

Plant the Moon Challenge
Brown Space Engineering – Team 2
Team Number: 13128
Undergraduate Challenge
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Introduction

With the development of the Artemis program and other long duration human spaceflight missions, it is becoming increasingly clear that implementing a renewable food source will be a critical challenge to overcome. Since the beginning of human history, agriculture and food propagation have been central to the development of our species, and as we expand to the Moon and beyond, the same will be the case. Forgetting the large variation in temperature, lack of atmospheric oxygen, minimal to no ground water, and intense incident radiation, the soil which covers the surface of the Moon is desolate and lacks the nutrients necessary to sustain any life. That being said, there are plants that can flourish in nutrient-poor environments on Earth. We aim to investigate those properties of cacti, one of Earth's most survivable plants, and determine whether they can succeed with lunar regolith as a replacement for Earth sand. We chose to use the *Opuntia* cactus because of its ability to produce fruit, flourish without significant nutrients, and propagate. Propagation is the process by which cacti and other succulents can grow from cuttings. This process can lead to higher crop success rates, which is a positive for extraterrestrial growth. Its ability to propagate also poses an interesting scientific challenge to explore.

While it is known that cacti are able to survive in arid and nutrient-poor soil, there has never been an analysis of cactus growth completed in Lunar regolith. With this experiment, we aim to begin to lay the foundation of this work. Our goal was to test if *Opuntia* cactus propagation in lunar highland simulant is affected by different soil and nutrient conditions. We compared cactus growth in a ratio of 55% Lunar simulant and soil mixture by volume to *Opuntia*'s ideal growth environment, the same volumetric ratio of sand and soil. Additionally, we tested the effectiveness of cactus fertilizer for increasing growth. We aim to show that cactus propagation in lunar soil is first possible, before attempting to tackle other goals which might

have an effect on optimizing other species' growth. In order to best prepare us for the reality of crop propagation, it is important to investigate all types of crops, both the traditional and the odd.

Theory

An interesting trait of succulents like cacti is their ability to reproduce both sexually via the creation of seeds and asexually through propagation. Propagation is the ability to asexually grow new cactus plants by planting the cutting of a mature cactus plant in ideal soil conditions. Past research has shown that a cacti population can be healthily grown and multiplied via this method (Finiti et al, 2013). Cactus plants ideally grow in a sandy loam, a type of soil composed of silt, sand, and clay. As sand and lunar regolith are similar in particulate matter size (Savio, 1989), we came to the conclusion it could serve as a replacement for sand in a loam like soil mixture. This conclusion has also been proven in the research environment, with Carrier et. al. showing that regolith can be a replacement for sand in a sandy soil mixture (Carrier, 2003).

Since cacti grow in a loam environment, and regolith has shown to be a viable replacement for sand in loam, we chose a cactus species as our test crop. However, to the best of our knowledge, no research has shown that cacti can reproduce through propagation in lunar regolith. The *Opuntia* genus has been shown to have success in a regolith environment with *Opuntia ficus-indica* (Barbary fig cactus) been seen to germinate in lunar regolith with the help of Endomycorrhizal Fungi (Konings-Dudin, 2014), which gave even more reason to pick a cactus from this genus. For this study, we chose to use the Cactaceae *Opuntia* (Prickly Pear), for its ability to grow in a low-nutrient environment, reproduce via propagation, and ability to produce an edible fruit (Savio, 1989). *Opuntia* cactus fruit has been successfully used in

medicine as anti-inflammatory agents, and even to treat Type 2 Diabetes (Ahmed 2005). In addition to producing fruit, Opuntia are extremely drought-resistant.

We designed this experiment to test the variability of success that Opuntia cacti would have when grown in a lunar regolith environment. Due to the similarities between the standard growth condition preferences of the prickly pear cactus and the conditions we fabricated for our experiment, we posit that the addition of an earth soil mix will speed up the growth process of these species. We predict the biomass of our samples to increase by different extents depending on the makeup of their growth substrate. We predict that the Opuntia will grow the best in the loam environment, as that is the natural and ideal environment. We expect the cacti in the regolith to grow, but to a lesser extent than the cacti in loam.

