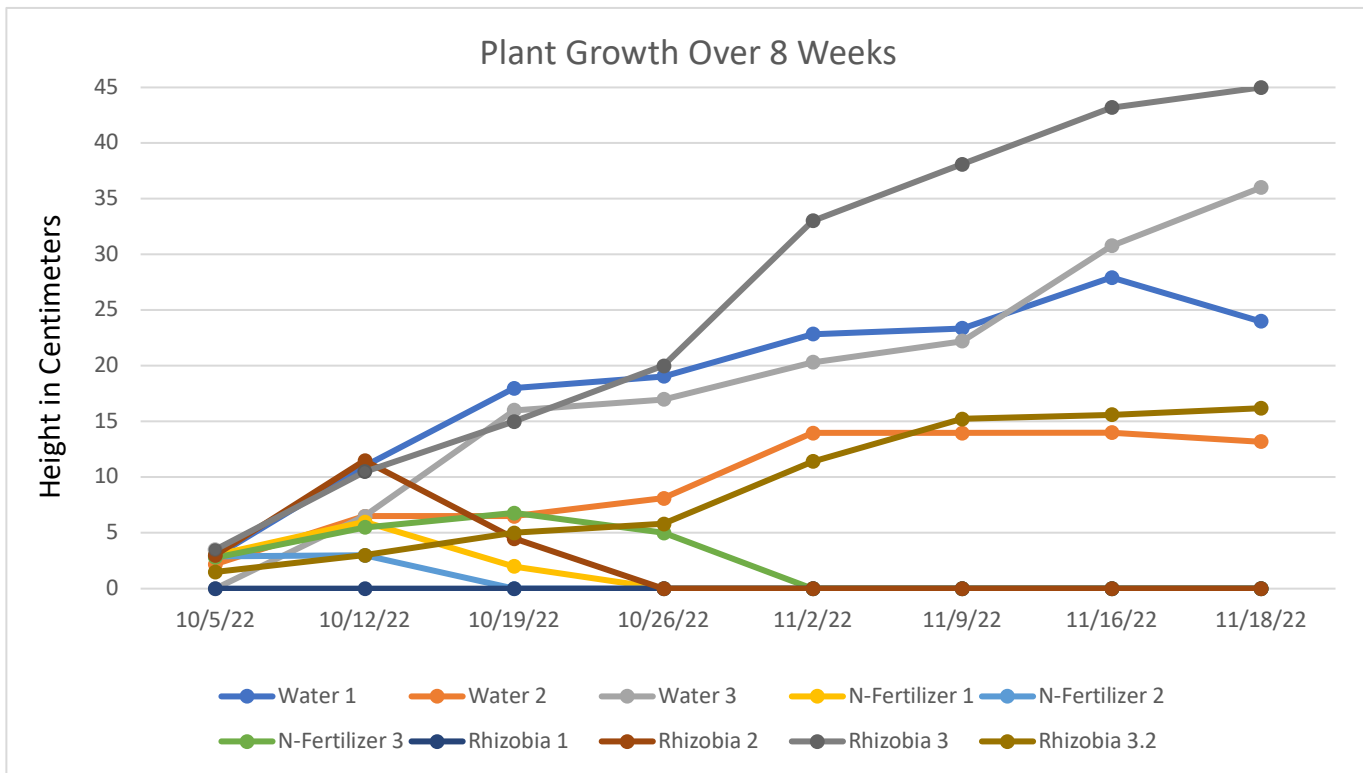


Chart 1: As shown in the chart above, plants that died out happened around week three and four. Water two was the plant that was uncontaminated, and it seemed to level out around week five. While the other two water plants (1 & 3) boot grew higher.



Figure 8: White, healthy roots of Plant 2, in the control group that only received water. No nodules were counted or seen on the roots, so an inference that there was zero cross contamination occurred.



Figure 9: Roots in this image are thicker, and more sturdy compared the roots of the non-cross contaminated roots. Pink nodules are also abundantly clear, indicating cross contamination. The roots in the above image are those of the Plant 1 and Plant 3 in the control group, that only received water

| Discussion & Conclusion |

The results from the overall experiment supported our original hypothesis. While excellent growth was also observed in the control group, as stated early, further examination of the plants root systems revealed there to be cross contamination of the bacterium. Two out of the three plants were contaminated, but the results could still be viewed in a positive light. When looking at the control group (water) a notable difference between the contaminated; plants 1 and plant 3, and non-contaminated; plant 2, is observed. Plant two shows stunted or less growth, than what is present in the rest of the pots in that group. Plants one and three both seemed to be well within the range of growth as the rhizobia treated group was, further supporting that plants with the Rhizobium Bacterium grew better with the helpful bacterium. Finding that this bacterium proves beneficial, brings hope and positive evidence that the amount of fertilizer that will have to go to the moon will be reduced.

