Sprayberry Plant Mars Fall 2023 Final Report:

Utilization of 3D-printed nutrient matrices to supplement plant growth in nutrient-devoid Martian regolith

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Background

The Sprayberry STEM Mars Explorers previously conducted experiments measuring the generational adaptations of royalty purple beans grown in Martian regolith. We experienced immense success in the development of an executable and sustainable method for cultivation and growth, but these previous designs were largely based on using large amounts of Earth soil to supplement the complete absence of nutrients from Martian regolith. In developing this year's experiment, the team endeavored to create a new, innovative design that reduces the reliance on Earth soil and instead focuses on compact nutrient reservoirs that can be embedded within the Martian regolith without vastly altering the actual soil's composition.

In consideration of this, the new experimental design centered around the implementation of 3D-printed nutrient matrices. Each matrix consisted of a lattice of hollow hexagonal structures that will ease the movement of both water and the plant roots through the soil, which were developed to prevent the dense and hydrophobic nature of the soil from being a major detriment to the plants' growth. Each matrix also contained a nutrient gel consisting of distilled water, gelatin, nitrogenous fertilizer, and crushed eggshells. Through housing nutrients within the matrix, the intent was to increase the absorption of vital ingredients for plant growth, and thus compensate for the complete absence of nutrients from the Martian regolith.

Hypothesis

Through the implementation of nutrient matrices, the black bamboo grown in the initially-nutrient-depleted Martian regolith will exhibit better growth as measured by height compared the control group.

Experimentation

Design

Phyllostachys nigra, or black bamboo, was chosen as our first subject crop because of its high growth potential. Bamboo is known for being an especially resilient plant, with black bamboo having a particular proclivity to clay – a type of soil whose crust-forming property the Martian regolith very closely mirrors, but in which most other plants struggle to survive (Flurry, 2020). Black bamboo also grows in soil with a pH ranging from about 5.5 to 7.5, with the pH of

Martian regolith falling at around 6 (Puisis, 2022). Black bamboo's versatility as both a nutritious food source and a strong building material also perfectly fits NASA's current focus on developing in-situ resource utilization.

The bamboo was planted in 3D-printed pots designed by the Sprayberry STEM Mars Explorers' technology team. We chose to create pots out of synthetic material to help the soil retain all of its moisture, as the terracotta pots used in previous years absorbed the water instead. Previous years of the Plant Mars Challenge also revealed that plastic pots prevented mold growth. The lack of water absorption secondarily served the purpose of preventing mold build-up on the pots. Utilizing the 3D-printed pots also allowed us to plan to drill a hole in the sides of the pots to create a route for a water-wicking irrigation system – a system in which each pot is connected to its own water reservoir via a piece of nylon water-wicking string, allowing the soil to autonomously absorb water through capillary action and consistently remain moist. However, this watering regiment was ultimately removed from the design in favor of manual watering via spray bottle. Not only would this allow us to measure the amount of water being given to the plants, but it also eliminated the risk of interrupting the structure of the nutrient matrices we would embed into the soil.

Additionally, it was a primary goal to mend other soil-related problems that the team had encountered in previous iterations of the experiment. The salt-rich composition of Martian regolith had continued to hinder plant growth regardless of the regolith's nutritional makeup, leading us to conclude that the issue needed to be addressed separately using a non-nutritional additive. Gypsum was chosen to be this additive; introducing Ca_2+ ions from the gypsum would displace any Na+ ions in the soil to be flushed out once water was run through it (Sawyer, 2003). This would help combat the salt saturation of the Martian regolith and make for a more fertile environment for the plants. Additionally, the limited amount of Martian regolith available would not provide enough depth to grow our desired quantity of crops. Sand was chosen to increase the total mass of our soil mixture because it would not supply any additional nutrients that would become a confounding variable in our experiment.

16 pots of bamboo were used in total. Originally, the pots were to be separated into groups of four (Groups A, B, C, and D) according to the following criteria:

> Group A was to consist of four pots filled with a 1:1 ratio of Martian regolith and sand/gypsum that would not possess nutrient matrices.
>
> Group B was to consist of four pots filled with a 1:1 ratio of Martian regolith and sand/gypsum that would possess nutrient matrices with a nutrient gel made in a 1:4 ratio of fertilizer to water.
>
> Group C was to consist of four pots with the exact same conditions as Group B, except possessing nutrient matrices with a nutrient gel made in a 1:8 ratio of fertilizer to water.
>
> Group D was to consist of four pots filled entirely with Earth soil that would not possess nutrient matrices to serve as the benchmark to which we would compare the growth of all plants grown in the experiment.

Using these groups, however, would not isolate our experiment from the effects of the nutrient matrices. The two groups possessing matrices would complicate our analysis by integrating different concentrations of the nutrient gel, which would require us to evaluate the impact of having larger or smaller amounts of nutrients on the plants alongside the actual presence of the matrix structures. Furthermore, the group of plants grown in Earth soil would not have served as a good comparison point. This group would only be useful in measuring the effectiveness of nutrient matrices in replicating observable Earth-like growth, whereas the purpose of our design was to stimulate plant growth in the Mars regolith without using nutrient-rich Earth soil, regardless of whether the growth would be comparable to that experienced on Earth or not.

As a result, we decided to divide the pots into two groups of eight instead – the Blue and Green Groups:

> The Blue Group pots were filled with a 1:1 ratio of Martian regolith and sand/gypsum that did not possess nutrient matrices.
>
> The Green Group pots were filled with a 1:1 ratio of Martian regolith and sand/gypsum that possessed nutrient matrices.

Uniformizing all growth conditions other than the presence of the nutrient matrices ensured that we could focus on the effects of the matrices themselves on plant growth and limit the effect of separate independent and confounding variables on our experiment.

Each nutrient matrix measured 10.16 x 10.16 cm, with each hexagonal structure at 2.54 cm wide, 1.27 cm tall, and 0.32 cm thick. Inspired by the structure of honeycombs, the hexagonal composition offers an efficient use of space to store the nutrient gel while using the least amount of 3D printer filament (*Figure 1*). The hexagons around the outer edges of the nutrient matrix were constructed to be thicker by an additional 1.27 cm, and each individual hexagonal layer was offset by 1.27 cm in a back-and-forth pattern, allowing for better structural integrity while providing maximum space for the plants to grow (*Figure 2*). It is the stackable nature of our structure that allows for effective compartmentalization and transportation of the soil.

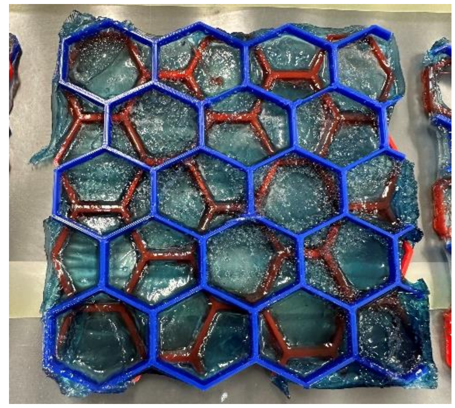

Figure 1

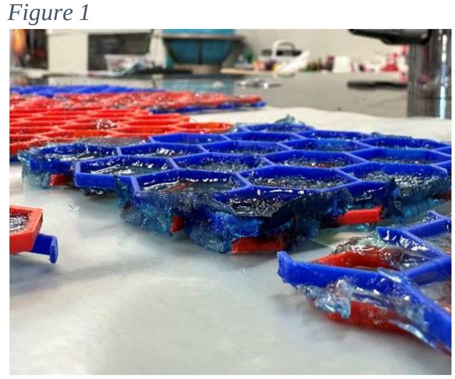

Figure 2

Its uniformity also makes it convenient to mass-produce, and the use of 3D-filament allows for a cheaper and lighter material that is easily accessible, compared to metals that would increase the resource cost. It is important to note that equally affordable, biodegradable filament options are available to account for sustainable alternatives, but we did not use them in this experiment due to time restraints when constructing our pots. Furthermore, a variety of plants require structural support to aid them in providing stability for the plants' shoots, especially in sandy soils (such as Martian regolith) where a strong hold is integral to a plant's longevity (Ryan et. al, 2016). Biodegradable materials would eventually decay into the soil and no longer provide the support needed; thus, the use of regular 3D-filament was decided to be the best choice.

The nutrient gel was made of gelatin, distilled water, and fertilizer tea. Each batch of the fertilizer tea consisted of 590 mL of distilled water and 100 g of Fitleaf Plant Energy Boost Fertilizer, with the resulting substance being strained twice to remove bulk particles from the final product. Due to its water-based composition, the gel is easily freezable allowing for ease of transportation in comparison to the tons of weight presented in moving fertilizer. Since most plants are unable to grow in freezing climates, there is an assumption that growing on Mars will require an additional heat source for plants to survive. Our nutrient gel will not only naturally stay solid within Mars' extreme temperatures but will also melt into the soil once planted with the crops. Once infused in the soil, plants can absorb the customized nutrient requirements created from the gel to get their exact needs. Since the nutrient matrix will be placed between layers of soil where a seed's roots will sprout (instead of the top layer of soil like most other fertilization techniques), our hope is that the germination process will be smoother for the early stages of plant growth when crops are most fragile.

We planned to monitor the plants' growth using the Dsoon Professional Time Lapse Trail Camera. The camera would take periodic images of the plants, whose height would be measured via a uniformly checkered backboard (each square measuring 5 x 5 cm). These measurements would also be verified by hand with a ruler.

The light source for all the plants was the VIPARSPECTRA P1000, which offered consistent heat distribution and a full-spectrum light which mimics the effects of natural sunlight. The light was turned on for approximately 9 hours per day, to recreate the effects of the natural sun during winter.

For the duration of the experiment, we used a simple space heater to keep the experiment room at 80° F (~27° C). This was done to ensure that the plants were kept at a temperature that would enable their growth most effectively, as well as providing a stable environment for germination. Another appliance used in a similar fashion was a humidifier filled exclusively with distilled water. The theory behind this was that it would provide extra moisture for the plants to absorb as well as keeping the air humid to suit the bamboo's optimal growing environment. Additionally, the distilled water would prevent any excess materials from entering the air or the plants via humidity.

To gather our data, we created online forms with QR codes where members could choose which days throughout the growth period they were available to go into the lab and record growth data. These included a Google Form for water intake (mL) and height (cm) which was to be measured every Tuesday and Friday. This data would then automatically be transferred onto a Google Sheet to simplify the process of organizing and illustrating any trends which would be seen. Similar methods were also used to record moisture (on a qualitative scale of dry, to moderate, to moist) and the general physical conditions of the plants themselves; along with water intake, these were not variables in our experiment, but were monitored as important factors in maintaining our plants' health and eliminating confounding variables from interfering with the experiment.

Growth setup

Our full growth setup can be seen in *Figure 3*.

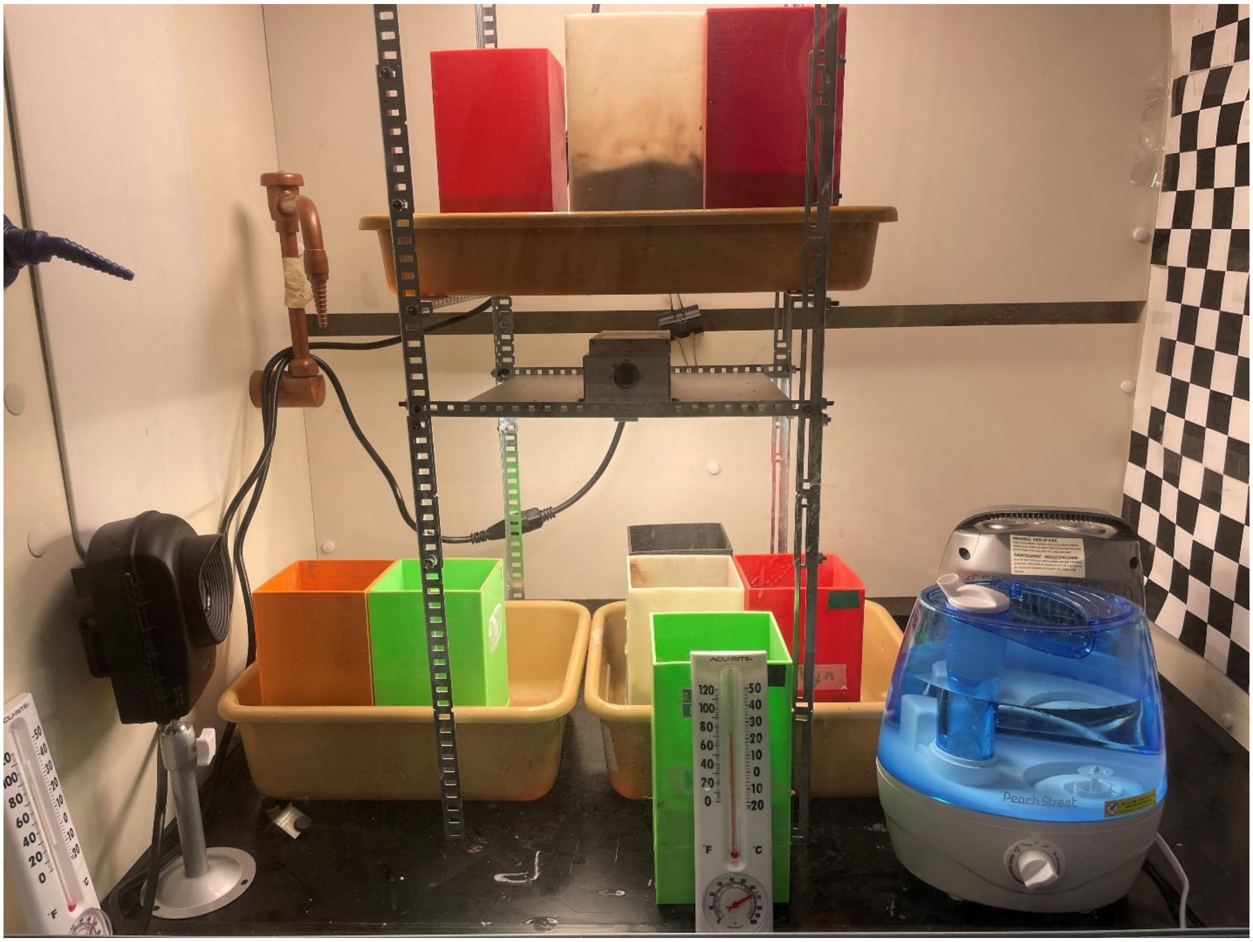

Figure 3

Variables

Independent: implementation of a nutrient matrix

Dependent: height (cm) of black bamboo, measured both from images taken against checkered backboard and by hand using a ruler

Constants: scheduled full-spectrum lighting, consistent heat distribution, consistent humidity regulation

Procedure

To create the nutrient gel, a measured initial quantity of water was heated to 90° C, then measured a second time after heating to minimize variability due to evaporation. Once the adjusted value was collected, the fertilizer was added in a teabag at a fertilizer-to-water ratio of roughly 1:50, where it was left to mix for 24 hours. It was then filtered, collected, and remeasured for the last step. The resulting mixture was heated to 75° C and the gelatin was mixed in at a gelatin-to-mixture ratio of 1:35, after which the final substance was allowed to sit until the powder was fully dissolved and distributed. The gel was kept at a minimum temperature of 50° C to avoid solidification within the container so that it could then be poured into any mold or receptacle – in this case, our nutrient matrices. The empty nutrient matrices were placed in a large container before the nutrient gel mixture was poured into them. These were cooled at room temperature for roughly 20-30 minutes to allow them to fully solidify. These complete nutrient matrices were then placed into the pots, where the gel would naturally melt into the soil.

Upon receiving the bamboo seeds, the team immediately set upon testing their viability. We put some seeds into a beaker of water and set them aside for about 20 minutes in a common “float test”; any seeds that sunk to the bottom of the beaker during this time were still viable, and any that floated were discarded because they would not sprout. To sprout them, we placed the seeds in a moist paper towel and sealed them in a plastic bag, similar to our previous attempts at the competition. However, these seeds which had, to our knowledge, been confirmed to be viable, showed no signs of sprouting even after numerous weeks.

Upon researching deeper into the seeds, we found that bamboo seeds sometimes also required a breaking of their dormancy cycle (Sparks, 2022). Because bamboo seeds are dropped very rarely, seeds that are not planted immediately after being harvested will harden to preserve their viability. To break the cycle, we placed the seeds in the refrigerator for one week to simulate “winter” before taking them out and leaving them to warm in open air, simulating “spring.” To ensure we did not simply have a defective patch of seeds, we also bought new black bamboo seeds from a different source, a select number of which was subjected to the aforementioned processes to test and cultivate their viability. Afterwards, we re-germinated both the refrigerated seeds and the newly bought seeds for another week, only to be left with no signs of sprouting once again.

An attempt was still made to grow the seeds in our Martian regolith Blue and Green Groups, following the lighting, heating, and humidifying regimens as outlined in the experimental design, as well as a watering regimen. Unfortunately, no crops were able to grow. After we had concluded that we would not be able to record any growth data, we found that the soil had turned hard and concrete-like after being mixed with water. We had already utilized all the allocated Martian regolith in creating our Blue and Green Groups, meaning that for any following growing efforts, all the soil would have to be chiseled out and aerated after already having absorbed the nutrients from the nutrient matrices.