Research has shown that the usage of fertilizer in the growth process increases the nutritive value of these plants (Gonzalez et al., 2018). Therefore, we decided to add separate groups of cacti that would be treated with fertilizer in both the regolith simulant and regular soil conditions of our experiment design. We predicted that the overall growth of plants would be higher in the fertilizer group than in the group cacti without fertilizer.

Measurement Methods

Our experimental setup consisted of 20 plants harvested in Southern California and shipped to Providence. 10 of the pots contained our “Ideal Soil” mixture which will be composed of 55% sand and 45% soil. The others consisted of a mixture of 55% regular soil and 45% lunar simulant. We split the group of regolith mixed with soil in two: one half having no additional nutrients, and one with a cactus fertilizer. We left the remaining 5 regolith-filled and 5 soil-filled pots unaltered. The plants receiving additional nutrients were treated twice a month, with 5

milliliters of liquid fertilizer per plant. At the end of the growing period of 8 weeks, we uprooted our samples and took quantitative measurements of root growth and plant weight changes.

Parameters	Group 1 (Control 1)	Group 2 (Control 2)
Soil Composition	45% dirt, 55% coarse sand (by volume)	45% dirt, 55% coarse sand (by volume)
Crop	5 Opuntia phylloclades	5 Opuntia phylloclades
Treatment	Watered and Fertilized	Watered

Table 1: Control group parameters.

Parameters	Group 3 (Control 3)	Group 4 (Control 4)
Soil Composition	55% regolith simulant, 45% dirt (by volume)	55% regolith simulant, 45% dirt (by volume)
Crop	5 Opuntia phylloclades	5 Opuntia phylloclades
Treatment	Watered	Watered and Fertilized

Table 2: Experimental group parameters.

Overall, not many measurements were needed since the only definitive goal was to get the cactus to successfully grow in a regolith soil composition. Before planting, we we measured the initial height and weight of each sample. The heights would provide an easy visual representation of growth, and the weight difference would prove whether or not the cactus had absorbed water, proving that the cacti weren't necessarily dead, and had the potential to grow given more time even if no visual growth was present. The weights were measured using a standard scale, and the heights were measured by taking photos on a blank background with rulers along the side as a reference.

Plant growth occurred in one of Brown's Plant Environmental Center Greenhouses, which was operating under their standard lighting and temperature protocols in order to optimize plant growth. Lighting was controlled using full spectrum growth lights, and the temperature was controlled using their system of fans and heating and cooling to keep ambient temperature between 68 and 80 degrees Fahrenheit, with moderate humidity of 30 - 40 percent.

During the planting process, to perfect the ratios of regolith to earth soil, ideally mimicking the soil-to-sand ratio cacti grow in, we ran density calculations of both to solve for the volumes to use in each pot. The soil which was used was ProMix HP Mycorrhizae High Porosity soil and sand, both provided by Brown's research greenhouse. After planting, the cacti were watered to field capacity and left to begin the rooting process. Wooden stakes were added to support cacti and were left in the potted plants until they were uprooted.

For the first three weeks, the plants were watered once per week, following the guidelines we found for general cactus transplanting. However, due to the visible dehydration in the cactus, this was changed after 3 weeks. During the first three weeks of growth is where we saw the most death in our plants. From the fourth week onward, plants were watered twice per week for the remainder of the experiment. After making the change to twice per week watering, we saw much less plant death and poor health. The reason for the dehydration is not exactly known, but we imagine the cross country travel, transplantation process, and small soil volume in each pot were factors that lead to increased evaporation.

Starting on March 10th, we began fertilizing cactus plants, providing each plant in those experimental groups with 5mL of Cute Farms Succulent, Cacti, and Aloe Fertilizer. This fertilizer contained nitrogen in the form of Ammonia and Nitrate Nitrogen. Fertilizer also contained available phosphorus in the form of Phosphoric Acid, as well as potassium in the form of soluble Potash.

