

Generational adaptations of royalty purple beans to Mars regolith

Final Report

Sprayberry STEM Academy

Challenge #10746

High School Division

Plant Mars Challenge

Written by: MyHan Phan, Kasey Cross, Daniel Alford, Rowan Beasley, Jackson Smoak, Izzy Carson,
Dylan Pojol, Briana Le, Clara Rometo, Alyna Dang

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Introduction

For over a millennium, humanity has had a thirst for exploration and the unknown. Real action on the interest died down in the early 2010s because of high costs and concerns on sustainability and safety, but the yearning to reach the stars has since intensified tenfold. The revived initiative for humans to return to space is exemplified by NASA's Artemis program, which specifically looks toward exploration and colonization of the Moon and Mars. Because the overall goal of the program is to establish long-term habitation of other planets, one of the fundamental questions NASA has been how to sustainably feed space crews while they are away from orthodox sources of food. NASA hopes for the Plant Mars Challenge to become a substantial resource for findings and revelations on how to solve this problem.

Background

Beans are one of the best crops in terms of their adaptability to any environment; royalty purple beans, our crop of choice, needed only around 5 to 10 days to germinate and roughly 2 months to reach maturity. Royalty purple beans are also nitrogen-fixing, meaning that once planted and growing, they deposit their own nitrogen into the soil as they grow, and presumably, require less fertilizer. Mars regolith is completely devoid of nutrients. Plants that will grow on Mars will need to adjust to life without many nutrients. This is the cornerstone of our project; we have access to a second generation of beans which we harvested from a previous performance of this experiment. In Spring of 2022, our team participated in the Plant Mars challenge testing with various ratios of Martian regolith and Earth additive to find what ratio would perform the best. We were fortunate to have produced fruit from our experiment and were able to use the seeds produced by the beans in our current experiment. Because they had been produced by plants grown in partly Martian soil, our theory is that they will be more accustomed to a lack of nutrients and be able to grow with less intervention. A new pack of beans were purchased; the same kind of beans used last year (further referred to as our 1st generation beans). This design was developed with the goal of seeing how a seed with potential genetic modifications and adaptations to Martian conditions would compare to a regular store-bought seed.

Experimental Design

The experiment was held at the Sprayberry STEM Academy's laboratory at Sprayberry High School, located in Marietta, GA. Our team grew our plants in a controlled indoor environment to accurately record data and monitor growing conditions.

Our plan prior to the midpoint was to grow our plants in 5x5 3D-printed pots made by our technology team. Our pots were designed to have a small window made of clear acrylic installed on the side of the pot to allow us to observe root growth. The pots are made of plastic which helps retain moisture in the soil. The design was made to utilize as little material as possible, seeing as Martian soil is hydrophobic and consistent watering on Mars could be difficult. Unfortunately, our pots ended up being too large to hold the amount of soil we had remaining as our team did not properly calculate the amount of soil to adequately fill our pots. Terracotta pots were used as our alternative for the entirety of the experiment in place of our 3D-printed pots.

The plants were not enclosed in the fume hood. Although our experiment was not held in a completely isolated environment, the fume hood provided walls that allowed us to easily set up our lighting system. For our lighting system, the VIPARSPECTRA P1000 (12'' x 11.2'' x 3'') was used as the main light source.

This system was modified to adjust the lighting at certain times of the day. This way, our planet receives a light cycle similar to the daylight cycle on Mars. Using a Raspberry Pi, the tech team wrote code that could simulate the day-night cycle autonomously by telling the light when to turn on and off.