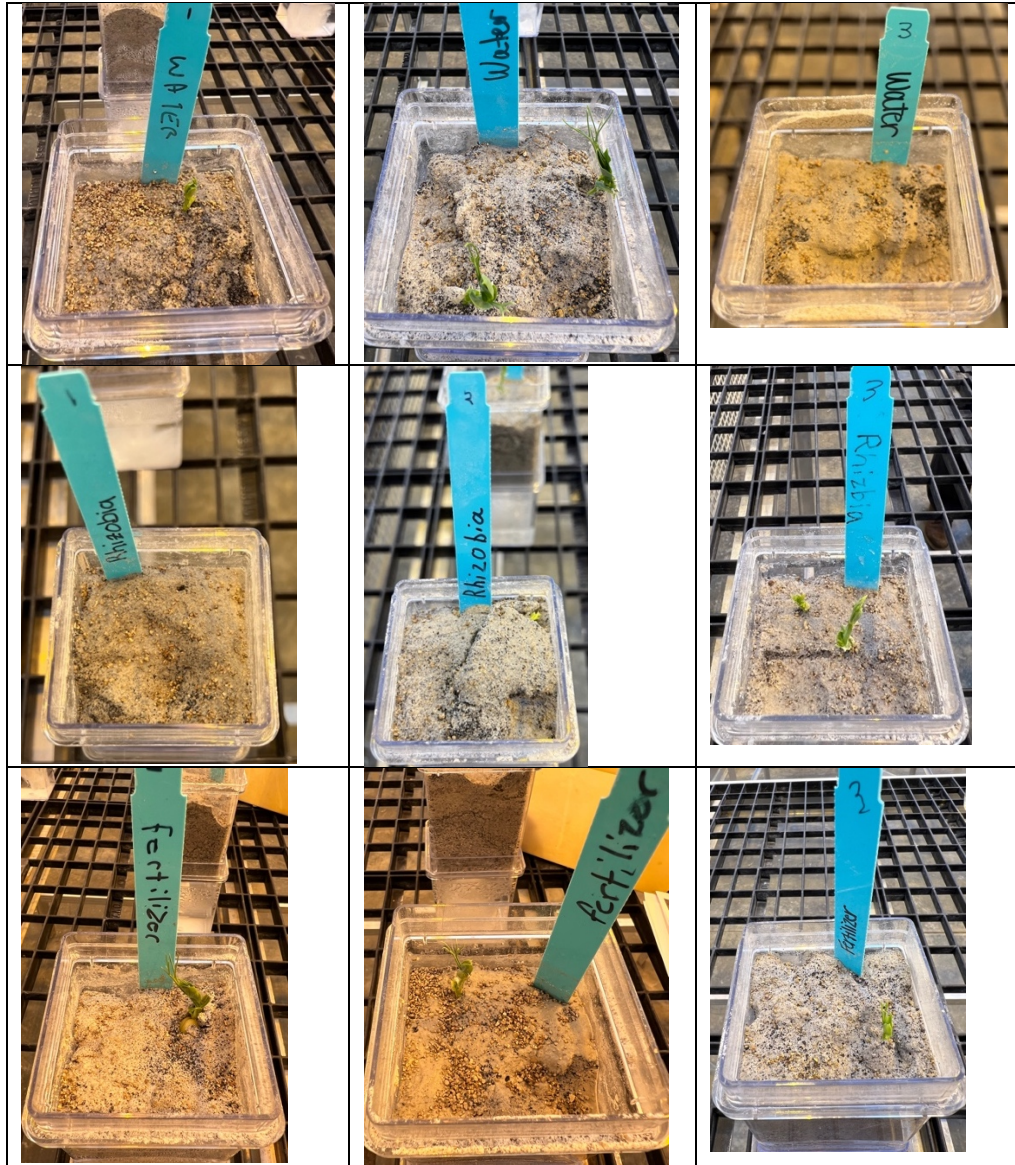
Multiple assumptions and speculations about what and why things were happening occurred and are possible hypothesis that may be look into further. These included but are not limited to: the reason why all of the Nitrogen Fertilizer treated plants died off within three to four weeks. Two possible ideas formulated. One idea that seemed plausible was that as the plant grew it required more nutrients, and the lunar regolith made it hard for the plant to get the needed nutrients. The denser regolith was predicted to make it harder for the roots to grow, which may have caused the earlier wilting of these plants. A second reason could have been that something changed chemically due to the long exposure to the regolith. This may have been something in the N-fertilizer treatment, or perhaps cross contamination also occurred, and the bacteria reacted badly to the treatment that was received. A third speculation might involve competition- as only one of the two plants survived in jars with two sprouted seeds. Perhaps the stronger, more established of the two plants absorbed all of the nutrients from the N-free rooting system, leaving next to nothing for the weaker one, eventually it dies out. In the future we would like to investigate the effects of gravity on plant growth and also compare LHS-1 particle size to that of our local clay soils.