During the growth phase, we collected the pH of each soil pod once per ounce after they were watered. We saw no variation in soil pH throughout the entire experiment. At the end of the growth period, plants were uprooted and the soil mixture was removed from the soil. Plants were then weighed and measured using the same setup as the initial measurements.

Results

In all groups, growth was observed. Phylloclades that start out larger grew more over the course of the growth period than those that started out smaller. To correct for the randomness in initial size, percent difference in weight was used to measure growth. As seen in Table 1, group 1 showed the highest growth on average, followed by group 4, group 2, and finally group 3.

Group Number	Percent Difference (Weight)
1	21.42
2	7.090
3	3.352
4	9.260

Table 3: Average percent difference by weight within each group.

Within each group, there were multiple deaths. Each death occurred early on, but the dead cacti remained in the experiment. These dead cacti lost weight over time as they decayed and ended with less weight than they started with. Due to this, their percent differences were negative. This impacts the values shown in Table 3 (as a negative value pulls down the average even more than a zero value would). Each group, except group 3, had 1 death. Group 3 had 2 deaths.

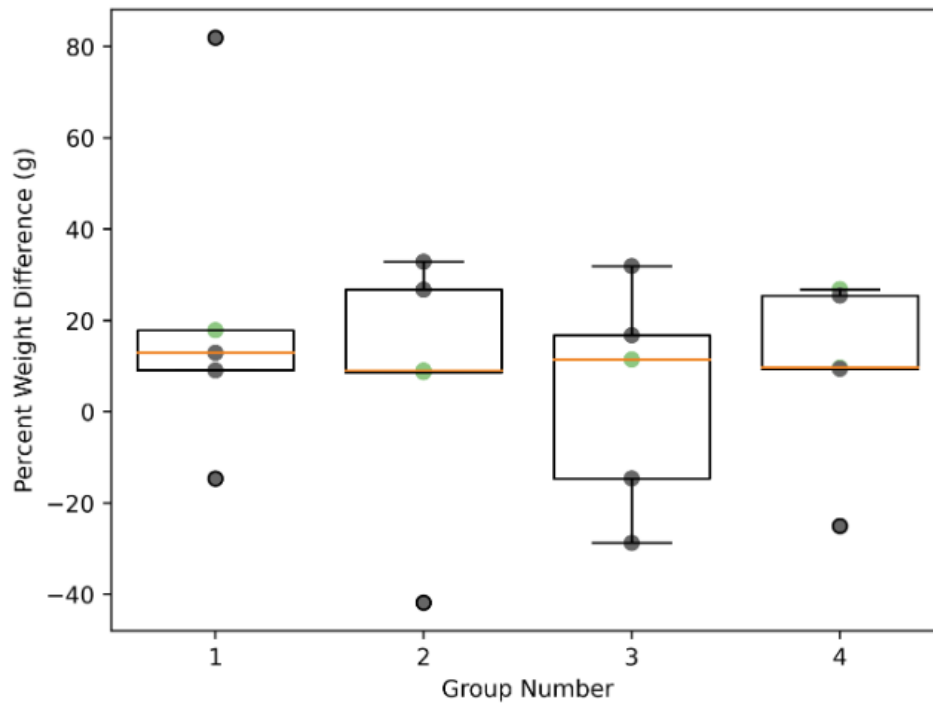


Figure 1. The distribution of percent weight difference shown for each group in a box plot. Boxes contain the middle two quartiles of data. Orange line is the median. Data points colored green represent cacti that flowered over the course of the growth period.

Figure 1 displays the full distribution of percent difference in weight. Those that died can be seen far below the other data points. Those that flowered are represented by a green data point. In both the sand-soil and regolith-soil groups, one cacti flowered while in both of the fertilized groups, two cacti flowered.

