

Effect of the compound used as a nitrogen source on Cherry Belle radish (*Raphanus sativus*) plant growth

Plant the Moon Challenge | Spring 2023 | Professional/Undergraduate Division

Minnesota West Bluejays | Team # 12926

Minnesota West Community & Technical College

Worthington, MN

Selam Alemayehu, Julian Bennett, Brandon Berghorst, Leidy Chavez Martin, Devaughn Demus, Taya Denroche, Irma Hernandez, Antonio Hesser-Leiva, Christian Lopez, Dylan Markus, Arlene Martinez Cambara, Irving Mendez, Emmot Nathan, Aaliyah Petersen, Taylor Pronto, Deante Porter, Lolita Ramirez Velasquez, Omar Robles, Simon Spittle, Paul Troe, Vant Tucker, Du'Vaughn Williams, Dr. Tyler Wadzinski, and Heidi Tarus

Advised by Heidi Tarus



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Introduction

As humans on Earth look towards future colonization of celestial bodies, we must be able to address the basic needs of colonizing humans including access to protective shelter, clean and safe water, and nutritious food. This project focuses on addressing the human need of access to nutritious food that can be grown on the Moon. In order for plants to be grown for food, they need access to water and essential nutrients including potassium, phosphorus, and nitrogen. Prior studies show that most plant essential nutrients are available in the composition of lunar regolith with the exception of useful nitrogen (Wamelink et al., 2014). Therefore, this study focuses on the most efficient source of nitrogen needed to allow plants to grow while reducing the amount of payload required on a spacecraft. Nitrogen is an essential macronutrient for plants and is used in the synthesis of proteins, nucleic acids, and some vitamins needed for growth (Clark et al., 2018). The main driving question for this project is to discover the trade-off between limiting the mass of nitrogen fertilizers brought to the moon while producing the highest yield of nutrient rich crops needed to feed individuals living on the moon.

Theory

The nitrogen sources tested (independent variable) were calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), ammonium nitrate (NH_4NO_3), and urea ($\text{CO}(\text{NH}_2)_2$). The three nitrogen sources have different molar masses and nitrogen percent compositions. They also fall into different parts of the nitrogen cycle, which makes comparing the trade-off between reducing the mass added to the spacecraft and the success of plant growth an important question. Calcium nitrate tetrahydrate has the lowest percent composition of nitrogen, requiring 8.4 g of compound per gram of nitrogen delivered. The nitrogen in calcium nitrate tetrahydrate is available

immediately to plants in the nitrate form; however, nitrate is water soluble and thus susceptible to leaching which is a major cause of nitrogen loss in soil. Ammonium nitrate has a higher percent composition of nitrogen and requires 2.9 g of compound per gram of nitrogen delivered. Ammonium nitrate provides a combination of 50% nitrate which is usable to plants right away and 50% ammonium which must be fixed by nitrogen fixing bacteria to nitrate before it is usable by plants (Isleib, 2017). Therefore, ammonium nitrate is a good source of short-term and long-term nitrogen. Urea has the highest mass percent of nitrogen and only requires 2.1 g of compound per gram of nitrogen delivered, which makes it the most preferred nitrogen source in regard to reducing rocket payload mass. However, urea must go through a three-step process where it is converted to ammonia, then ammonium, and then nitrate before plants can use it (Mengel n.d). Similar to nitrate, urea is water soluble prior to being converted, making it susceptible to leaching. An additional benefit of urea is that it can be locally sourced and extracted from urine produced by individuals living on the moon. Since the calcium nitrate tetrahydrate and the ammonium nitrate nitrogen sources being used in this experiment need to be fixed by nitrogen fixing bacteria the lunar soil will be amended with 25% top soil which will contain the nitrogen fixing bacteria needed for this process to occur.

The team decided to plant Cherry Belle radishes (*Raphanus sativus*) because they are container friendly, have a fast germination time of four to six days with 22 days to harvest, and retain their taste quality for a long time after harvest to prevent food waste. The nutritional value of Cherry Belle radish bulbs is high. According to the U.S. Department of Agriculture (2019), one medium (3/4" – 1" diameter) cherry belle radish bulb (4.5g) contains 0.72 calories, 0.031g of protein, 0.072g of fiber, 0.153g of carbohydrate, and 0.004g of fat. In addition, *R.*

sativus are an excellent source of many essential vitamins and minerals for humans including vitamin C, Vitamin B6, riboflavin, niacin, thiamine, folate, potassium, iron, manganese, and calcium (Begum, 2022). Not only are the radish bulbs able to be used for food, but so are the stems, leaves, and roots, making it an efficient plant to produce. *R. sativus* have many uses in foods like salads, chicken sandwiches, and many other foods. *R. sativus* also has medicinal value and can be used as an antibacterial when you extract its alcohol (Perera, 2022). Due to the plant choice by the team, a second soil amendment of 25% vermiculite was added. Vermiculite is a lightweight magnesium-aluminum-iron silicate that is used for water and nutrient retention (Gwmag, 2021). In addition, vermiculite can help aerate the soil to reduce the amount of compaction that occurs when the lunar regolith is watered. Making sure the soil is not compact is important radish bulbs will only develop in loose soil which allows the roots to thicken into bulbs (Grant, 2021).

The data collected in the experiment (dependent variables) will include the primordial leaf emergence for germination rate, plant height, final leaf surface area, radish bulb mass, and pH of the pots. Past studies show that *R. sativus* plant height, final leaf surface area, and radish bulb mass are directly related to the amount of nitrogen added to the soil (Jiliani et al, 2010 and Yousaf et al, 2021). Therefore, we conclude that the listed dependent variables will be good proxies to show nitrogen uptake in the treatment groups.

We hypothesized that (1) the nitrogen source will not influence the germination (primordial leaf emergence) rate of the *R. sativus* plants, (2) the *R. sativus* plants grown in pots containing a nitrogen source will produce a higher leaf height growth rate, a larger leaf surface area, and the largest bulb compared to the control group and (3) out of the three nitrogen

sources being tested, ammonium nitrate (NH_4NO_3) will produce the highest leaf height growth rate, the largest leaf surface area, and the largest bulbs due to the ability of ammonium nitrate to provide both fast acting short-term nitrate and slower acting long-term nitrate that goes through fixation.

Experiment & Measurement Methods

Initial Set-Up

The independent variable for this experiment was the type of nitrogen-containing compound used as a nitrogen source. The experimental set-up included four treatments, (1) control (no nitrogen source), (2) calcium nitrate tetrahydrate, (3) ammonium nitrate, and (4) urea with each treatment having 8 replicates (Figure 1). Each pot (7cm x 7cm x 7.7cm) contained the same soil composition of 25% top soil, 25% vermiculite, and 50% lunar regolith by volume. Rational for this soil mixture is addressed in the theory section above. Each nitrogen source was weighed as a pure solid and then mixed into the soil to achieve the recommended fertilizing loading rate of 0.5 lb N per 1000 ft² (Swift & Hammond, 2020; Kokemuller 2016; Table 1). Each pot was watered with 25ml of water.

The larger team of 22 students formed eight sub-teams that contained one to three students per team. Each sub-team was responsible for one pot per treatment. The pots were identified by a colored popsicle stick placed in the back of the pot with the team's name written on it. The eight team names were Tarus, DIL, DO, ATT, ND, Agricultural Space Scientists (A.S.S.), I.M., and Ambiguity. The team planted each treatment pot with 3 Burpee Cherry Belle radish (*Raphanus sativus*; Packed for 2023, Lot 7) seeds and watered with 25ml of tap water. Over the course of the eight-week grow period, the set-up was kept indoors with a temperature of 22°C

with the set of pots placed under a grow light containing 2-18" GE plant and aquarium bulbs on a 12-hour light: 12-hour dark timer. The grow light was placed 12 cm from the top of the pots (Figure Set 1).

Figure 1. Diagram of experimental set-up showing the four treatment groups (control, calcium nitrate tetrahydrate, ammonium nitrate, and urea) each with eight replicate pots for a total of 32 pots.

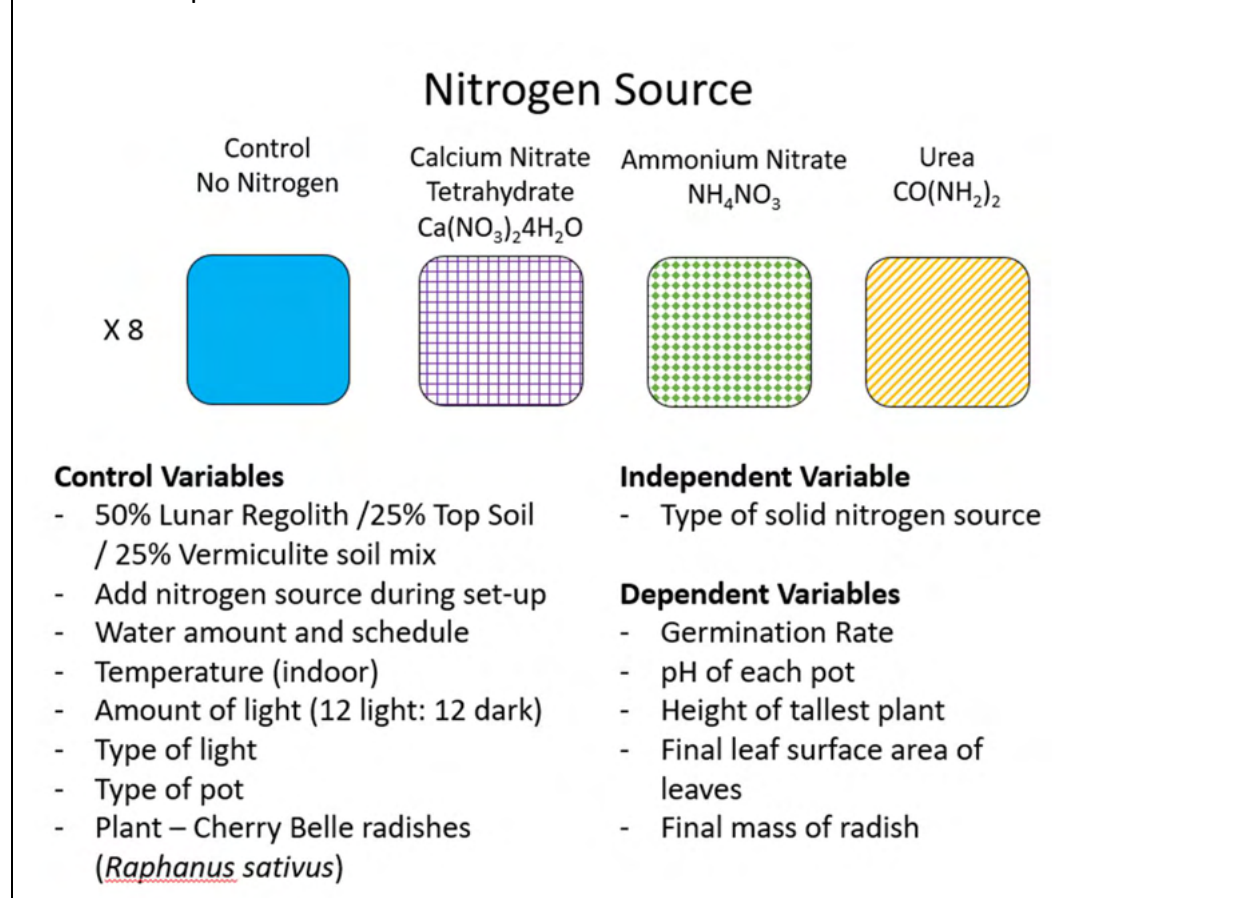


Table 1. Plant the Moon Spring 2023 Fertilizer Application Calculations

formula	molar mass (g/mol)	mass % comp N	g of cpd per ft ²	area of pot in ft ²	g of cpd per pot	moles N per ft ²
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	236.15	11.9	1.912	0.054	0.104	0.0162
NH_4NO_3	80.043	35.0	0.648	0.054	0.035	0.0162
$\text{CO}(\text{NH}_2)_2$	60.06	46.6	0.486	0.054	0.026	0.0162

Image Set 1. Pictures of the initial plant growth set-up on February 16, 2023.



Plant Care

Each pot was top watered with 25ml of distilled water on Tuesday and Thursday each week during the grow period. All pots were watered on the same schedule independent of the presence of seedling or no seedlings. According to the seed packet, the *R. sativus* should be thinned to be at least 5.08 cm (two inches) apart; therefore, as the pots are 7cm in width, the plants were thinned to one seedling on March 9, 2023 by keeping the tallest plant in the pot.

Thinning is a common practice in gardening and was done to give the tallest (strongest) seedling in each pot the best chance to grow. The plants were kept in the same room through the duration of the experiment and were moved away for the light source for a maximum of 30 minutes on Thursday at 1pm to take measurements and then replaced back in the same location after measuring.

Measurements

The dependent variables for this experiment were primordial leaf emergence for germination rate, plant height, final leaf surface area, radish bulb mass, and pH of the pots. Primordial leaf emergence was used to collect data on germination rate of the plants. Germination rate was collected once by visually observing of the number of germinated seeds 14 days after planting. The soil pH measurements and plant height measurement were collected by sub-team members once a week each on Thursday using the soil pH meter sent with the lunar regolith and a 15-cm ruler. Leaf surface area was collected during the final week on Tuesday, April 4, 2023 using the LeafByte App, <https://zoegp.science/leafbyte>. This was done by holding up a scale (a white piece of paper with four black dots in the shape of a square) behind the leaf, taking a picture with a smart phone, identifying the scale, and having the app calculate the square centimeters of the leaf (Image Set 2).

Image Set 2. Process of using the LeafByte App.

Step 1. A picture is taken with a known scale made up of four black dots arranged in a square.



Step 2. The scale is indicated using red 'x's.



Step 3. The app calculates the surface area:



Final radish bulb size data was collected by carefully removing the soil out of the pots by pouring the soil into a black plastic growing tray, removing the plants from the soil in the tray, washing off excess soil from the roots, and placing the plants on a paper towel labeled with the treatment and the popsicle stick containing the team name. Unfortunately, radish bulbs did not develop in the soil (Appendix B: Photos) so no quantitative data was collected on radish size. Images were taken of the plants to document the qualitative results.

In addition, a top-view and side-view picture of the entire set-up was taken twice a week on Tuesday and Thursday after watering the pots (Appendix B: Photos). Records of the data collected was kept on paper next to the set up and was entered each week into a Microsoft Excel spreadsheet each week for data analysis.

Analysis & Results

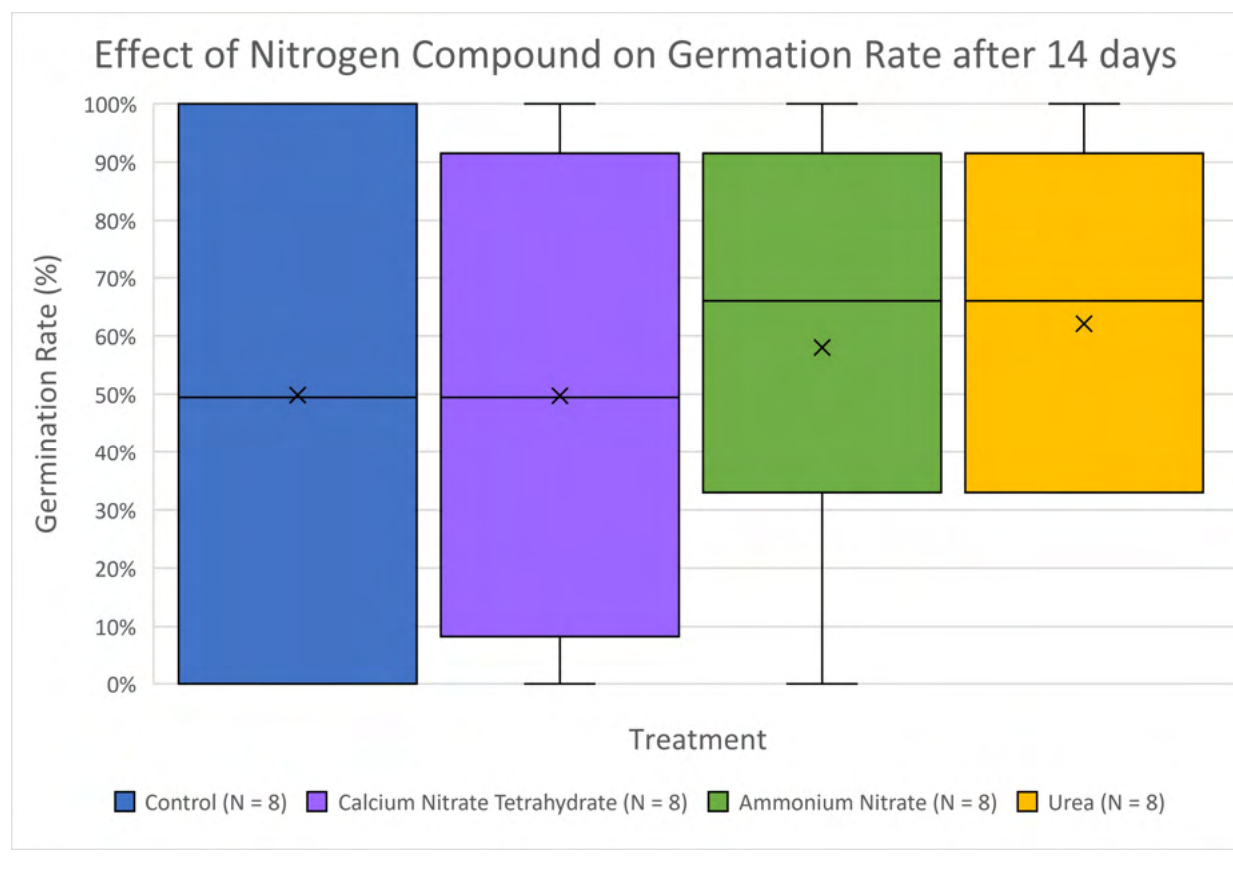
Data for each sub-team was written down on paper data collection sheets and entered into a Microsoft Excel spreadsheet each week. The paper data collection sheets were stored in the biology lab and electronic copies of the data was stored on Tarus' laptop computer. The data was organized and graphed using Microsoft Excel. Statistical tests for the means, standard errors, rates using linear fit, Shapiro-Wilk tests for normality, Kruskal Wallis H tests, and ANOVA tests were performed using the free online website Statistics Kingdom

(<https://www.statskingdom.com/>).

Soil pH & Germination Rate

The soil pH of each pot did not change over the course of the eight weeks and stayed at a pH of 8 (Appendix A Table 5). Because the pH did not change over time statistical analysis was not done on this data. The germination (primordial leaf emergence) data collected on day 14 shows that the seeds had a mean $\pm$ standard error germination rate of $50\% \pm 16\%$ in the control group, $50\% \pm 13\%$ in the calcium nitrate tetrahydrate group, $58\% \pm 11\%$ in the ammonium nitrate group and $62\% \pm 9\%$ in the urea group (Appendix A Table 2 and Figure 2).

Figure 2. Box plot with whiskers showing the effect of the compound used as a nitrogen source on average germination rate (primordial leaf emergence) at 14 days after planting on March 2, 2024. Each treatment group contained eight pots (N = 8) where three seeds were planted per pot.