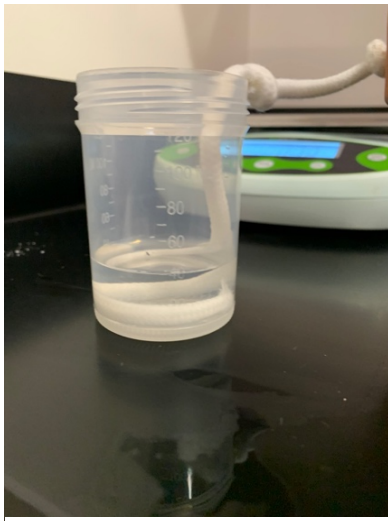


Figure 1

The team planned to be able to increase and decrease the brightness of the light to be more accurate, but due to insufficient equipment, the code was not able to be implemented to the light at all. Our team did not have the resources to hardwire the light and the Pi together due to insufficient funding to afford a power converter due to the input/output ports on the Pi being different than the input/output ports on the light. For the experiment that we ran, we decided that our day-night cycle would be based off the school day. Sprayberry's school day lasts for 8 hours and 10 minutes, from 8:20 AM – 3:30 PM. We had team members, or our team sponsor take turns manually turning on and off the light at the start or end of each day.

One way we tested pH is to run water through the plant and test the water after it has been through the soil. Plants tend to grow best in soil with a pH of 6 to 7. This would have been a problem, as the pH of the Mars ranged from 8 to 9, but we figured out that the small gap in pH could easily be counteracted with additives and fertilizers, while simultaneously allowing the introduction of nutrients lacking in the soil based off our last project. The only way to do this was to pour enough water into the soil so that it flows out of the bottom of the pot. This would essentially drown a plant. To avoid this, we wanted to operate our data collection with a SONKIR soil pH meter stick with a built-in sensor to measure soil moisture, pH, and light intensity of the plant's soil and environment.

Most of our materials and experimental processes were drawn from the successes of last year's experimental design. We continued to have 12 pots in total; 6 as our 1st generation beans (new seeds straight from the packet/control) and 6 as our 2nd generation plants (seeds collected from last year's growth grown in a 70/30 soil ratio).

We implemented a water-wicking system that we also used for the previous experiment, which produced successful germination and sprouting, and provided adequate for the early adolescent stages of growth. As well as a 70/30 mixture of Martian regolith and soil additives such as garden soil and fertilizer. We continued this method based on our observations of our last experiment and because of the minimal maintenance it required. In our previous experiment, we found that the Martian soil was hydrophobic and overhead water wasn't efficient in providing water to the roots; the soil would repel the water and the water would not be absorbed into the lower levels. In our current wicking system, each pot is connected to a piece of water-wicking string made of nylon as shown in *Figure 1*. Because the water wicking system is self-acting via capillary action, we only had to regularly supply the water reservoirs with water throughout the semester. Each pot had its own separate beaker, and the amount of water taken from each beaker was recorded. At the beginning of each week, we recorded the amount of water absorbed by the plants (including how much was absorbed over the weekend) and refilled the amount of water in each beaker accordingly to reach a uniform amount of 100 ml, shown in *Figure 2*.



Figure 2

Additionally, we measured our plants' growth through mass. This is mainly because we have found that if we measured by height, it may become inconsistent as some people might straighten out the plant when measuring, some will consider the base of the plant differently compared to others, etc. Since we are a big team and would prefer operations to be as consistent as possible, we plan to use a scale as the primary way to measure growth as fewer technical issues could occur. We plan to remedy these possible inconsistencies in the future by utilizing time-lapse footage and digital measuring software.

Hypothesis

The 2nd generation beans will experience more plant growth due to their adaptation to Martian soil. The second generation will be less reliant on additives because they are acclimated to the Martian soil. Prior to our experiment, we did an informal growth test for both generation 1 and generation 2 beans in a Ziplock bag with a damp paper towel. After waiting a couple of days for germination, we saw a noticeable difference in the growth of generation 1 compared to generation 2 beans. The 2nd generation beans germinated noticeably faster than the 1st generation bean. Since the test was purely out of curiosity and not a formal experiment, we cannot fully trust the results we saw. Furthermore, we only grew one of each generation since we only had a limited number of the 2nd generation seeds. Our goal for this experiment is to produce such results that can be used in tandem with our own previous findings to solidify our theory on the increased performance of the 2nd generation plants and further enhance our knowledge on generational crop development.

Variables

Control

- Soil Ratio – All pots contain 70% Martian soil and 30% soil additives

Independent

- Generation 1 royalty purple seeds (new beans from packet)
- Generation 2 royalty purple seeds (offspring beans harvested from spring semester's experiment)
- Lighting system (controlled on a timed schedule)
- Water-Wicking system

Dependent

- Weight of plant
- Fruit/ budding of plant
- Water – through our water-wicking system, the plants acquire water based on their own needs, preventing them from being overwatered. The amount absorbed by our plants will be measured by subtracting the initial amount of water in each beaker from the amount of water left at the next data collection period.
- Leaf condition

Procedures

Plant measurement

Pot #	Mass of Pot (g). FOR TEMPORARY POTS	Mass of Soil Mixture – Earth, Mars, Fertilizer (g) FOR TEMPORARY POTS	Mass of Beaker + Wick (dried and empty) (g)
1	266.65	182.69	18.61
2	266.29	197.05	18.69
3	265.90	192.37	19.70
4	266.41	199.44	19.53
5	266.25	207.15	19.38
6	266.36	189.49	19.16
7	266.12	190.86	19.56
8	266.22	204.07	19.09
9	266.87	212.57	19.09
10	266.72	193.67	18.69
11	267.10	200.32	18.75
12	266.41	195.71	19.50

Figure 3

We measured our plants and recorded observations every other day for water intake and plant weight. Although we weren't consistent with our initial measurement days due to scheduling conflicts, we were still able to obtain measurements every 2-3 days a week to monitor signs of growth. Our team has a rotational schedule among our members to take measurements during school days.

Before we started taking measurements, we made sure to take the mass of each individual beaker and wick (as one measurement), pot, amount of soil mixture, and initial plant mass (bean stage). The pots, beaker, and wick mass will be subtracted from the total weight recorded of each pot to leave the plant/soil mass where we can see the change of the mass.

At the end of our measurement period, we will use the

individual masses for calculations. All data is concluded in *Figure 3*.

We start the measuring process by removing a plant from the fume hood and bringing it to our scale station. To start off, we first measure the water intake. Then we remove the wicking string from inside the beaker. Next, we pour the remaining water from the beaker into a graduated cylinder, to measure the amount of milliliters that wasn't absorbed by the plant. We then subtract the amount by 100 (the initial amount of water refreshed in each beaker every measurement session) and record that in our excel sheet. From there, we take the empty beaker, wick, plant, and weigh it on the scale, shown in *Figure 4*. The reason we include the wick and beaker with the plant is because the wick is inside the soil and taking the wick out before and after every measurement period would be inefficient and had a chance in stressing out the roots of the plants. The process is repeated for all 12 pots. These measurements were used to assess the change in weight since the initial weight was measured, because not every pot was identical in initial weight.



Figure 4

Results

We found that the germination rates of the 2nd generation beans were much faster compared to the 1st generation beans. For initial germination, we wrapped the beans inside wet paper towels and enclosed them inside plastic Ziplock bags for 1 week to help trigger the germination process. The 1st generation had development of roots and germinated first. Soon after, the 2nd began to germinate and showed better sign of growth such as increased number of roots, shown in *Figure 6* (generation 1) and *Figure 5* (generation 2).



Figure 6



Figure 5

In the early stages of growth after potting the sprout, we found that the 2nd generation plants had adapted to the regolith significantly better than the 1st generation beans. The 2nd generation plants had an increase in overall height and began to develop mature leaves, shedding their seed coat in the 1st week of potting, shown in *Figure 7*.

At the time of potting, we didn't have our 3D-modeled pots completely printed so we had to rely on terracotta pots that eventually became permanent. As time passed, the growth rate of the 2nd generation beans gradually declined. The 1st generation continued growing until it matched the maturity of the 2nd generation, but the growth rate eventually also flattened and started to decrease. We can see in the graph in *Figure 8*, the plants reached their peak of growth on the 10th and the mass of all the plants begin to decline after. Although the mass of the plant decreases with each measurement day, we can observe that leaves, buds, and flowers are still moving towards maturity, meaning that growth is still happening. Our data shows for both generations of plants, that there is a steady increase in mass until the 7th Day. We related its high period of growth to the germination period mentioned on the beans seed package of a suggested 14 days. About 6 days from our first measurement day (Day 4, 10/18/22), the beans were germinating in the plastic bags and not in their permanent pots. Although we didn't take measurements of mass until the 3rd day after planting due to the weekend, we can see that the increase in mass from both the 1st and 2nd generation occurred until the 7th day (mark of the full 14 days from initial germination) After the 7th day, the rate of growth starts to decline until the 10th day and reach a steady influx of subtle increases and decreases. Even so, we can see that there is a slow decline of mass overall as the growth period continues until the 3rd day. Along with the decreasing mass, the condition of the plants began to deteriorate after the 3rd day shown in *Figures 8-10*.



Figure 7

Although we didn't take measurements of mass until the 3rd day after planting due to the weekend, we can see that the increase in mass from both the 1st and 2nd generation occurred until the 7th day (mark of the full 14 days from initial germination) After the 7th day, the rate of growth starts to decline until the 10th day and reach a steady influx of subtle increases and decreases. Even so, we can see that there is a slow decline of mass overall as the growth period continues until the 3rd day. Along with the decreasing mass, the condition of the plants began to deteriorate after the 3rd day shown in *Figures 8-10*.

In *Figures 8-10* shown below, we used the graphs to compare each of the pots to itself and measured the change of mass from the initial weight of each of the plants in their permanent pots (Day 4)

First Generation Graph

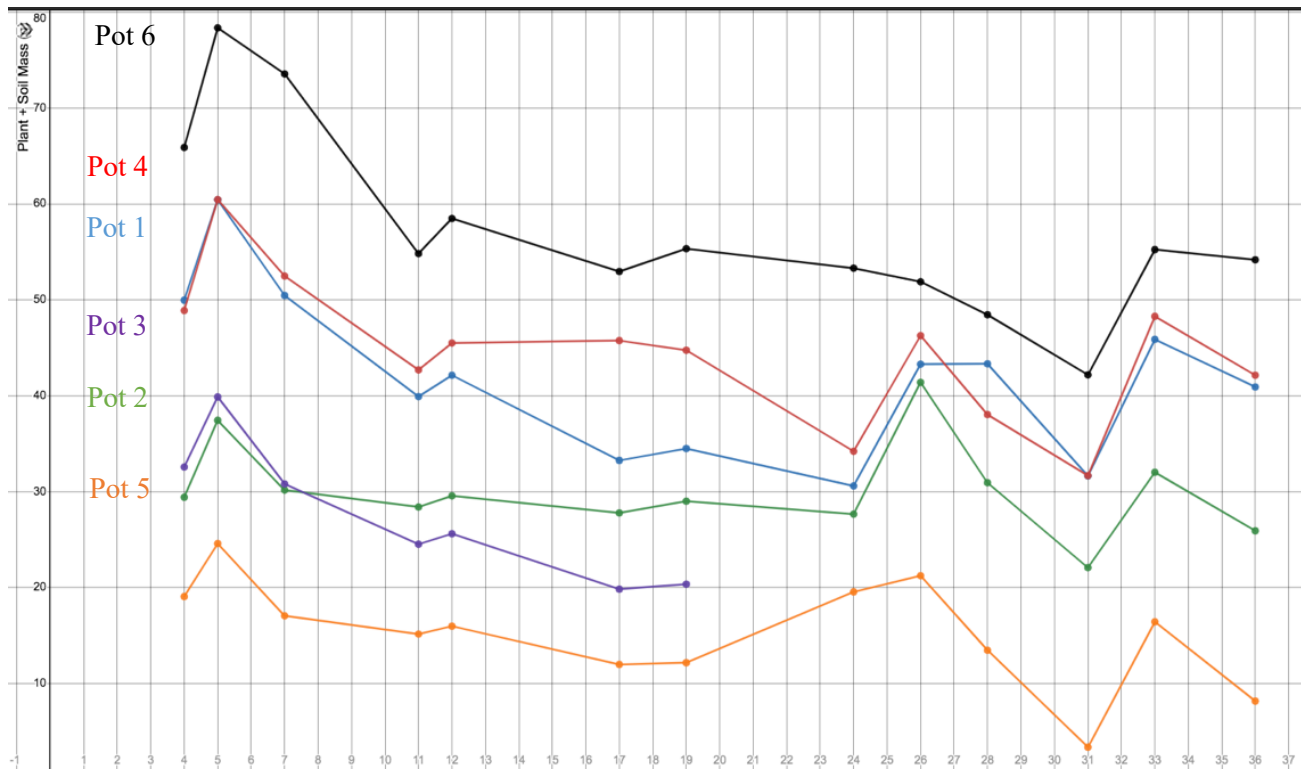


Figure 8

Second Generation Graph

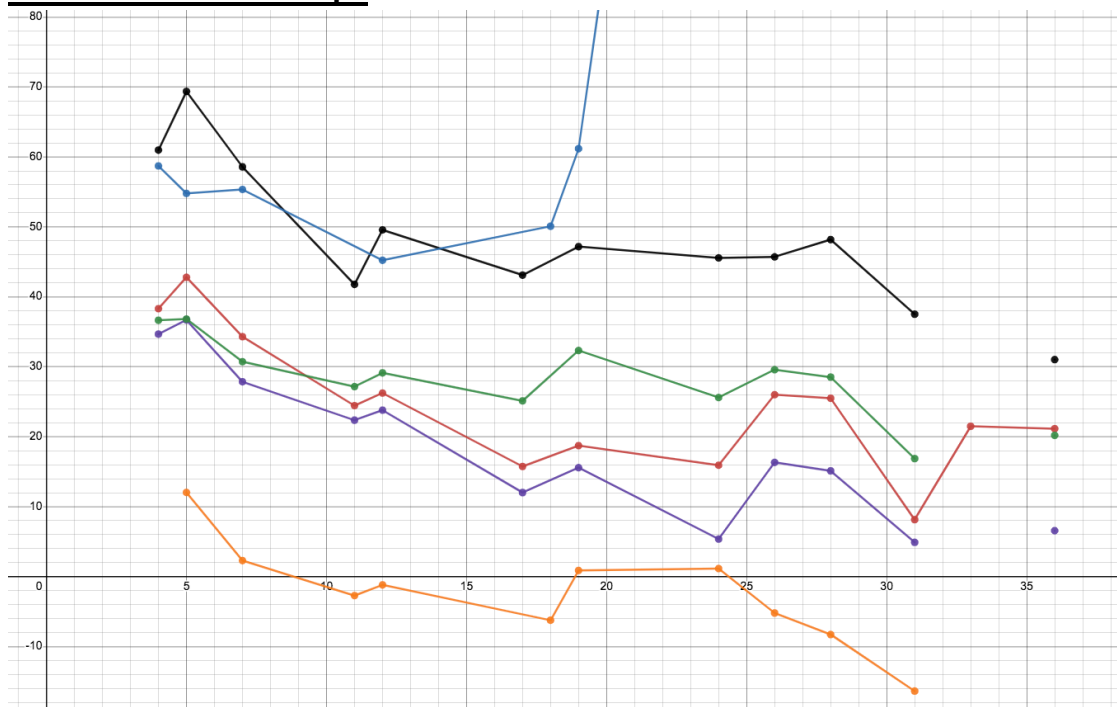


Figure 9

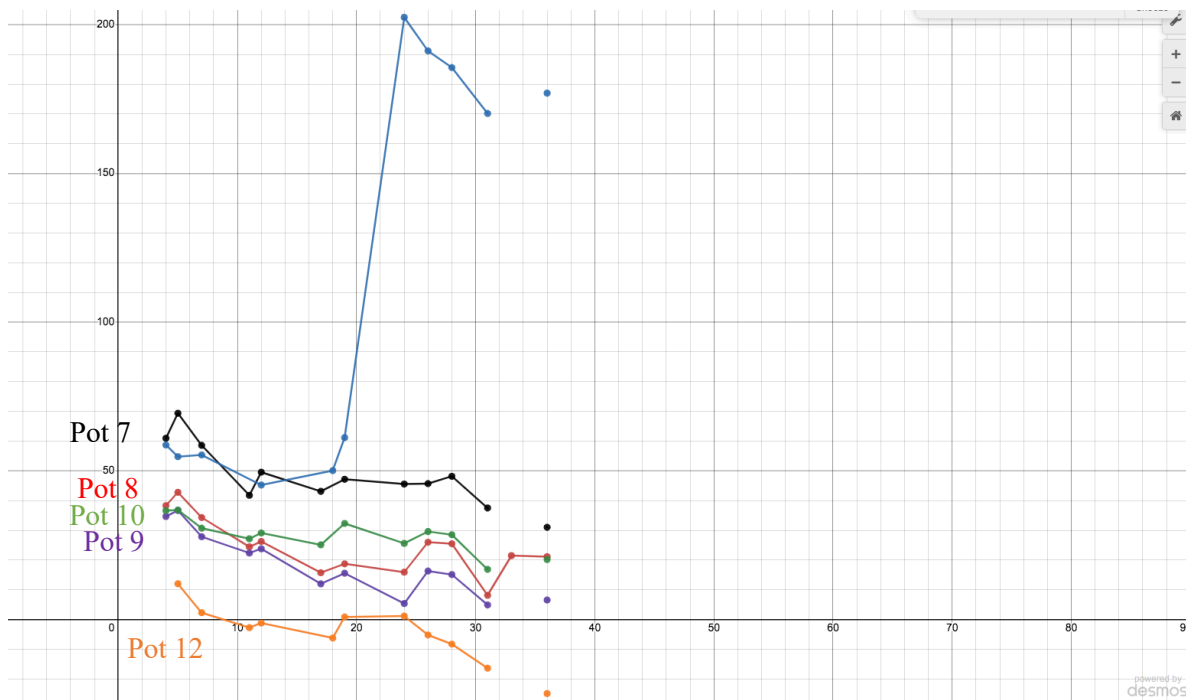


Figure 10

In the 1st generation graph, *Figure 8*, we can see that the decrease in mass ranges from about 10-20 grams. In the 2nd generation graph, *Figure 9*, the difference is much greater. The drop of mass ranges from 20 – 30 grams, except for pot 11, further explained on page 17. Although this may seem like an issue, we learned that the decreasing mass is a positive sign of growth. This is because soil itself is composed of many elements such as nitrogen