Analysis

The first variable in our experiment was lunar regolith versus soil without fertilizer. Our experiment demonstrates the viability of cacti growth in our regolith mixture without fertilizer, though a larger sample size in the future could give us more concrete information on growth conditions in soil versus lunar regolith. Both the regolith mixture and soil groups without

fertilizer had one flowering plant. While the soil group had one cacti death, the regolith group had two. Although the growth rate of plants in simulant was significantly lower than those in the soil and sand mixture, the plants in simulant still had a positive growth rate. Given the small sample size of our experiment, these results reflect comparable success across soil and regolith without fertilizer. As seen in Figure 1, the growth of plants in this regolith mixture group, Group 3, had much more variation in percentage weight difference and a slightly lower average growth than that of Group 1, the soil control. Though soil provides more reliable growth conditions, Lunar regolith is certainly viable for *Opuntia Cactus* growth.

Fertilizer proved to be an effective way to increase cacti growth. Relative to the growth rate in the simulant without fertilizer, fertilized cacti in the simulant experienced nearly 6% higher growth on average. Furthermore, an additional cactus flowered in Group 4—regolith mixture and fertilizer—and one fewer died than those in Group 3—regolith without fertilizer. Fertilized cacti appeared to grow in a regolith mixture nearly if not as effectively as they did in a soil-sand mixture. Their average percent growth was slightly higher (9.3% relative to 7.1%) than Group 2 and the same number of cacti flowered and died in both Groups 2 and 4. These results tentatively indicate that cactus fertilizer is an effective way to boost cactus growth in lunar simulants.

While our experiment demonstrates the potential for prickly pear growth in simulant, increasing the sample size could dramatically deepen experimental findings. A two-way analysis of variance (ANOVA) was used to measure the impact of our two experimental factors. The result was that neither factor (regolith and fertilizer) had a statistically significant impact on growth of the cacti. This is most likely due to the low sample size of our experiment. It is difficult to find a statistically significant result with low power.

While the solid growth rates in cacti could indicate that cacti can be effectively grown in lunar regolith, it may also indicate that the simulant we used, Exolith Lab's LHS-1, did not effectively model the conditions that might inhibit plant growth in lunar soil. While germination rates for simulant and true regolith were both nearly 100% in Paul et. al. 2022, their JSC-1A simulant controls demonstrated significantly stronger root growth and survivability than the Apollo soil counterparts (4). Just like we cannot completely separate the cacti's success from experimental constraints, cacti deaths may be partially due to them as well. The fact that cacti died across all four groups indicates the challenge of propagating phylloclades, especially in light of the extended period of time that cacti spent out of the soil while traveling from California and lack of watering in the early weeks of the project.

Conclusion

Our experiment demonstrated that *Opuntia* cuttings can be successfully propagated in a majority Lunar regolith soil environment. We saw phylloclade growth, including bud and flower development, on plants in all groups, including both experimental groups. While the plants grown in *Opuntia*'s ideal environment without fertilizer grew more than the corresponding regolith group, samples in both experimental groups showed growth from the beginning of the experiment. Additionally, plants in both the ideal soil and regolith groups showed bud and flower development, an indication of good plant health. There was plant death, but this is most likely attributable to issues with watering within the first weeks of the experiment as we saw no plant death after the watering schedule was changed.

While the initial goal of our work was achieved, there are limitations to our work. These come mostly from the small sample size and issues with watering at the beginning stage of the

growth period. Put simply, 20 plants are not enough to gather statistically significant data on plant growth as there is too much plant-to-plant variation for trends to establish themselves. Additionally, the issues that we encountered with watering within the first weeks could have had an effect on the overall results. Death, or stress that the cacti were under during the first few weeks of the growth period, could have had effects on the overall growth of the plants throughout the entire experiment. Because of a limited supply of *Opuntia phylloclades*, we were unable to replant cacti that died during this period. Other uncertainties come from aspects of our experimental design. Since our goal was a proof of concept, we did not set up systems to measure every aspect of plant growth, nor did we complete advanced plant health analysis. Our results show that cacti can survive, and even grow, in a majority lunar regolith soil environment. This discovery allows for further research questions to be investigated.