Based on the results and the problems faced, improvements would deal with finding ways to prevent cross contamination between the pots. Finding a faster way to test the pH of the soil would also be something to look closer into next year.

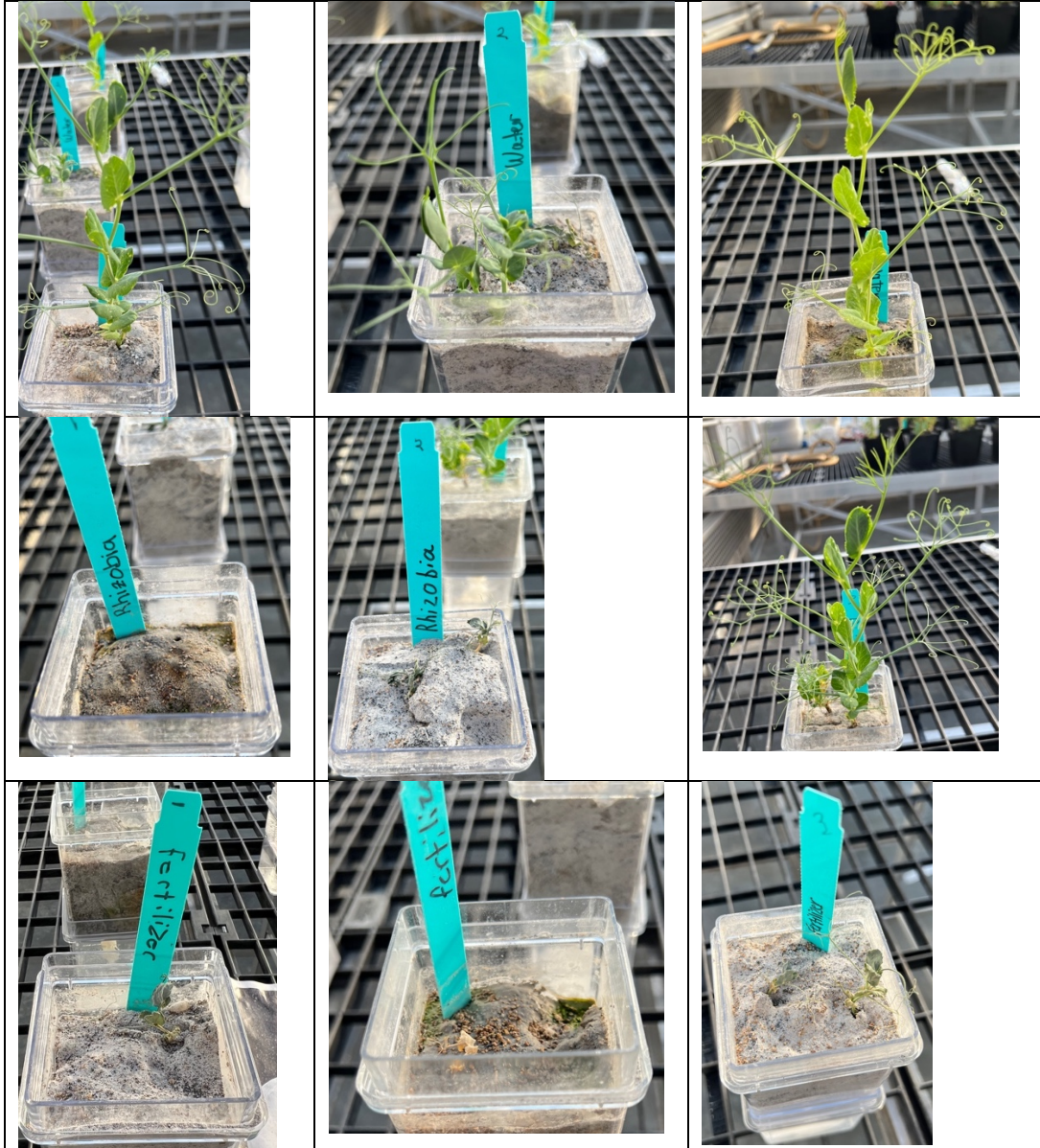
| Weekly Images |

Order goes Water, Rhizobia, Fertilizer until week 4, then proceeds as Water, Rhizobia

Week 1:



Week 4:



Week 8:

