Developing a Model In-Situ Resource Utilization System for Oxygen Sustaining Life Support and Launch Cost Reduction for Mars

Ariella Blackman

Abstract

Martian agriculture may be the most cost-effective means to develop a sustainable human life support system on Mars by employing in-situ resource utilization to convert atmospheric CO_2 into O_2. However, launching the necessary Earth soil is prohibitively expensive, and Eichler et al. (2021) failed to germinate seeds in MGS-1, one of the most accurate Martian regolith simulants available. This study determined whether *Phaseolus acutifolius* could grow in ratios of MGS-1 and Earth-based potting soil and which substrate resulted in maximum O_2 while reducing Earth-based launch mass. Plants were grown in incremental substrate ratios, and an original mathematical model was created to estimate the number of plants required to produce enough O_2 to support human life while minimizing total Earth-based soil mass. Plants germinated in ratios with 0%, 25%, and 50% MGS-1. Results suggested that MGS-1 limited plant growth due to its water-retention properties. A significant difference existed between wet biomasses of plants grown in 50% MGS-1 and 0% MGS-1 ($p<.05$), with no such significant difference for the dry biomasses ($p>.05$). Plants in 50% MGS-1 allocated more resources towards obtaining water with significantly more below-ground biomass than the control ($p<.05$). Model calculations demonstrated a trend from 0% to 25% MGS-1: estimated number of required plants increased (867 to 1003 plants), but the total amount of Earth-based soil decreased (101kg to 87.2kg). This trend potentially holds between 25% and 50% MGS-1 but is unclear because of large amounts of below-ground biomass. Results imply that the ideal regolith content of a growth substrate is between 50-75% MGS-1 since the cost benefits of decreasing the Earth-based soil used per plant outweigh the need for more plants due to decreased O_2 production.

All images/graphs created by Ariella Blackman (2021)

Introduction

Human Spaceflight to Mars:

A human mission to Mars has the potential to vastly increase scientific knowledge and provide the world with new technologies. It is considered to be the most feasible option for human deep space exploration due to its proximity to Earth and relatively mild surface conditions. For future Mars exploration, an eventual human presence is advantageous to fully robotic missions, as humans are capable of shifting focus and creating unique solutions to problems, increasing the output of scientific data (Ehlmann et al., 2005).

Despite these benefits, there are a multitude of challenges with human mission infrastructure. This includes the high cost of a mission and the extreme environments a human would need to withstand. As the infrastructure for a Mars mission is largely undecided, it is difficult to determine the exact cost. However, estimates are between 20 billion USD and 450 billion USD (Ehlmann et al., 2005). One component of this is launch cost. Current launch cost estimates are around $45,000 USD per kilogram launched to Mars (Hinterman, 2022). Therefore, a human Mars mission could be made more economically feasible by reducing the Earth-based launch mass. This can be done by using more reusable systems or by utilizing resources already on Mars.

Another aspect to consider is the harsh environment that humans would need to withstand during a mission. Mission infrastructure would need to address Mars' lack of known liquid surface water, thin atmosphere of 95% CO_2, lack of atmospheric O_2, and higher radiation levels than Earth (Lotto et al., 2018). To send humans to Mars despite these obstacles, systems must be designed that can support human life in this environment.

Life Support Systems:

Life support systems (LSS) are the systems required to sustain human life while living and working in space. Some purposes of LSS are to produce O_2, remove CO_2, provide food and water, and remove waste (NASA, 2017). For a human Mars mission, LSS would be required to provide astronauts with a safe environment, as none of these needs are met naturally on the Martian surface.

The sustainability and reliability of LSS for human Mars missions are important to consider. The current LSS onboard the International Space Station is unlikely to result in the loss of a crew, as there is the option for an emergency return to Earth in several hours. Due to the proximity to Earth, this LSS incorporates resupply missions from Earth and waste disposal. However, the trip from Earth to Mars would take approximately six months (Jones et al., 2014). Therefore, the LSS must be more sustainable in the case of a failure, as there is no emergency return capability. In addition, the distance between Earth and Mars means that resupply missions are not feasible (Jones et al., 2014). Therefore, a successful LSS must provide sufficient resources for humans without receiving any Earth materials post-launch. This can be done through the use of closed-loop systems, which will have the capability of recycling all resources so no resupply is necessary, or through in-situ resource utilization.

In-Situ Resource Utilization:

In-situ resource utilization (ISRU) is the use of materials already existing in a location as resources. For a Mars mission, this could mean using the atmospheric gasses, subsurface water ice, Martian regolith, and other materials found on the planet to support human life (Lotto et al., 2018). As resources would be more readily available, the need for resupply missions would decline. This would also increase the reliability of a LSS, as the increased accessibility to resources means a failure of the LSS may not be catastrophic to the mission. Also, materials could be obtained later in the mission, so there would be a reduction of the launch mass, and therefore the launch cost.

One way ISRU can be used to reduce launch mass is by converting the CO_2 that makes up 95% of the Martian atmosphere into O_2 that can be used as a resource (Lotto et al., 2018). The Mars Oxygen In-Situ Resource Utilization Experiment (MOXIE) is a part of the Mars 2020 rover that is designed to use electrochemical processes to convert the CO_2 of the Martian atmosphere into O_2 at 0.5% of the scale that would be required for a human mission. If the system is expanded, this O_2 could be used as rocket propellant for a Mars Ascent Vehicle or for breathing (Hinterman & Hoffman, 2020). In April 2021, MOXIE was tested for the first time and it produced 5.8g of O_2 in one hour (Kotary & Cody, 2021). It has been shown that humans have the technology to convert the CO_2 of the Martian atmosphere into O_2 and that ISRU is an effective way of producing resources.

While mechanical systems such as MOXIE can be beneficial, they are limited to a single purpose. If multifunctional systems could be developed, it could allow for cheaper life support. Therefore, another potential method of O_2 production is plant growth. Such a system could use ISRU by growing in Martian regolith and converting the atmospheric CO_2 into O_2 via photosynthesis. In addition, plants would likely already be included in the mission infrastructure as a food source. Therefore, the total required systems would be minimized, decreasing the launch mass.

Growing Plants with Martian Regolith:

Martian regolith is similar to soil on the Martian surface. While there has been no sample return to Earth, Martian regolith simulants (MRS) have been produced to allow for scientific research. One of these simulants, the Mars Global Simulant (MGS-1), is considered to be the most accurate MRS to date. It was developed based on the mineralogy determined by the Mars Curiosity rover and was created by combining the individual components, rather than utilizing Earth material from one location as was done with previous MRS. MGS-1 is recommended to be used in studies where the mineralogy is an important factor, such as with plant growth (Cannon et al., 2019). Growing plants in MGS-1 presents more challenges than growing in soil on Earth, as many of the characteristics of the MRS are not conducive to plant growth. For example, MGS-1 is alkaline (pH > 9.0), aggregates when watered, and lacks nutrients like nitrogen that plants need to grow (Eichler et al., 2021).

Eichler et al. (2021) grew plants in MGS-1 with limited success. When seeds were placed in MGS-1, they failed to germinate, even when given additional nutrients. The seeds were recovered from the MGS-1, and still failed to germinate after 14 days on filter paper, suggesting that MGS-1 is potentially toxic. When seeds were germinated on rockwool beds, allowed to grow for 5 days, and transferred into MGS-1, they all died within 5 days. When the same was done with one-month old plants, they died within 7 days (Eichler et al., 2021). Overall, this suggests that plant growth in 100% MGS-1 is not feasible.

However, it is possible that if the MGS-1 were mixed with another growth substrate that counteracted the challenging qualities, plants may be able to grow. Even if plants could not be grown in 100% regolith on a mission, the ability to grow in a mixture of Martian regolith and another growth substrate would still be beneficial, because any amount of ISRU could reduce launch costs.

Fitchett et al. (2020) grew plants in growth substrate mixtures using the Mojave Mars Simulant (MMS-2), a different type of MRS. Plants were grown in a control of 100% Earth soil, a mixture of 50% MMS-2/50% Earth soil, and a mixture of 50% MMS-2/25% coffee grounds/12.5% Earth soil/12.5% vermiculite. The plants grown in 50% MMS-2 and 50% Earth soil were capable of growth (Fitchett et al., 2020).

Gap in the Knowledge:

The study done by Fitchett et al. (2020) demonstrated that it was possible to grow plants in ratios of an MRS and Earth soil. However, the study used MMS-2, a less accurate MRS than MGS-1, and did not study O_2 production. Plants were unable to grow in 100% MGS-1, the most accurate MRS (Eichler et al., 2021). However, it was unclear whether the plants could successfully grow in ratios of MGS-1 and Earth soil.

Even if the plants could grow in a ratio of MGS-1 and Earth soil, it was unknown whether this growth substrate could impact O_2 production. Understanding how a substrate ratio could impact both plant growth and O_2 production informed about the potential success of a plant-based Mars LSS.

Purpose:

The purpose of this study was to model a sustainable, plant-based O_2 production LSS that used ISRU of Martian regolith. Various substrate ratios using MGS-1 and Earth soil were tested to determine which allowed for maximum plant growth, and therefore O_2 production. Then, a model was created to determine if the plants produced enough O_2 to support human life and to determine which of the substrate ratios allowed for the use of the least Earth soil, which would ultimately lower launch cost. By modeling a system that could use ISRU to produce O_2 for a sustainable LSS while reducing the cost of such a system, steps were taken towards the ability to send humans to Mars.

Research Question:

Which substrate ratio of potting soil and MGS-1 allows tepary beans *(Phaseolus acutifolius)* to produce enough O_2 for a life-support system while reducing the Earth-based mass?

Hypothesis:

A threshold exists where *Phaseolus acutifolius* will be capable of growing and producing O_2 in a mixture of MGS-1 and Earth soil, as measured by biomass produced.

Goal:

Determine an ideal substrate ratio to model a sustainable, plant-based LSS that optimizes high O_2 production and low launch mass, and therefore launch cost.

Methodology

This study contains two parts: the growth of plants in multiple substrate ratios and the creation of an original mathematical model to determine the feasibility of a LSS.

Part A: Growth of Plants in Various Substrate Ratios

Plant Species Selection:

Tepary beans *(Phaseolus acutifolius)* were obtained from Adaptive Seeds and used because of their drought tolerance and ability to grow in a harsh environment with Mars-like characteristics. Twenty-five seeds were used, five for each of five substrate ratios.

Substrate Selection:

This study utilized combinations of Martian regolith simulant and potting soil. The simulant was Mars Global Simulant (MGS-1) obtained from the Exolith Lab at the University of Central Florida. MGS-1 has similar chemical and mineralogical properties as Martian regolith, as it was developed based on samples from the Mars Curiosity rover (Cannon et al., 2019). This makes it one of the most accurate Martian regolith simulants developed to date. The potting soil was MiracleGro Potting Mix, obtained from a local Home Depot, because it can retain water well and contains a high ammonium nitrate content (.21%) (The Scotts Company, 2021). The substrates were mixed in five different ratios: 0%, 25%, 50%, 75%, and 100% MGS-1 by percent volume.

Setup:

The setup, depicted in Figure 1, was designed to maintain a controlled environment which utilized grow lights on timers, thermometers, thermostats, heating mats, and growing containers on trays. The cart was exposed to little ambient light and had limited unnecessary human contact. Each tray contained a different substrate ratio with five samples per ratio. The lights were on for 12 hours per day, simulating day and night. Each tray contained one "VIVOSUN 10"x20.75" Seedling Heat Mat and Digital Thermostat Combo Set" obtained on Amazon and five 3.5" x 3.5" x 5" planting containers. The heat mat's temperature probe was placed into one of the containers on the tray. This caused the temperature mats to stay near the set temperature: 26°C during the light cycle and 22°C during the dark cycle.

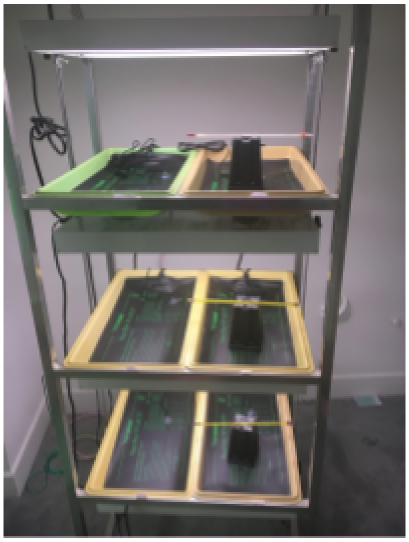

Figure 1. Grow cart setup for plants

Variables:

Independent Variable: Ratio of MGS-1 and MiracleGro Potting Mix in growth substrate

Dependent Variables: Plant growth (Throughout study: height, stem height, width, stem diameter, number of branch points, number of leaves. End of study: number of roots off main stem, wet total biomass, dry total biomass, dry above ground biomass, and dry below ground biomass)

Controlled Variables: Temperatures maintained at 26°C and 22°C, light 12 hours/day, total substrate volume of 560cm^3, all *Phaseolus acutifolius* seeds from Adaptive Seeds, MGS-1 obtained from Exolith Lab, Miracle-Gro Potting Mix, watered with 160mL distilled water

Control group: Plants grown in 0% MGS-1

Treatments: 25%, 50%, 75%, and 100% MGS-1

Creating Substrates:

Each growth substrate ratio had a total volume of 560cm^3. Because the regolith simulant had very fine particles and can cause lung damage with prolonged inhalation, it was measured and poured under a fume hood. A surgical mask, safety goggles, and gloves were worn when handling. The two substrates were thoroughly mixed under the fume hood and then treated to prevent too much aggregation of the regolith simulant. As depicted in Figure 2a, each container was watered with 160mL of distilled water, as this volume was qualitatively determined to make the control “damp, but not wet” (Pima County Public Library, 2019). Then, each substrate was spread out to air dry, as shown in Figure 2b. The substrates dried into large aggregates, shown in Figure 2c, and were then crushed to break up these aggregates, shown in Figure 2d. The process was repeated three times for the 0%, 25%, and 50% MGS-1 and four times for the 75% and 100% groups. This was due to the 75% and 100% groups continuing to aggregate after three repetitions. The final treated growth substrates for each ratio are depicted in Figure 2e.


Figure 2a. Watered growth substrate


Figure 2b. Dried growth substrate


Figure 2c. Growth substrate aggregates


Figure 2d. Crushed growth substrate

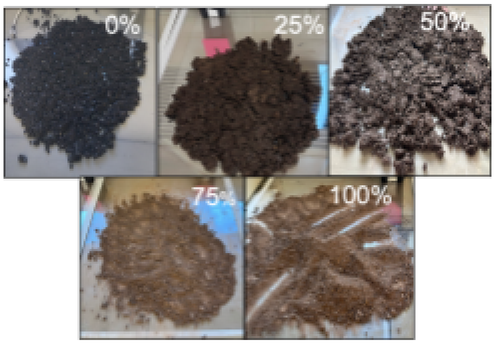

Figure 2e. Treated growth substrates

Planting:

The dry *Phaseolus acutifolius* seeds were placed into the treated substrates approximately 2.5cm deep in 560cm^3 of substrate. This ensured the roots had sufficient space to grow. The soil was initially watered with 160mL of distilled water and the containers were placed on trays in the setup.

Growth:

Every morning, the heat mats were switched to the light cycle temperature, the lights turned on, and the dark cycle temperature of the heat mat and each sample was recorded. Twelve hours later, the light cycle temperatures were measured and the heat mats and lights were switched to dark cycle settings. The plants were watered with 50mL distilled water daily until germination. After seedlings sprouted, they were watered with 50mL of distilled water every three days. The watering plan was adjusted for individual plants if the soil seemed too moist or dry by qualitative human observation. Due to pooling of water, the substrates with a higher percentage of MGS-1were watered less often. The plants were rotated into a new configuration to change their tray's height and their positions on the tray. This helped mitigate effects of a potential heat gradient. The plants were measured daily for total height, stem height, total width, stem diameter, branch points, and the number of leaves, as depicted in Figure 3. Some plants began to hit the lights at 20 days post-germination, which may have begun to inhibit growth, so this was chosen as the growth period.

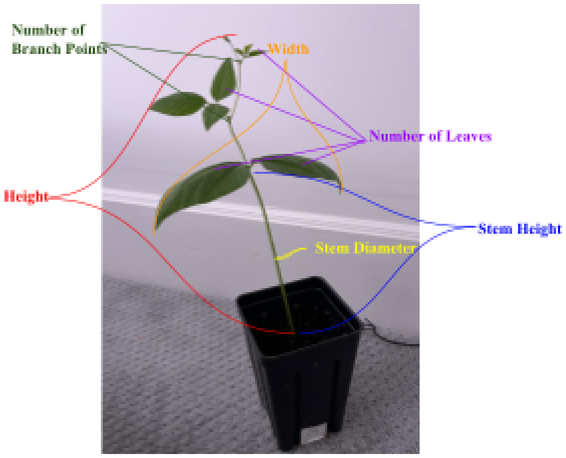

Figure 3. Diagram of plant measurements

Measuring Biomass:

The biomass of each plant was measured at 20 days post-germination. The plant roots were extracted from the substrate and the excess substrate was brushed away from the roots. The total wet biomass was massed with a Scout Pro SPE202 model scale, and the plants were dehydrated in a Quincy Lab Model 12-140 Incubator for 24 hours at about 55°C. Then, the total dry biomass was massed. The plant was cut where the roots met the stem, and above and below ground dry biomasses were massed individually.

Data Analysis

Bar graphs were created to compare the percent germination of plants, mean wet total biomass, dry total biomass, dry above ground biomass, and dry below ground biomass between growth substrates.

A Z-test for proportions was used to test for significance between germination rates, and one-way ANOVA tests and Tukey's HSD Tests for multiple comparisons were used to test for significance between the control group and treatment groups for the biomass measurements. Scatter plots were created for all measured growth parameters, and linear regressions were run to determine relationships between different parameters and to extrapolate growth trends beyond the growth period.

All graphs and tests were created and conducted in Graph Pad Prism Version 9.3.1 (350).

Part B: Creation of a Model

An original mathematical model was created to estimate the amount of O_2 produced per plant, the number of plants required to produce enough O_2 to support life, and the mass of the potting soil required to create a system of this scale. Height was extrapolated to 75 days post-germination, the estimated time of plant maturity, and was used to extrapolate biomass at 75 days. The model utilized the biomass carbon fraction of a dry bean to determine C content of each sample and the photosynthesis equation to convert C content to O_2 production. The amount of O_2 required to sustain life per person per day was used to determine the number of plants required in each substrate ratio. Based on the potting soil density, volume percentage, and number of required plants for the substrate, the total required potting soil mass was calculated for every substrate ratio. The economic implications of using different substrate ratios was determined by examining the Earth-based mass required for each substrate and the estimated cost per kilogram launched to Mars. This ultimately allowed for the ideal substrate ratio to be determined.

Results

Part A: Plant growth

Figure 4 illustrates that the control and 50% MGS-1 substrate ratios had 80% germination rates, and the 25% ratio had a 60% germination rate. No seeds in the 75% or 100% MGS-1 ratio germinated within 18 days of planting.

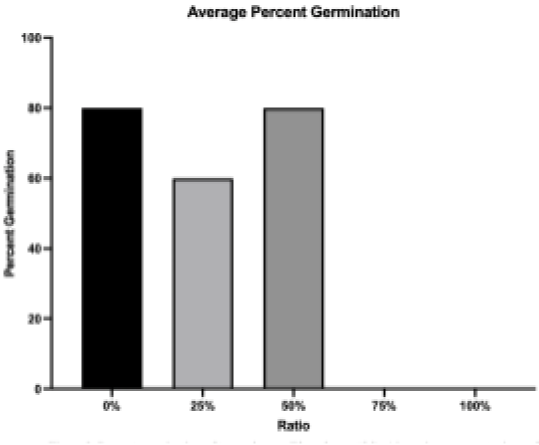

Figure 4. Percent germination of tepary beans (*Phaseolus acutifolius*) in various concentrations of MGS-1. n=5

Figure 5a depicts the wet total biomass for each plant, measured at the end of the study (20 days post-germination). The control had an average of 3.08g, the plants grown in 25% had an average of 1.22g, and the plants grown in 50% had an average of 1.32g. The biomasses for 25% MGS-1 and 50% MGS-1 were both significantly less than that of the control (p<.05).

Figure 5b depicts the dry total biomass for each plant, measured at the end of the study (20 days post-germination). The control had an average of 0.32g, the plants grown in 25% had an average of 0.19g, and the plants grown in 50% had an average of 0.33g. Neither ratio produced significantly less dry biomass than the control (p>.05).

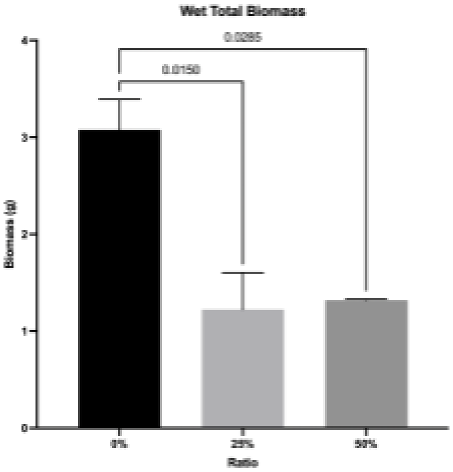

Figure 5a. Mean wet total biomass (+SEM) of tepary beans *(Phaseolis acutifolius)* after growing for 20 days in various concentrations of MGS-1.

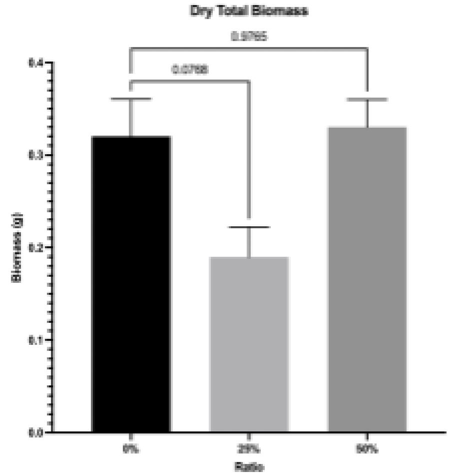

Figure 5b. Mean dry total biomass (+SEM) of tepary beans *(Phaseolis acutifolius)* after growing for 20 days in various concentrations of MGS-1.

The dry below ground biomass for each plant was measured at the end of the study (20 days post-germination), as depicted by Figure 6. The control had an average of 0.07g, the plants grown in 25% had an average of 0.07g, and the plants grown in 50% had an average of 0.18g. There was an upwards trend in dry below ground biomass as the concentration of MGS-1 increased, and the plants grown in 50% MGS-1 had significantly more biomass than the control (p<.05).

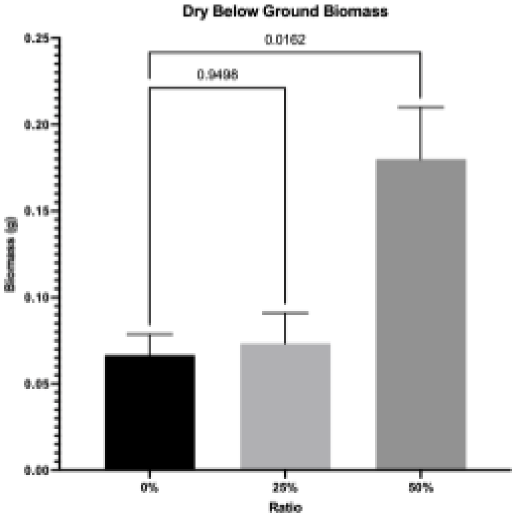

Figure 6. Mean dry below ground biomass (+SEM) of tepary beans *(Phaseolus acutifolius)* after growing for 20 days in various concentrations of MGS-1.

Part B: Modeling

A model was created to estimate the amount of O_2 produced per plant, the number of plants required to produce enough O_2 to support life per person per day, and the mass of the potting soil required to create a system of this scale. Plants were extrapolated to 75 days post-germination, as this is considered to be the average time for *Phaseolus acutifolius* to reach maturity on Earth (San Diego Seed Company, n.d.).

1. Biomass was measured at one time, so a relationship was determined between biomass and a secondary variable to extrapolate biomass at 75 days. Linear regressions compared the biomass and the variables measured over time. Similar slopes between substrates suggested that the trend will continue to hold true regardless of the ratio. A linear relationship existed between wet biomass and total plant height as demonstrated by Figure 7. The slopes were not significantly different between ratios by an ANOVA test, so there was a consistent relationship between the two variables (0%: r=.979, 25%: r=.925, 50%: r=.996) ($p<.05$).