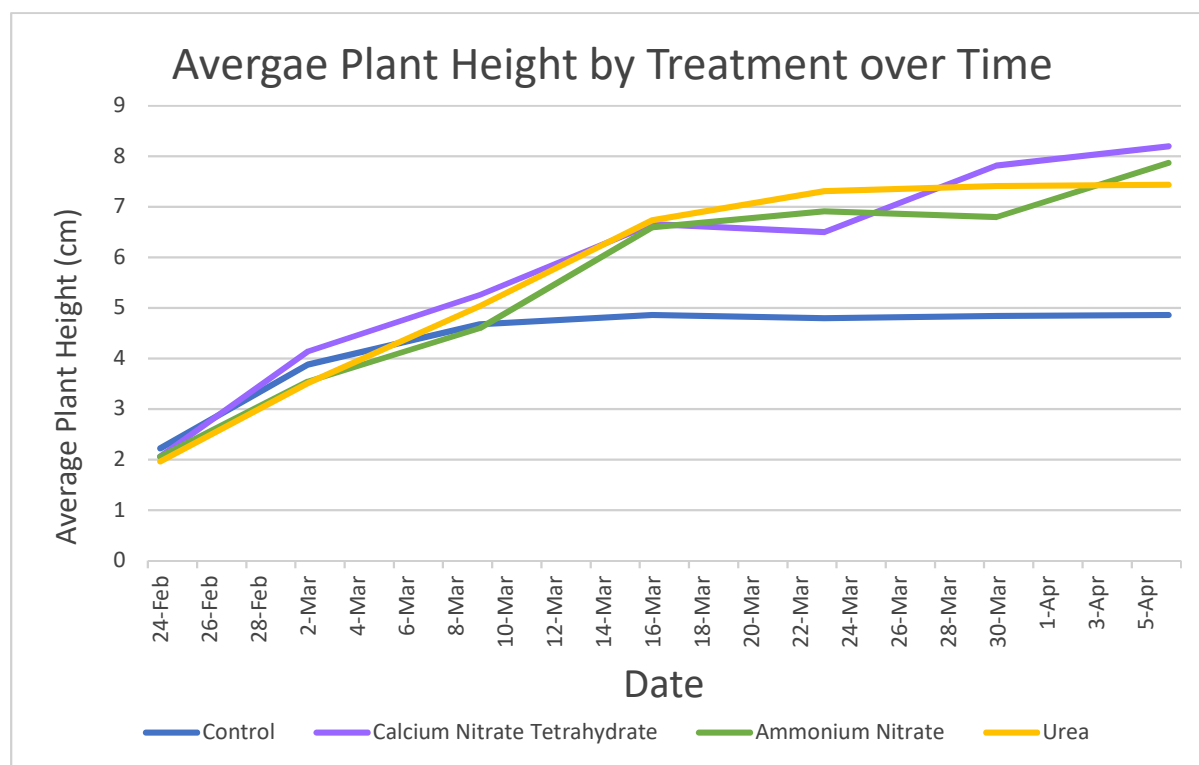
A Shapiro-Wilk tests showed a significance departure from the normality for the control group, $W(8) = .799$, $p = .035$, but did not showed a significance departure from the normality for the calcium nitrate tetrahydrate group, $W(8) = .897$, $p = .297$, the ammonium nitrate group, $W(8) = .918$, $p = .456$, or for the urea group, $W(8) = .835$, $p = .075$. Because the data did not show normality for all four groups, a Kruskal-Wallis H test was used to analyze the between group data. The Kruskal-Wallis H test indicated that there was a non-significant difference in the germination rate between the four groups, $\chi^2(3) = 0.53$, $p = .912$, with a mean rank score of

15.5 for the control group, 15.25 for the calcium nitrate tetrahydrate group, 17.19 for the ammonium nitrate group, 18.06 for the urea group.

