Evaluating the Impact of Mycorrhizae Inoculants on Tomato and Kale Growth in Various Martian and Earth Soil Mixtures

Team St. Margaret's Episcopal School

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Background

Earth soil (standard Miracle Grow potting soil) is integral to the growth of plants because it contains organic material that provides limiting nutrients and natural fertilizers. Earth soil is also, as demonstrated in the experiment that follows, more aerated and porous, allowing water to more easily permeate through the soil and saturate plant roots. Mars regolith is much more compact (almost like clay), making it more difficult for water to move through and for fragile roots to establish (and, as stated above, no organic material, which comes from life). As such, Earth soil (or organic matter equivalent) will most likely have to be transported by future Mars-colonizers if they intend to cultivate crops. Minimizing the weight of this soil will be ideal, which is why this experiment attempted to measure the minimum amount of Earth soil necessary to grow crops. This experiment also sought to observe the impact of microbial inoculants (specifically, mycorrhizae fungi purchased from Dynomyco) on plant growth. Mycorrhizae is a fungi which forms a symbiotic relationship with plant roots and helps the plant uptake more nutrients from the soil to promote plant growth. The use of this inoculant goes with the assumption that plants will be grown indoors on Mars, where the microbes are protected from potentially damaging UV-radiation. It is hoped that the inoculants, which weigh less than Earth soil, will help the plants take up adequate nutrients and counteract the impact of the Martian regolith in potting mixtures with higher concentrations of the regolith. We also designed and 3D-printed our own self-watering pots to help prevent under- and over-watering. Research is currently being done on how humans could someday 3D-print materials from Martian dust to build bases and tools (and, perhaps, pots) in order to reduce the amount of supplies future colonizers would have to bring from Earth. We were curious about 3D-printing and figured that the pots would ensure that the plants had access to water even if we were not there every day. We also wanted to see what it would be like to create our own pots from scratch, similarly to how future colonizers will need to use innovation, technology, and creativity to build their own supplies on Mars.

The nine members of our all-girls team during our first meeting on the day we received our soil simulant! From left to right: Rainbow L., Tina M., Alexis L., Eva D., Sophia K., Charlotte Q., Michaela M., Tina M. (x2), Charlotte Q. (x2), Emma G.
Not pictured: *Charlotte N. (she was on Zoom due to quarantine).*

Experimental Design

As described above, one aspect of our experiment was evaluating the impact of different concentrations of Martian regolith on plant growth. In the experiment, we grew plants in a 50% Martian regolith by volume (and 50% Earth soil), a 75% Martian regolith by volume (and 25% Earth soil), and 100% Earth soil for the control pots. We made the assumption that anything higher than 75% regolith would not be able to support significant plant growth and that the control pots would exhibit the most growth. In tandem with varying the concentrations of Mars regolith, we also evaluated the impact of microbial inoculants. For each of the three concentrations (50%, 75%, and 0% Mars regolith) we had two pots, one inoculated with mycorrhizae and one that was not. This would enable us to see the impact of the microbial inoculants alone by comparing pots with the same percentage of Martian regolith. Our layout also enabled us to see if the addition of microbial inoculants in potting mixtures with more Martian soil could exhibit the same amount of plant growth as in potting with less Martian regolith, but no inoculants. (The short answer, no.) Lastly, we chose to grow kale and tomatoes. We researched prior professional martian-growing experiments, and saw that their results often favored kale and tomato growth over other plants. Both plants are also known to be tolerant of extreme conditions. This research and planning was done before we received our soil simulant and supplies.

Note: *Originally this experiment was also planned to evaluate the impact of ammonium nitrate (a soil acidifier) on the growth of tomato plants (since they prefer more acidic soils), and Martian regolith is naturally basic at a pH of 8-9). However, we ultimately decided not to use the soil acidifier in order to focus on the other variables and aspects of our experiment.*

#	Name / Plant	Earth/Regolith %	Soil Additives/Amendments
1	50/50 Kale	50% Earth / 50% Regolith	Potting soil
2	50/50 Tomato	50% Earth / 50% Regolith	Potting soil
3	25/75 Kale	25% Earth / 75% Regolith	Potting soil
4	25/75 Tomato	25% Earth / 75% Regolith	Potting soil
5	Acidic Tomato 50/50	50% Earth / 50% Regolith	Ammonium Nitrate, potting soil
6	Acidic Tomato 25/75	25% Earth / 75% Regolith	Ammonium Nitrate, potting soil
7	Myco Tomato 50/50	50% Earth / 50% Regolith	Myco, potting soil
8	Myco Kale 50/50	50% Earth / 50% Regolith	Myco, potting soil
9	Myco Kale 25/75	25% Earth / 75% Regolith	Myco, potting soil
10	Myco Tomato 25/75	25% Earth / 75% Regolith	Myco, potting soil
11	Control Pot - Kale	100% Earth soil	Potting soil
12	Control Pot - Tomato	100% Earth soil	Potting soil
13	Control Pot - Acidic Tomato	100% Earth soil	Ammonium Nitrate, potting soil
14	Control Pot - Myco Tomato	100% Earth soil	Myco, potting soil
15	Control Pot - Myco Kale	100% Earth soil	Myco, potting soil

Figure 1: *Our so-called "plant map" describing the contents of each of our 15 pots. "Myco" is our shorthand for mycorrhizae.*

Initially, we filled Solo cups (with holes in the bottom to allow for drainage) with the soil mixtures. The plan was to transfer the germinated sprouts to larger pots (the 3D-printed ones) once they established some roots. The Solo cups were then placed in a large plastic bin, which was then loaded into our chemistry teacher's (Ms. Chou's) fume hood. The fume hood had LED lighting, a ventilation system, and a clear front window to prevent curious freshmen from tampering with the experiment. In addition to the fume hood's lighting, we purchased red, blue, and purple colored (the color wavelengths that are most efficient for photosynthesis) grow lights and clipped them to the rim of the clear bin, so that the light source was closer to the plants. In the first few weeks, the lights were left on all day and night.

Figure 2: *The first day of the grow period, depicting the unplanted soil mixtures in the labeled cups.*

Figure 3: *A few weeks into the challenge, the plants are shown from a birds-eye view with the grow lights and some green sprouts!* ***Note:*** *full-spectrum (white) grow lights are shown in the image because the colored ones had not yet arrived, and we thought it would be better for the plants to receive full-spectrum initially, then switch to colored lights.*

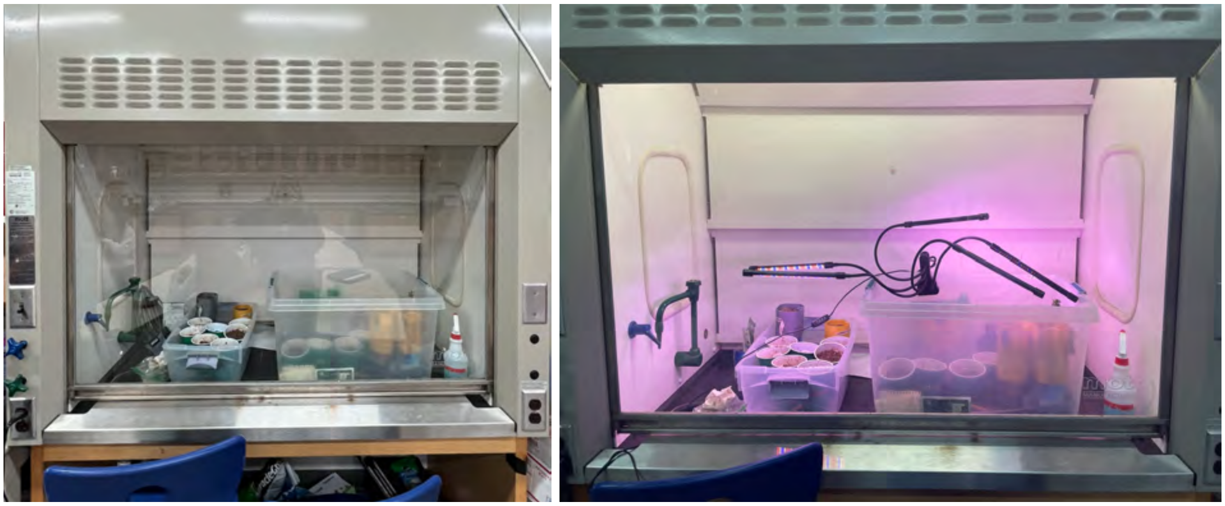

Figure 4: *The outside of the fume hood and the setup, with red and blue LED grow lights.*

3D-printed pots

Another key part of our setup was the 3D-printed pots. The design features a reservoir on top of which rests a funnel with holes in the narrower portion. The reservoir is filled with water, and the planted soil mixture goes into the funnel portion. The plant roots can uptake water through the holes in the bottom of the funnel. Originally, both the reservoirs and the funnels were going to be 3D-printed, but we had trouble getting the right density of lattice to prevent leakage and wanted to generate less plastic waste, so we used recycled Fiji water bottles instead. Because of this, the size and shape of the funnel portion was designed to fit the water bottle. The printer we had access to was relatively small, so we had to model the designs in multiple sections in order to maximize the size of pots we could create on the small printer.

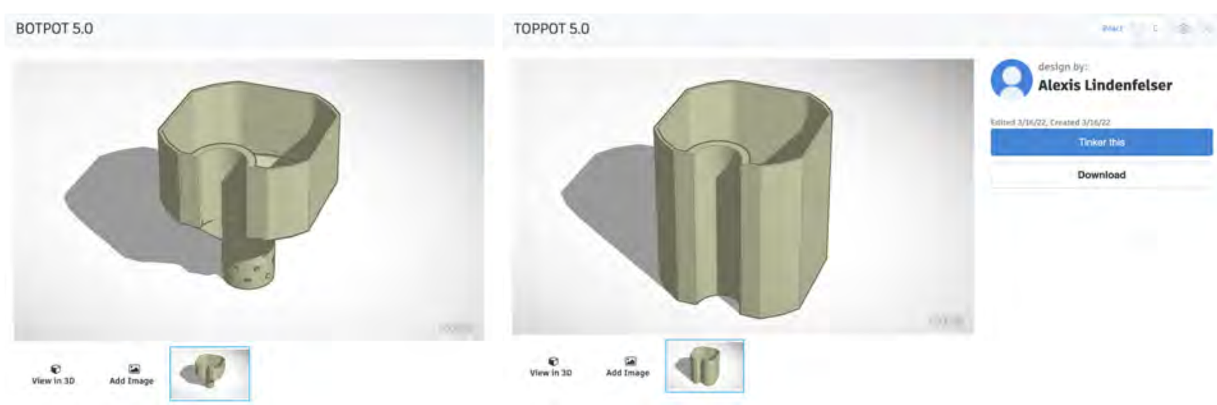

Figure 5: *The final 3D-models (in Tinkercad) of both pieces of our pots. They would be later glued together and placed on top of the reservoir. The semicircle cutout in the side enables water to be poured into the reservoir to refill it without lifting up the funnel and disturbing the plants.*

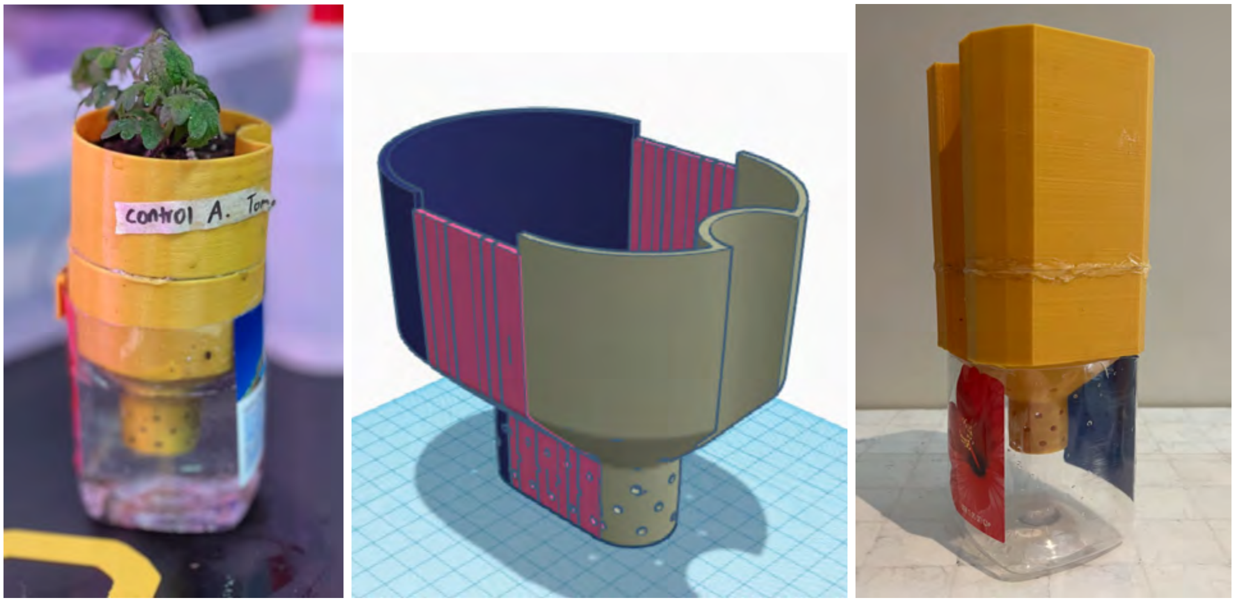

Figure 6: *Multiple iterations of our pots.* ***Left:*** *The second iteration was redesigned because it was too small.* ***Middle:*** *The third iteration, oblong to maximize volume, but impractical for a reservoir.* ***Right:*** *The final iteration, finding a perfect balance between size and shape.*

Hypothesis

We predicted that plants will grow more (have a greater height) in pots that have a lower percent-by-volume of Martian regolith, since Earth soil is generally better for growing plants. We also predict that the pots receiving microbial inoculants will exhibit more growth because of the benefits that mycorrhizae has on nutrient uptake.

Variables

- **Independent Variable**
  The independent variables in this experiment was the ratio of Earth soil to Martian soil as measured in percent-by-volume and the presence or absence of mycorrhizae.
- **Dependent Variable**
  The dependent variable was the height of the plant measured after growing in the soil mixture.
- **Controls**
  The controls for this experiment was that each pot received the same amount of water and all were equally exposed to the grow lights. Temperature was also kept constant.

Measurements

We monitored the height of the plants in centimeters (based on the height of the tallest leaf/sprout) weekly, and also wrote down observations on the appearance of the soil and plants. Additionally, we added to the observations log everytime we made a change to the potting mixtures contents. We attempted to measure the pH of the potting mixtures using the provided pH strips, but our data kept coming out the same for every single pot (at a pH of around 7). These readings seemed inaccurate, which was part of the decision to not add the soil acidifier, since we did not want to risk killing the already-fragile tomato plants in a hostile growing environment. We later learned that leaving pH strips to dry on white paper (to better see the color) caused them to take on the pH of the paper, not the soil. As a result, we unfortunately had to void all our pH data.

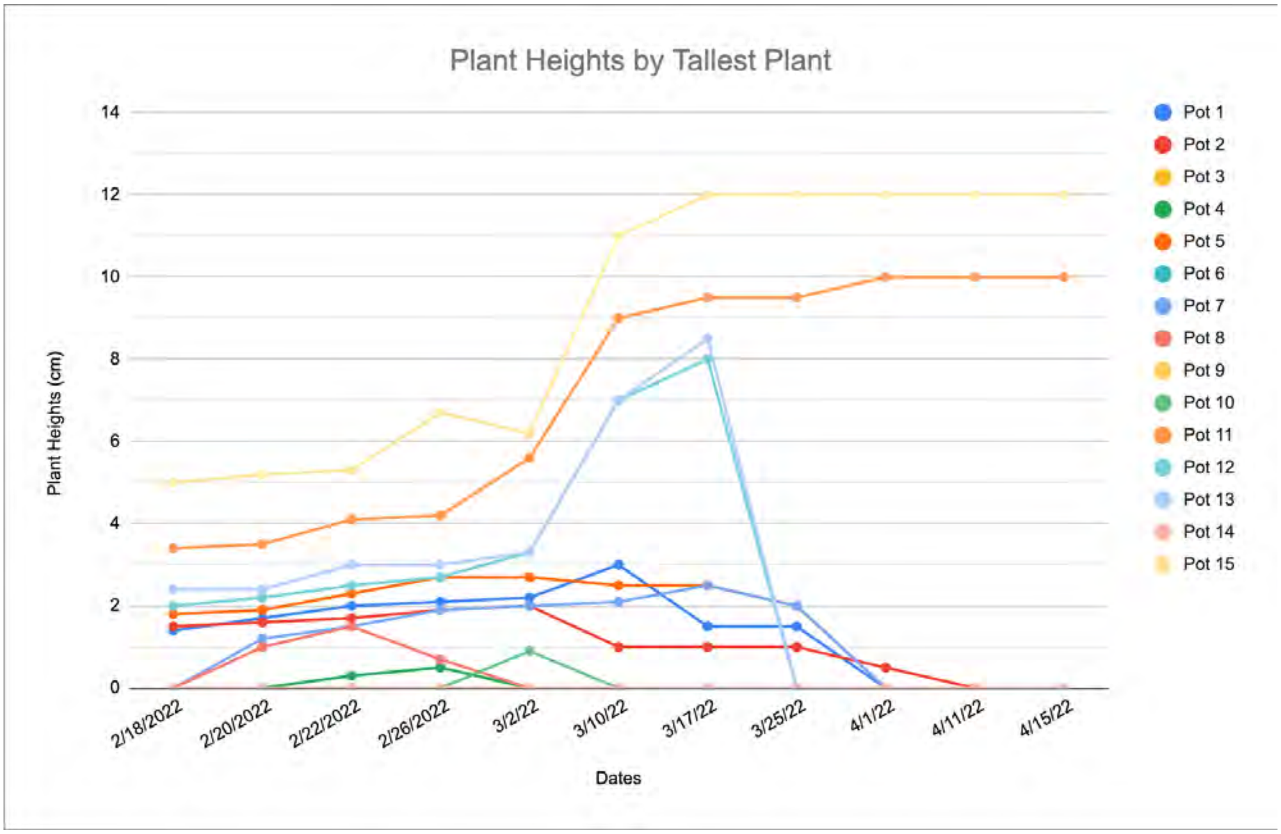

Figure 7: *Tracking of the plant heights. A height of 0 represents a plant that was not exhibiting growth.*

Pot #	Name
1	50/50 Kale
2	50/50 Tomato
3	25/75 Kale
4	25/75 Tomato
5	Acidic Tomato 50/50
6	Acidic Tomato 25/75
7	Myco Tomato 50/50
8	Myco Kale 50/50
9	Myco Kale 25/75
10	Myco Tomato 25/75
11	Control Pot - Kale
12	Control Pot - Tomato
13	Control Pot - Acidic Tomato
14	Control Pot - Myco Tomato
15	Control Pot - Myco Kale

Figure 8: Left: *A picture of the plants from 2/15 (pre-self watering pots).*
Middle: *This legend matches the pot number (as shown in* ***Figure*** *7) to the contents of each pot. Control pot represents a 100% Earth soil mixture.* ***Right:*** *A picture of the plants from 3/2 (when growth was at its peak).*

Date	Observations	Adjustments
2/7-2/8	-Martian regolith is red and has very fine particles -Martian regolith is much softer to the touch, but much denser compared to Earth soil -Earth soil remains relatively light, more rough, dark brown in color -Wet Martian regolith forms a very muddy texture on top, but remains dry underneath -Wet Earth soil is moist and not nearly as muddy → water distribution throughout soil is even, the underneath is wet	-Plant the seeds and add water -Do **not** add inoculants yet (per box instructions)
2/10	-Martian regolith appears to be extremely dry → Dries out much quicker compared to Earthen soil -Martian regolith dries in the shapes of how water moves the regolith → forms small, hardened peaks and divots -Earth soil is also relatively dry, but not nearly as much as Martian regolith -Earth soil is also significantly softer than the Martian regolith when dry → dried Martian soil is rock hard and difficult to break apart	-Aerate soil by mixing
2/11	-Martian regolith is dry → soil is not very permeable as water gathers on top and takes a while to filter down through the cup -Earthen soil absorbs water with relative ease -After Martian regolith dries it looks like clay, becomes extremely hard and jagged	-Water, aerate soil by mixing, turn down light intensity for the weekend (hope that they survive)
2/14	-Small, flat, white formations along the sides of cups appearing (mold? calcium deposits?) -Some germination in lower regolith concentration pots → small, green sprouts around 1-2 cm in height -Martian soil still very dry → more consistent watering methods required (consider piercing the soil and watering to place water into lower levels of soil)	
3/10	-Plants are either not growing at all or are so big that they are growing outside of cup the cup (need to transfer to bigger pot) → the controls are experiencing the most growth currently around 3-5 cm -Some Martian plants are looking like slimy and wilting; slight yellow discoloration → might be due to Martian soil's inefficient water distribution/drainage -Many of the plants that grew in Martian regolith showed mixes of green and red inside the plant itself → discoloration -Small, white, dry calcium build up/salt deposits still present on the interior walls of the cups	-Plant more seeds in pots that exhibit no growth, in case the original seeds were infertile -Need to add acidic additive and myco as per instructions
3/17	-Last week we added new seeds to the pots that are not growing in case the seeds are infertile → experiencing a lack of growth -Currently working on transferring the crops to the bigger self-watering pots → Plants reached around 6-8 cm in length and need more space -Control plants are experiencing the most growth and need transfers → Strong, green, & healthy -The white calcium deposits are have disappeared -Some plants that we replanted in the higher concentration of regolith appear to have wilted or dried out -Regolith soil is still very dry and hard	-5g of mycorrhizae added to each myco-designated pot