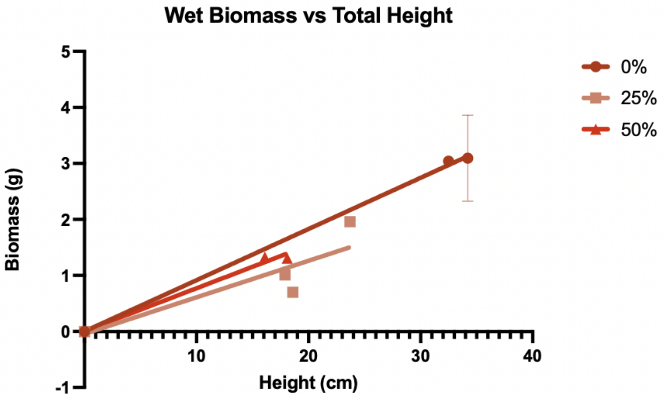

Figure 7. A linear regression comparing wet total biomass and height between tepary beans *(Phaseolus acutifolius)* grown in various concentrations of MGS-1 over 20 days.

2. Figure 8 depicts a linear regression of plant height vs time (0%: r=.891, 25%: r=.809, 50%: r=.785). Equations 1a, 1b, and 1c, the linear regression equations, were used to extrapolate plant height at 75 days. The rate of change of plant height was found to be significantly different between ratios by an ANOVA test ($p<.05$).

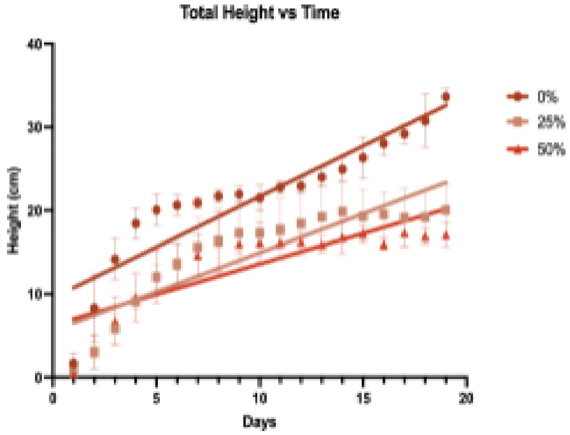

Figure 8. A linear regression of the height of tepary beans (*Phaseolus acutifolius*) grown in various concentrations of MGS-1 over time.

(Equation 1)

1a. Height of Plants in 0% MGS-1 at 75 Days

$$y = 1.213x + 9.549$$

$$y = 1.213(75) + 9.549$$

$$y = 100.524 \text{ cm}$$

1b. Height of Plants in 25% MGS-1 at 75 Days

$$y = 0.9346x + 5.592$$

$$y = 0.9346(75) + 5.592$$

$$y = 75.687 \text{ cm}$$

1c. Height of Plants in 50% MGS-1 at 75 Days

$$y = 0.7308x + 6.289$$

$$y = 0.7308(75) + 6.289$$

$$y = 61.099 \text{ cm}$$

3. A linear regression of wet biomass vs height was done, depicted in Figure 7. Equations 2a, 2b, and 2c, the linear regression equations between wet biomass and total height by the pooled slope and y-intercept values, were used to estimate wet biomass at 75 days. There was no significant difference in the slopes between different substrate ratios ($p>.05$), suggesting that this relationship was conserved for the different ratios.

(Equation 2)

$$y = 0.08351x - 0.04809$$

2a. Wet Biomass of Plants in 0% MGS-1 at 75 Days

$$y = 0.08351(100.524) - 0.04809$$

$$y = 8.35 \text{ g}$$

2b. Wet Biomass of Plants in 25% MGS-1 at 75 Days

$$y = 0.08351(75.687) - 0.04809$$

$$y = 6.27 \text{ g}$$

2c. Wet Biomass of Plants in 25% MGS-1 at 75 Days

$$y = 0.08351(61.099) - 0.04809$$

$$y = 5.05 \text{ g}$$

4. Calculations relating O_2 production to biomass utilized dry biomass. A linear regression determined the relationship between wet and dry biomass, as depicted in Figure 9 (0%: r=.999, 25%: r=.908, 50%: r=.993). Equations 3a, 3b, and 3c were used to find the dry biomass values based on each substrate's linear regression equation, as the slopes were significantly different between ratios ($p<.05$).

(Equation 3)

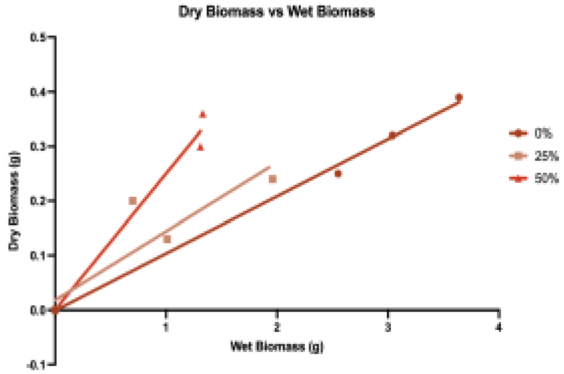

Figure 9. A linear regression comparing dry total biomass to wet total biomass of tepary beans (*Phaseolus acutifolius*) after being grown in various concentrations of MGS-1 over 20 days.

3a. Dry Biomass of Plants in 0% MGS-1 at 75 Days

$$y = 0.1059x - 0.004289$$

$$y = 0.1059(8.35) - 0.004289$$

$$y = 0.880 \text{ g}$$

3b. Dry Biomass of Plants in 25% MGS-1 at 75 Days

$$y = 0.1212x + 0.006319$$

$$y = 0.1212(6.27) + 0.006319$$

$$y = 0.766 \text{ g}$$

3c. Dry Biomass of Plants in 50% MGS-1 at 75 Days

$$y = 0.2525x - 0.0004166$$

$$y = 0.2525(5.05) - 0.0004166$$

$$y = 1.27 \text{ g}$$

5. Equations 4a, 4b, and 4c were used to calculate the carbon content of each plant assuming that each plant has a biomass carbon fraction in which 45% of its dry total biomass is carbon (Anderson et al., 2018).

(Equation 4)

$$y = 0.45x$$

4a. Carbon Mass of Plants in 0% MGS-1 at 75 Days

$$y = 0.45(0.880)$$

$$y = 0.396 \text{ g C}$$

4b. Carbon Mass of Plants in 25% MGS-1 at 75 Days

$$y = 0.45(0.766)$$

$$y = 0.345 \text{ g C}$$

4c. Carbon Mass of Plants in 50% MGS-1 at 75 Days

$$y = 0.45(1.27)$$

$$y = 0.572 \text{ g C}$$

6. The photosynthesis equation, Equation 5, was used to determine a 1:1 ratio between moles of C and moles of O_2. Therefore, dimensional analysis was used in Equations 6a, 6b, and 6c to convert grams of carbon found in Equations 4a, 4b, and 4c to kilograms of O_2.

(Equation 5)

$$6CO_2 + 6H_2O \rightarrow \mathbf{C_6}H_{12}O_6 + \mathbf{6O_2}$$

$$1\, mol\, C = 1\, mol\, O_2$$

(Equation 6)

6a. Grams C to Kilograms O_2 for 0%

$$0.396\, g\, C \cdot \frac{1\, mol\, C}{12.011\, g\, C} \cdot \frac{1\, mol\, O_2}{1\, mol\, C} \cdot \frac{2(15.999)\, g\, O_2}{1\, mol\, O_2} \cdot \frac{10^{-3}\, kg\, O_2}{1 g\, O_2} = 1.06 \times 10^{-3}\, kg\, O_2$$

6b. Carbon Grams to Moles for 25%

$$0.345\, g\, C \cdot \frac{1\, mol\, C}{12.011\, g\, C} \cdot \frac{1\, mol\, O_2}{1\, mol\, C} \cdot \frac{2(15.999)\, g\, O_2}{1\, mol\, O_2} \cdot \frac{10^{-3}\, kg\, O_2}{1 g\, O_2} = 9.18 \times 10^{-4}\, kg\, O_2$$

6c. Carbon Grams to Moles for 50%

$$0.572\, g\, C \cdot \frac{1\, mol\, C}{12.011\, g\, C} \cdot \frac{1\, mol\, O_2}{1\, mol\, C} \cdot \frac{2(15.999)\, g\, O_2}{1\, mol\, O_2} \cdot \frac{10^{-3}\, kg\, O_2}{1 g\, O_2} = 1.52 \times 10^{-3}\, kg\, O_2$$

7. Assuming the average human requires 0.92 kg O_2 per day to survive (Anderson et al., 2018), Equations 7a, 7b, and 7c were used to calculate the number of mature plants required to support one person for one day. Values were rounded up to the number of whole plants.

(Equation 7)

$$y = \frac{0.92\, kg\, O_2}{kg\, O_2\, per\, plant}$$

7a. Number of Plants for 0%

$$y = \frac{0.92}{1.06 \times 10^{-3}}$$

$$y = 867\, plants$$

7b. Number of Plants for 25%

$$y = \frac{0.92}{9.18 \times 10^{-4}}$$

$$y = 1003\, plants$$

7c. Number of Plants for 50%

$$y = \frac{0.92}{1.52 \times 10^{-3}}$$

$$y = 606\, plants$$

8. Equations 8a, 8b, and 8c were used to calculate the amount of Earth-based potting soil required to grow enough plants to produce enough O_2 for sufficient life support by using the density of the potting soil, the volume used for each ratio, and the number of required plants.

(Equation 8)

$$density = \frac{total\, potting\, soil\, mass}{(volume\, for\, ratio)(\#\, of\, plants)}$$

8a. Mass of Soil for 0%

$$0.207 g/cm^3 = \frac{m}{(560 cm^3)(867)}$$

$$m = 101,000\, g = 101\, kg$$

8b. Mass of Soil for 25%

$$0.207 g/cm^3 = \frac{m}{(420 cm^3)(1003)}$$

$$m = 87,200\, g = 87.2\, kg$$

8c. Mass of Soil for 50%

$$0.207 g/cm^3 = \frac{m}{(280 cm^3)(606)}$$

$$m = 35,100\, g = 35.1\, kg$$

Discussion

The hypothesis, a threshold exists where *Phaseolus acutifolius* will be capable of growing and producing O_2 in a mixture of Martian regolith simulant and Earth soil, as measured by biomass produced, was supported by the study. The plants were able to grow in both the 25% and 50% MGS-1, yet did not germinate in 75% and 100%, demonstrating that the threshold for growth exists somewhere between 50% and 75%. For example, plants grown in 0%, 25%, and 50% MGS-1 had at least a 60% germination rate. Meanwhile, no plants grown in 75% or 100% MGS-1 germinated. In addition, the plants that germinated supported the hypothesis because their biomasses allowed for the O_2 production rates to be calculated. The plants grown in 0%, 25%, and 50% MGS-1 had an average of 0.32, 0.19, and 0.33 grams of dry biomass, respectively. This was calculated to determine an O_2 production of 1.06×10^{-3}, 9.18×10^{-4}, and 1.52×10^{-3} kilograms of O_2 per plant per day. Despite the biomasses of the plants grown in MGS-1 being close to or less than that of the control, they still grew in up to 50% MGS-1 and required much less Earth-based potting soil. Therefore, even though plants grown in less productive ratios require more plants to produce enough O_2 for life support, the decreased amount of potting soil per plant causes the overall Earth-based soil mass to be less than that of the control. For example, there would need to be approximately 1003 plants grown in 25% MGS-1 to produce enough O_2 to support one person per day, compared to the control group's 867 plants. However, only 87.2 kg of potting soil would be required to grow this large number of plants, while the control would require 101 kg to grow fewer plants. Therefore, the study suggests that the ideal substrate ratio for a LSS to produce sufficient O_2 and reduce Earth soil mass would likely contain the largest amount of regolith possible that does not suppress germination. While increased Martian regolith leads to issues such as decreased O_2 production, the decreased mass of Earth-based soil per plant still accounts for utilizing less total Earth-based mass .

One potential reason for the decreased germination rates, heights, and biomasses in ratios with more MGS-1 is the lack of organic material. Plants require organic matter and nutrients to survive, so the higher ratios of MGS-1 likely did not contain enough of this for the plants to germinate or grow well. In addition, untreated MGS-1 aggregates when watered, turning it into a hard, cement-like block. Therefore, all substrate ratios were treated before planting by being watered, dried, and broken up to produce smaller aggregates that would prevent this. However, organic material stabilizes the aggregates created by the treatment (Irons, 2021). Therefore, the substrates with higher ratios of MGS-1 had less stable aggregates that may have broken apart when watered, leading to a more compact whole that prevented water and air from accessing seeds or roots.

The water retention properties of the growth substrates, and therefore plant uptake of water, therefore likely impacted the growth of the plants. MGS-1 has a smaller pore structure, which causes water to absorb less quickly, but be held for longer. Meanwhile, potting soil has a larger pore structure, so the water flows through more easily and dries out more quickly. The aggregates in substrates with large amounts of MGS-1 also contributed to these characteristics. This was supported through qualitative observations, as pooling was observed when higher ratios of MGS-1 were watered with the same amount of water as the control. The varying water retention properties were also supported by biomass observations, as the control had significantly more wet total biomass than any of the ratios containing MGS-1, yet not significantly more dry biomass. This indicates that the differences between wet biomasses was due to water content. The amount of water uptake impacts the ability of the plant to grow and produce biomass, so if uptake was impacted by the water retention properties of the growth substrate, biomass production would be impacted too.