Plant Height Growth Rate

Plant height was measured each week and the data was analyzed using a linear regression model to show plant height growth over the grow period. The linear regression model calculated the average rate of plant height growth during the measurement period was 0.05082 cm/day for the control group (X predicted Y , $R^2 = .59$, $F(1,5) = 7.23$, $p = .043$), 0.1393 cm/day for the calcium nitrate tetrahydrate group (X predicted Y , $R^2 = .92$, $F(1,5) = 56.68$, $p < .001$), 0.1356 cm/day for the ammonium nitrate group (X predicted Y , $R^2 = .91$, $F(1,5) = 48.13$, $p < .001$), and 0.1366 cm/day for the urea group (X predicted Y , $R^2 = .86$, $F(1,5) = 31.5$, $p = .002$; Appendix A Table 3 and Figure 3).

Figure 3. Effect of compound used as a nitrogen source on plant height.



Shapiro-Wilk tests did not showed a significance departure from the normality for plant height growth rate in the control group, $W(5) = .937$, $p = .808$, the calcium nitrate tetrahydrate group, $W(5) = .832$, $p = .178$, the ammonium nitrate group, $W(7) = .842$, $p = .116$, or the urea group, $W(8) = .848$, $p = .100$. Because the data show normal distribution, a one-way ANOVA test was used to analyze the between group data.

A one-way ANOVA was performed to compared the effect of the compound used as a nitrogen source on plant height growth rate. The test revealed that there was a statistically significant difference in the plant height growth rate between at least two groups ($F(3,21) = [8.1784]$, $p = 0.0008508$). Tukey's HSD Test for multiple comparisons found that the mean value of plant height growth rate was significantly different between the control and the calcium nitrate tetrahydrate group ($p = 0.003207$, 95% C.I. = $[0.02713, 0.1416]$), the control and the ammonium nitrate group ($p = 0.001572$, 95% C.I. = $[0.03052, 0.1411]$), and the control and the urea group ($p = 0.003207$, 95% C.I. = $[0.02713, 0.06135]$). There was no statistically significant difference in plant height growth rate between the calcium nitrate tetrahydrate and ammonium nitrate group ($p=0.9978$), the calcium nitrate tetrahydrate and urea group ($p=0.9991$) or between the ammonium nitrate and urea group ($p=0.999$).

ANOVA for Plant Height Growth Rate

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	3	0.02971	0.009904	8.1784	0.0008508*
Within Groups	21	0.2543	0.001211		
Total	24	0.5514	0.002298		