Despite the limited time we had left in the competition, we were confident that our experimental design could produce real results if only our seeds were viable for growth. With no leftover Mars soil which had not already absorbed our specific nutrient mixture, a new gel having a different nutritional composition could not be put into the soil without compromising the validity of our experiment or risking nutrient oversaturation. Through further research, however, we found that the specific combination of nutrients in the gel we crafted for the bamboo seeds was also suitable to oats (Kaiser & Piotrowski, 2023). So, we used the remainder of the growth period to germinate *Avena sativa*, or naked oats, whose nutritional requirements closely matched the composition of our nutrient gel. We used the same germination method of placing the seeds in warm, damp paper towels and sealing them inside plastic bags to wait for them to sprout. Two days of this process initially produced promising results; the seeds had sprouted very well and were viable for planting (*Figure 4*). However, since we were not at school for the county-

Figure 4

scheduled Thanksgiving break, our seeds were only briefly able to be examined by our team and left too long inside the damp towels, developing a mold infestation before we had the chance to plant them (*Figure 5*).

Figure 5

Data collection and analysis

Unfortunately, the numerous struggles lasting the entirety of the growth period meant that we were unable to collect any quantitative data.

Discussion

The design was developed to focus on measuring the growth benefits brought by the presence of the nutrient matrices in each pot, along with the expected adverse effects caused by their absence. Our own experiment, however, faced many disadvantages.

While not discussed above, our nutrient gel had an intense odor of manure due to the continued exposure of fertilizer to air while in gel form. Good ventilation and the usage of high-quality masks and gloves will play a significant role in mitigating the effects of the smell on researchers and increasing the overall cleanliness of the lab.

Viable bamboo seeds, while having a relatively short germination period, are notoriously difficult to find and even more difficult to cultivate into sprouts (Wilson, 2016). Due to our oversight in this department, we did not have any bamboo seeds that we were able to grow and failed to acquire any data during the limited time remaining. In an exact replication of our experiment, bamboo of any type should be avoided to prevent any similar consequences. Instead, a focus on oats would be beneficial, especially with the advantage of their quick growth period and ability to grow within the parameters of the lab setup created for bamboo. During the germination period, more consistent check-ups of seeds should also be conducted to prevent mold from growing.

Additionally, choosing sand as our non-nutritional additive proved maladaptive to our experiment; while using sand did eliminate the confounding variable of external nutrient sources, we neglected to acknowledge the unwanted effects that would be produced by watering a mixture of sand and gypsum, a substance with a tendency to form cementitious material when coming into contact with water. Deeper research must be done to find a better non-nutritional substance to supplement the lack of physical soil substance in growing a large number of plants.

While we do not have results from our experiment to back up the material effectiveness of the nutrient matrix, we firmly believe in its unexplored capabilities. Not only does their hexagonal structure reduce the amount of 3D-filament needed to construct each matrix, but its sturdiness also offers foundational support for the plant roots – an essentiality of growing crops in the sandy Martian regolith. This design also allows it to be mass-produced and transported due to its uniform, compact, and stackable nature. Due to the nutrient gel being water-based, it will freeze easily in the temperatures of Mars and space, allowing for transportation without the risk of it falling out of the nutrient matrix. Since plants on Mars will require the use of an external heat source to support plant growth, the nutrient gel will be able to melt in between the layers of soil where the roots are located and ensure plants can get adequate nutrients. Additionally, all resources used in building the nutrient matrices are relatively affordable.

The main point of interest for our experimental design is that it can easily be used to grow other types of crops because the process of creating our nutrient gel is not exclusive to any one type of fertilizer, and its nutritional composition can be customized to any plant species. Although our attempt at growing black bamboo failed due to extenuating circumstances, the application variability of the experimental design lends itself perfectly to NASA's efforts towards in-situ resource utilization, where Martian settlement would sustain itself on resources grown and collected on Mars rather than brought from Earth. The experiment's customizability provides the potential for this design to be the foundation for producing any type of plant-based resource on Mars, whether it be food, building materials, textiles, or even medicine. The Sprayberry STEM Mars Explorers plan to pass down the knowledge and experience gained from this experiment to the next team to allow them to expand upon research in the field and take full advantage of the tremendous opportunity to shape the future of Martian exploration and horticulture efforts.

Resources

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