A lack of water uptake was also demonstrated through the below ground biomass, which was significantly higher in the 50% MGS-1 substrate than in the control. If the plants in this substrate had more difficulty obtaining resources, they would have increased root growth in an attempt to access enough water and nutrients. Therefore, fewer resources would be spent on other variables such as height, which was demonstrated when the rate of growth was significantly different between ratios. However, the increase in below ground biomass could potentially be a confounding variable. The growth substrate could not completely be removed from the roots of any of the plants. Since the MGS-1 aggregates when watered, it is possible that the 50% MGS-1 substrate aggregated more, making it more difficult to remove from the roots of these plants.

This study agrees with the study done by Eichler et al. (2021), as both demonstrate that growing plants in 100% MGS-1 is not possible. In both studies, the seeds planted in 100% MGS-1 were unable to germinate at all (Eichler et al., 2021). This study further finds that the threshold for growth is likely between 50% and 75% MGS-1 when combined with potting soil.

Evaluation:

This was a pilot study and the model produced a first-order approximation for the amount of O_2 produced per plant and the number of plants required to support life. There was a small sample size, which was due to the prohibitive cost and limited availability of the MGS-1. In addition, the plants were grown in a home environment rather than a lab due to impacts from the COVID-19 pandemic. Therefore, while the environment was maintained to be as constant as possible, there were still some variations in conditions such as temperature, humidity, and light. In addition, while a surgical mask was worn to minimize the impacts, the plants were handled by the researcher, which may have led to uncontrolled CO_2 exposure for the plants.

A limitation of the model is that values such as the biomass carbon fraction came from NASA's Life Support Baseline Values and Assumptions Document, which contains previously determined values.

These values were generic to a “dry bean,” and not specific to tepary beans. In addition, the document does not base the values off plants that have been grown in Martian regolith. Therefore, values like the amount of O_2 production per amount of biomass may have changed based on the growth substrate, but these changes were not accounted for in the model.

This may also lead to inflated predicted O_2 production of plants grown in 50% MGS-1, as the model used total dry biomass. However, the plants in this substrate contained mostly below ground biomass, which did not photosynthesize like the leaves and stems of the plants. Since the O_2 production values were based on total biomass but above ground biomass was limited in these plants, it is possible that the O_2 production was overestimated.

Finally, the linear regression of the height graph was used to extrapolate biomass at 75 days. However, plant height is often closer to an exponential increase rather than linear, making the estimate of biomass and O_2 production a lower limit for the plant.

Overall, while the exact values of the model may not be precise due to the necessity of assumptions in its creation, the trends, and therefore ideal substrate ratio, will likely hold true with further study.

Future work:

Future work in this field involves improving on the pilot study to make estimates more accurate and examining the implications of the study to see how this impacts the efficiency and ideal design of an LSS. To improve the accuracy of the study, it should be repeated with a larger sample size in a closed, controlled environment where gas exchange can be measured directly rather than as a function of biomass. More substrate ratios should be tested, particularly between 50% and 75% MGS-1, to further examine where the threshold for plant growth lies. Also, different species of plants should be used to determine if this changes the results of the study.

It should be noted that legumes were used in this study, which have nitrogen-fixing root nodule symbiosis with the rhizobium bacteria typically found in soil. This process fixes atmospheric nitrogen into ammonia, which fertilizes plants. In this study, the plant growth was stopped before visual root nodules formed, so there was likely no major impact. However, if the plants were grown to a more mature stage, the decreased amount of bacteria in substrates with higher amounts of regolith could negatively impact plant growth, as the rhizobium bacteria would not be found in Martian regolith. However, if Martian regolith can be successfully inoculated with the rhizobium bacteria, legumes may be able to fix nitrogen more effectively in MGS-1, reducing the amount of required Earth soil.

The model must be expanded to consider the implications of an actual Mars mission. Further work should be done into the mass estimates in the model, as not only Earth-based soil mass will be impacted. Other variables, such as light, energy, space, and water requirements, as well as the methods used for regolith collection, will be affected too. This can be used to estimate launch costs for different LSS configurations, and a cost-benefit analysis should be done to compare the LSS that grows plants to the ideal substrate ratio with various mechanical means of O_2 production to determine which is most efficient for a mission.

Implications:

This study has demonstrated that the amount of organic matter in a growth substrate has implications on the plant's ability to grow effectively in that substrate. Therefore, if more organic material is added to Martian regolith, plants may be able to grow in higher concentrations of it. This can potentially be done through the use of more robust pioneer species such as weeds or mosses. If these plants can be grown in higher concentrations of Martian regolith than more complex plants, they can decompose and release organic material and nutrients into the regolith. Another option is the use of biochar, which would add organic carbon to the growth substrate and has the potential to be produced in-situ on Mars. This may allow complex plants that will be used as an LSS to grow in higher concentrations of MGS-1 than in this study, further decreasing Earth-based mass and launch costs.

This research has major implications on the future of space travel and the ability to send humans to Mars. Since current launch cost estimates are approximately 45,000 USD per kilogram (Hinterman, 2022), the research determined that using even 25% MGS-1 could save over 600,000 USD per astronaut in launch costs compared to only potting soil.

Machines are being created to produce O_2 on Mars, and this study will help determine whether these are necessary, or if plants can be used for O_2 production. A plant-based system would contribute to food production as well as O_2 production, which may make it more cost-effective than machines. Even if a full O_2 production plant-based LSS is found to be unfeasible, this study helps to determine whether plants can be used to contribute to part of the O_2 production. Therefore, the mass of the machinery may be reduced or plants may be used for redundancy in case of a failure of a mechanical O_2 production LSS. Ultimately, the research is a first-order approximation that will allow for future studies of Martian regolith remediation and sustainable Martian LSS, providing humans the resources and knowledge they will need to survive on Mars at a non-prohibitive cost.