With a higher sample size, a more detailed set of analysis procedures could be performed. While Paul et. al. 2022 performs a thorough analysis of the comparison between the growth of *Arabidopsis Thaliana* in lunar regolith and simulant, their experiments could be extended by performing a similar procedure comparing simulant to soil. An experiment like this one, in comparison to Paul et. al. 2022, could help determine the efficacy of simulant as an environmental model for plant growth in *Arabidopsis Thaliana* or other plants like those of the *Opuntia* genus. While our experiment demonstrates the capacity for cacti to be grown in simulant, testing a variety of regolith to soil ratios in both mare and highland simulants could determine the minimum soil required to optimize cactus growth. While typical NDVI analysis is not effective on cacti, the health of samples could still be computed spectroscopically by performing spectral measurements in the near infrared and calculating the cacti indexes discussed in Hartfield et al., 2022.

While imperfect, the growth of cactus even in regolith and additional conditions of distress demonstrates the potential role *Opuntia* cacti could play in space exploration and discovery. We for one cannot wait to eat some prickly pear grown fresh on the Moon.

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Appendices

Data

<https://docs.google.com/spreadsheets/d/1wC9g-M1nmHUcQboe-8pvusaziOASdt8-TJrdTb7KfGU/edit#gid=1262125351>

Cactus Initial and Final Weight and Height Data:

Specimen Numb	Group Number	Initial Weight (g)	Final Weight (g)	Weight Differenc	Percent Differen	AVGs	Final Height (cm)	Flowered?
3	1	82.5	97.215	14.715	17.83636364		29.5	Yes
4	1	32.5	59.131	26.631	81.94153846		25.9	No
5	1	180.5	154.099	-26.401	-14.6265928		21	No
7	1	36	40.647	4.647	12.90833333		19	No
13	1	44	47.98	3.98	9.045454545	21.42101944	17.4	No
2	2	117	127.139	10.139	8.665811966		30.5	Yes
10	2	46	61.106	15.106	32.83913043		22	No
12	2	118	128.633	10.633	9.011016949		34.7	Yes
15	2	31.5	39.917	8.417	26.72063492		19.8	No
17	2	96.5	56.174	-40.326	-41.78860104	7.089598647	16.9	No
8	3	64	71.321	7.321	11.4390625		24.2	Yes
14	3	82.5	70.45	-12.05	-14.60606061		18	No
18	3	12	14.011	2.011	16.75833333		11.6	No
19	3	138	98.334	-39.666	-28.74347826		17.8	No
20	3	7	9.234	2.234	31.91428571	3.352428536	10.7	No
1	4	56	71.035	15.035	26.84821429		23	Yes
6	4	107.5	117.868	10.368	9.644651163		33	Yes
9	4	70	76.581	6.581	9.401428571		26	No
11	4	54.5	68.374	13.874	25.45688073		30.6	No
16	4	30	22.484	-7.516	-25.05333333	9.259568284	12.7	No

ANOVA Data:

Initial Weight (g)	Final Weight (g)	Weight Difference	Percent Difference	Fertilizer	Regolith
56	71.035	15.035	0.2684821429	1	1
117	127.139	10.139	0.08665811966	1	0
82.5	97.215	14.715	0.1783636364	0	0
32.5	59.131	26.631	0.8194153846	0	0
180.5	154.099	-26.401	-0.146265928	0	0
107.5	117.868	10.368	0.09644651163	1	1
36	40.647	4.647	0.1290833333	0	0
64	71.321	7.321	0.114390625	0	1
70	76.581	6.581	0.09401428571	1	1
46	61.106	15.106	0.3283913043	1	0
54.5	68.374	13.874	0.2545688073	1	1
118	128.633	10.633	0.09011016949	1	0
44	47.98	3.98	0.09045454545	0	0
82.5	70.45	-12.05	-0.1460606061	0	1
31.5	39.917	8.417	0.2672063492	1	0
30	22.484	-7.516	-0.2505333333	1	1
96.5	56.174	-40.326	-0.4178860104	1	0
12	14.011	2.011	0.1675833333	0	1
138	98.334	-39.666	-0.2874347826	0	1
7	9.234	2.234	0.3191428571	0	1

Photos