and phosphorus and as a plant grows it needs to absorb all the nutrients, taking away mass from the soil itself. While they both had the same mixture, we can see the similar differences by generation. Although the 1st generation plants took a while to grow accustomed to the soil, they were eventually able to catch up to the growth stages of the 2nd generation plants. In contrast, the 2nd generation easily grew habituated to the Martian regolith and surprisingly, grew more with the fertilizer and soil nutrients. What we can tell based on our data is that the 2nd generation plants performed better than the 1st generation plants based on the plant's mass. This data demonstrates the strength of our hypothesis that the 2nd generation plants were able to adapt and germinate better than the 1st generation plants. The higher difference of mass loss demonstrated the inverse relationship between the mass and height of the plants and further supports our theory to generational crop development.

Below are analysis of growth through *Figures 11- 14*.



Figure 11

10/11/22 - The 2nd generation plants have shown rapid results of growth while the 1st generation are showing little to no signs of additional growth past germinating in the plastic bag.



Figure 12

10/18/22 - We can see that the 1st generation have adapted to the Martian regolith and have started to enter the sprouting stage. On the other hand, the majority of the 2nd generation have continued to significantly mature compared to 10/11/22 shown above in *Figure 12*. The stems have increased in height and their leaves continue to broadly grow with a deep green hue. They are thriving on the wicking system.



Figure 13

10/28/22 - Both generations are showing significant growth in height, leaf quality, and hue of the leaves. The 1st gen plants are starting to catch up with the condition of the 2nd gen



Figure 14

11/4/22 - At this point, the growth of the 2nd gen has declined, we estimate that it's due to the limited soil and room of the pots that the growth has slowed. The 1st gen is still showing signs of growth, but it has also began to slowly decline. Both generations have reached a plateau in their growth. We estimate that it could be that the roots have outgrown the soil/ pot, the wicking system does not satisfy the current water requirements of the plants, or it is approaching the period to give our plants additional nutrition. One of the plants, pot 11, served as our trial plant to weigh the plant at the midpoint of the grow period. We removed the plant from the pot and the remaining soil attached to the roots to measure the plant by itself to compare it to its beginning mass. Unfortunately, we were unaware of the detrimental impacts of what stress can do to our plants. This has caused Pot 11 of the 2nd generation to die around the 3/4s into the midpoint. We took this trail to recalculate our plans of weighing the plants and continued without the midpoint weight checkup.