References

Andrews, R. G. (2019, September 6). *Can spaceflight save the planet?* Scientific American. https://www.scientificamerican.com/article/can-spaceflight-save-the-planet/

Cannon, K. M., Britt, D. T., Smith, T. M., Fritsche, R. F., & Batcheldor, D. (2019). Mars global simulant MGS-1: A Rocknest-based open standard for basaltic martian regolith simulants. *Icarus*, *317*, 470-478. https://doi.org/10.1016/j.icarus.2018.08.019

Cartier, K. M. S. (2018, January 12). *Tests indicate which edible plants could thrive on Mars*. American Geophysical Union. https://eos.org/articles/tests-indicate-which-edible-plants-could-thrive-on-mars

Ehlmann, B. L., Chowdhury, J., Marzullo, T. C., Collins, E., Litzenberger, J., Ibsen, S., Krauser, W., DeKock, B., Hannon, M., Kinnevan, J., Shepard, R., & Grant, D. (2005). Humans to Mars: A feasibility and cost–benefit analysis. *Acta Astronautica*, *56*(9-12). https://doi.org/10.1016/j.actaastro.2005.01.010

Eichler, A., Hadland, N., Pickett, D., Masaitis, D., Handy, D., Perez, A., Batcheldor, D., Wheeler, B., & Palmer, A. (2021). Challenging the agricultural viability of martian regolith simulants. *Icarus*, *354*. https://doi.org/10.1016/j.icarus.2020.114022

Exolith Lab. (2021). *MGS-1 Mars Global Simulant*. Exolith Simulants. https://exolithsimulants.com/products/mgs-1-mars-global-simulant

Fitchett, H., Ford, R., Ray, C., Tukei, L.K., Beedle, D., García-Espinosa, J., Kostic, G., Enevoldsen, A.A., Pikul, J.L., Aragon, K., Rivas, J.G., & Orneas-Mendoza, M. (2020). Kale Growth in MMS-2 Enhanced Mars Regolith Simulant During Indoor Earth Conditions.

Furfaro, R., Sadler, P., & Giacomelli, G. A. (2016). Mars-lunar greenhouse (M-LGH) prototype for bioregenerative life support systems in future planetary outposts. *Proceedings of the International Astronautical Congress, IAC*.

Furfaro, R., Gellenbeck, S., Giacomelli, G., & Sadler, P. (2017). Mars-Lunar greenhouse (MLGH) prototype for bioregenerative life support systems: current status and future efforts. *International Conference on Environmental Systems*. https://ttu-ir.tdl.org/bitstream/handle/2346/73105/ICES_2017_347.pdf?sequence=1

Gaskill, M. (2019, April 26). *Building better life support systems for future space travel* (M. Johnson, Ed.). NASA. https://www.nasa.gov/mission_pages/station/research/news/photobioreactor-better-life-support

Geer, K. (2019, February 25). *Growing tepary beans: The most heat-tolerant crop in the world*. Countryside. https://www.iamcountryside.com/growing/growing-tepary-beans/

Grant, A. (2021, November). *What are tepary beans: information on tepary bean cultivation*. Gardening Know How. https://www.gardeningknowhow.com/edible/vegetables/beans/what-are-tepary-beans.htm

Hinterman, E. (2022, April 13). [Personal interview].

Hinterman, E., & Hoffman, J. A. (2020). Simulating oxygen production on Mars for the Mars Oxygen In-Situ Resource Utilization Experiment. *Acta Astronautica*, *170*, 678-685. https://doi.org/10.1016/j.actaastro.2020.02.043

Irons, M. (2021). [Personal interview].

Jones, H. W., Hodgson, E. W., & Kliss, M. H. (2014). Life support for deep space and Mars. *International Conference on Environmental Systems*. https://ttu-ir.tdl.org/handle/2346/59729

Kleinhenz, J. E., & Paz, A. (n.d.). An ISRU propellant production system for a fully fueled Mars Ascent Vehicle. *AIAA SciTech Forum*. https://doi.org/10.2514/6.2017-0423

Kotary, N., & Cody, S. (2021, April). *Aboard NASA's Perseverance rover, MOXIE creates oxygen on Mars*. MIT News. https://news.mit.edu/2021/aboard-nasa-perseverance-mars-rover-moxie-creates-oxygen-0421

Lotto, M. A., Klaus, D. M., & Hynek, B. M. (2018). Operational conditions and in situ resources for Mars surface exploration [PDF]. *New Space*, *6*(4), 320-334. https://doi.org/10.1089/space.2018.0019

NASA. (2017, August 3). *Life support systems*. NASA. https://www.nasa.gov/content/life-support-systems

NASA. (2018, January). *Life support baseline values and assumptions document* (M. S. Anderson, M. K. Ewert, & J. F. Keener, Authors). STI, NASA. https://ntrs.nasa.gov/api/citations/20180001338/downloads/20180001338.pdf

Native-Seeds-Search. (2021). *Tepary*. Native Seeds Search. Retrieved June 2, 2021, from https://www.nativeseeds.org/collections/tepary-beans

Organic tepary bush bean seeds. (n.d.). San Diego Seed Company. Retrieved September 31, 2021, from https://sandiegoseedcompany.com/product/vegetables/beans/organic-tepary-bush-bean-seeds/

PimaLib_SeedLibrary. (2019, July 1). *Pima county public library*. https://www.library.pima.gov/blogs/post/now-sowing-tepary-beans-mar15aug10/

Pultarova, T. (2018, November 7). *This space station air recycler could help astronauts breathe easier on Mars*. Space.com. https://www.space.com/42362-space-station-air-recycler-for-mars-astronauts.html

Sanpete Ready. (2020, May 21). *Gardening in Sanpete: Tepary beans*. Sanpete Ready. https://sanpeteready.org/index.php/2020/05/21/gardening-in-sanpete-tepary-beans/

The Scotts Company LLC. (2021). *What goes into Miracle-Gro Potting Mix?* Miracle-Gro. Retrieved June 2, 2021, from https://www.miraclegro.com/en-us/miracle-gro-potting-mix-ingredients

Temming, M. (2020, July 15). *What will astronauts need to survive the dangerous journey to Mars?* Science News. https://www.sciencenews.org/article/astronauts-mars-space-health-survival

Weigel, B. (2018, January 4). *Our first Martian plants*. Medium. https://medium.com/our-space/our-first-martian-plants-e03baad32ee9

Zitter, L. (2020, February 18). *This scientist grows plants for Mars*. Food and Farming Technology. https://www.foodandfarmingtechnology.com/news/harvesting-technology/this-scientist-grows-plants-for-mars.html