	-The plants that have grown are doing really great!	
3/28	-Many plants are struggling to grow in the Martian soils → little to no growth -A few sprouts present in the 50/50 and 75/25 samples, but the sprouts are small and wilting → indicating plant death soon -Starting to see some discoloration in the tomato plants for the controls → wilting at ends of leaves + yellow leaves -Control kale plants are still green, strong, and healthy -Earth soil is slightly dry, but remains relatively soft -Martian soil is still very dry and very jagged and hard	-Realized that the colored lights are killing plants, switched back to white/full spectrum
4/14	-Most plants are no longer growing, with the exception of the control pots -Kale control pots were the most successful and remain green, strong and healthy -One control tomato has completely died → the heat from growth lights caused the leaves to wilt, turn yellow, then it shriveled up and died -The other control tomato remains around 5 cm and has a slight wilting, but is still green -Earthen soil appears dry, but still soft and airy -Martian soil remains rockhard and without growth → the underbelly of the Martian soil is also dry but much softer and fine like from Week 1 -White deposits have largely vanished	

Figure 9: *Observations over the course of the experiment when we made major changes. Note: The self-watering pots are sprinkled in as we gradually re-planted the larger plants. We did not end up printing a full set of pots because it would not make sense to replant pots with no growth.*

Figure 10: *Close-ups of the soil and some sprouts early on. The white deposits mentioned in the observations are seen in the bottom right corner of the middle image.*

Results

Honestly, life is quite brutal for an Earth plant trying to establish roots in a Martian environment. Overall, the data shows that, ultimately, kale was better at surviving than the tomatoes. This might have had something to do with the pH, as we predicted that the increased alkalinity of Martian soil could be detrimental to acid-loving tomatoes. It was difficult to evaluate the impact of the mycorrhizae on our plant growth because many of the plants did not survive the entire Grow Period. However, the data shows that the kale control pot inoculated with mycorrhizae grew about 3 inches taller than the kale control pot that was not inoculated. The self-watering pots seemed to have a positive impact on the growth of the plants, because they enabled the plants to uptake water both through the roots (wetting the bottom half of the soil) and we could sprinkle water on the plants from above, helping mitigate some of the negative impact that the impermeable Martian soil had on growth. When we cleaned up our experiment after the end of the Grow Period (after a few days neglecting to water the plants) and poured out the potting mixture from those pots, we saw that the top layer of dirt was dry and crackly, while the lower layer was more moist.

Figure 11: *A final snapshot of our plants and experimental setup.*

Discussion & Conclusion

In exploring the growth of plants in Martian conditions, there are still a few factors we chose to omit from the experiment. We assume that in a situation where plants are being grown on mars, it would be done indoors in an environment with normal gravity. Gravity is said to affect how water permeates into the soil, but because of this assumption, we decided not to test the impacts of reduced gravity on plant growth. Wind storms, which would also be a concern in outdoor growing environments, are not an issue in indoor conditions. Another concern in relation to Mars regolith are perchlorates. Perchlorates have a strong attraction to water and lower its freezing point. But while a concern for real Martian dust, perchlorates are not included in any of Exolith's simulants because they are dangerous to humans.

In this experiment, we were probably too ambitious with the amount of variables we wanted to evaluate. The complicated nature of our experimental design probably made our experiment more hypothetical than actually realistic for a group of non-gardener students to figure out in a 10-week period. In other plants, our plants probably would have been more successful if we simplified our experiment and focused more on cultivation and tending to our plants than manipulating variables. If we ever try this challenge again in the future, we would have focused our experiment on manipulating just one variable (e.g., Martian to Earth soil ratio) and making sure to check up on our plants as frequently as possible.

But, I suppose they say "Shoot for the moon, even if you miss, at least you'll still land amongst the stars." In our case, we shot for Mars, maybe missed, but still learned a lot on designing and conducting experiments and how to work together. Thank you to the Plant the Moon Challenge for this amazing experience!

Figure 12: *"We planted Mars!"* ***From left to right:*** *Charlotte Q., Michaela M., Sophia K., Alexis L., Charlotte N. (on Zoom), Tina M., Emma G.*

Sources

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