Limitations

pH

Our team planned to use the SONKIR soil pH meter stick with a built-in sensor to measure soil moisture, pH, and light intensity of the plant's soil and environment. Unfortunately, the pH and soil moisture readings that were produced by this proved to be inaccurate. We would not be able to get reliable readings unless we used the method that would drown our plant. For that reason, we were unable to provide consistent and reliable pH readings. Sadly, we were not able to test the nutrients either due to the expense of buying phosphorus, nitrogen, and potassium nutrient tests in bulk.

3D-printing

As aforementioned, our technology team designed a 3D model of a pot that we would print and use for our experiment. The first version of this file was far too large and lacked the structure to hold the thin acrylic sheet that would be added as a window to the pot. The second version of the file solved both

issues, but the walls proved to be far too thick and wasteful of our filament. The final design was the optimal pot that solved all of the major issues listed above. However, given their size, each pot took 18 hours to print, meaning that to print all 12 pots we would need nearly two weeks of time allotted just for printing. We did produce the full amount needed before the deadline, but due to a calculation error, we didn't have enough soil to fill the pots, meaning that we could not utilize them for this semester's project. This did not mean that they were completely useless, as they did provide valuable information – each iteration showed us how we could fix each flaw in design.

Conclusion

While we did notice that the 2nd generation grew better compared to the 1st generation due to unexpected events and lack of equipment, we were unable to keep the plants in healthy condition by the end of the growing period. Initially, the 2nd generation had noticeably better and faster growth in comparison to the 1st generation; we observed more root development and height compared to the 1st generation. This can be represented by the higher number of 2nd generation seeds that germinated within the first week, in juxtaposition with the lower number of 1st generation seeds that did. This gap in growth continued to be shown with the 2nd generation developing leaves before the 1st generation. The growth could also be seen through the water intake of each group, with the 2nd generation having consistently higher intakes of water in comparison to the 1st. Greater height was also observed in the 2nd generation plants. However, the gap in growth between each generation gradually lessened and it became more visually difficult to notice which generation was which. Eventually, due to the hydrophobic nature of the Martian soil, it caused our plants to slowly go back and forth between almost dying and going back to good health. Because of this, it is hard to accurately evaluate whether the gap seen at the end of the experiment was due to the generational differences between the seeds naturally causing growth to plateau or due to external factors causing both generations to suffer from it. From these results as well as further research that we have done during the experiment, we can conclude that our focus should switch toward measuring height, weight, leaf development, and fruit development.

Implications

The results of this experiment show an opportunity for generational crop use in creating sustainable methods of horticulture for Mars exploration. Breeding the second-generation Martian plants to produce a 3rd generation crop and then ultimately a 4th, can create a version of this plant that has been better equipped to thrive in the foreign Martian environment. If this process continues, the beans will become even more accessible and cost efficient to produce. Less fertilizer can be transported from Earth to Mars, as well as less Earth soil. In addition to reducing mass and resources, storage on a vessel is of concern, and custom 3D printed pots would allow that volume to be maximized. Our bean's generational acclimation to Martian soil shows a clear path for future crops, even of different varieties. NASA's Science Advisory Board is currently conducting research on the same topic, generational crop development. The results and process that we have received directly correlate to the growing research contributing to developing sustainable sustenance on Mars. This experiment serves as a foundation for producing a sustainable food source on Mars, without the need for transporting excessive amounts of supplies from Earth. Large-scale farming practices such as crop rotation might even be viable strategies for adding nutrients to Martian soil, and conserving usable soil, while keeping the agricultural land intensive to produce the highest crop output. This opportunity has tremendous potential to develop to lead the future generations to sustainable Mars exploration and horticulture. The Sprayberry STEM Academy will take this initiative to continue this experiment to pass it down to future generations of student scientists who will take part in the experiment for many years to come. We plan to further develop our plants to multiple generations and enhance our research along with NASA.