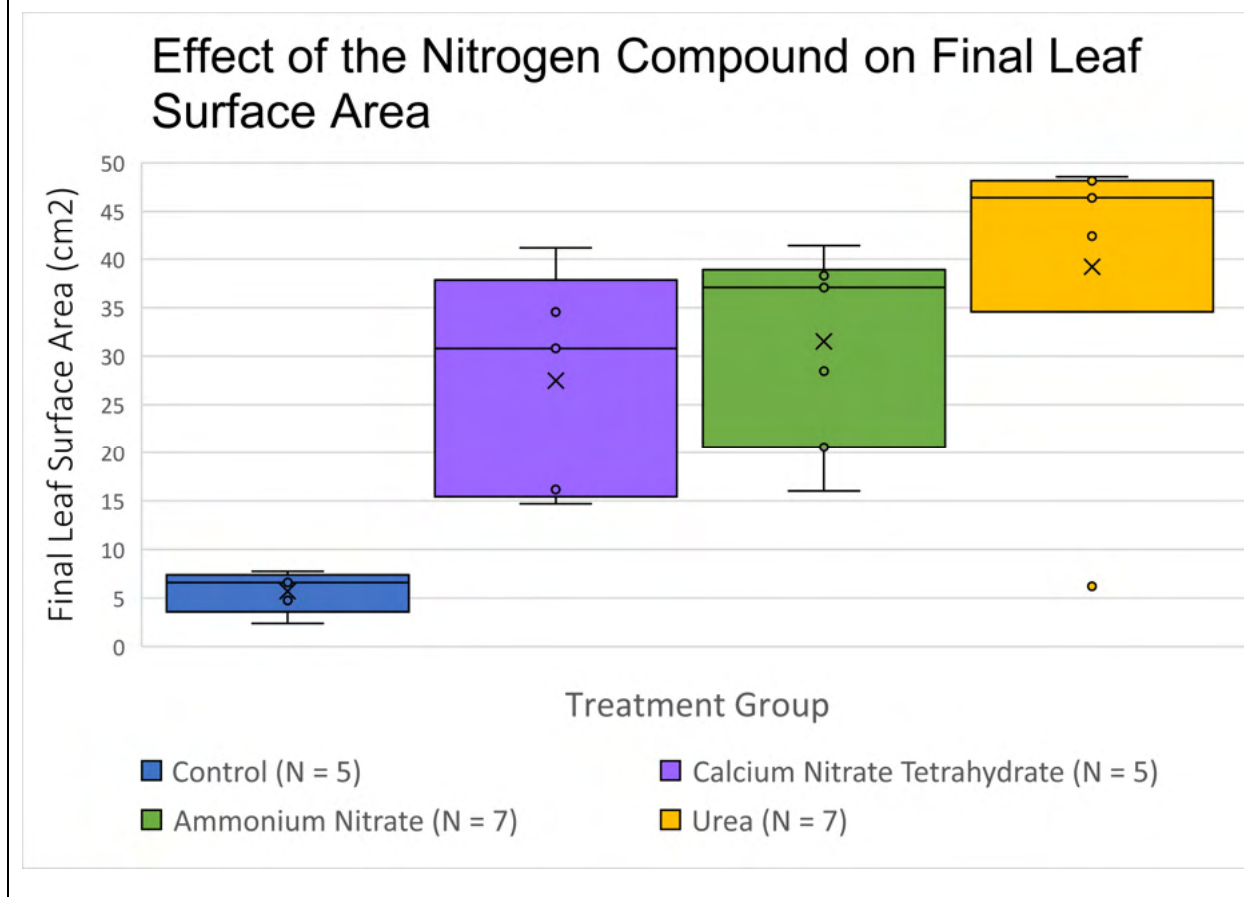
Multiple Comparisons - Tukey HSD

		Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	calcium nitrate tetrahydrate	0.08848	0.01556	0.003207*	0.02713	0.06135
	ammonium nitrate	0.0848	0.01441	0.002308*	0.028	0.1416
	urea	0.08582	0.01403	0.001572*	0.03052	0.1411
calcium nitrate tetrahydrate	control	0.08848	0.01556	0.003207*	0.02713	0.06135
	ammonium nitrate	0.003683	0.01441	0.9978	-0.05311	0.06048
	urea	0.002663	0.1403	0.9991	-0.05263	0.05796
ammonium nitrate	control	0.0848	0.01441	0.002308*	0.028	0.1416
	calcium nitrate tetrahydrate	0.003683	0.01441	0.9978	-0.05311	0.06048
	urea	0.00102	0.01274	0.9999	-0.04918	0.05122
urea	control	0.08582	0.01403	0.001572*	0.03052	0.1411
	calcium nitrate tetrahydrate	0.002663	0.1403	0.9991	-0.05263	0.05796
	ammonium nitrate	0.00102	0.01274	0.9999	-0.04918	0.05122

Final Leaf Surface Area

The final leaf surface area data collected on day 47 shows that the plants in the control group had a mean $\pm$ standard error leaf surface area of $5.715 \pm 0.86 \text{ cm}^2$ in the control group, $27.489 \pm 4.64 \text{ cm}^2$ for the calcium nitrate tetrahydrate group, $31.543 \pm 3.49 \text{ cm}^2$ for the ammonium nitrate group had, and $39.223 \pm 5.38 \text{ cm}^2$ for urea group (Appendix A Table 4 and Figure 4).

Figure 4. Box plot with whiskers showing the effect of the compound used as a nitrogen source on final leaf surface area (cm²).



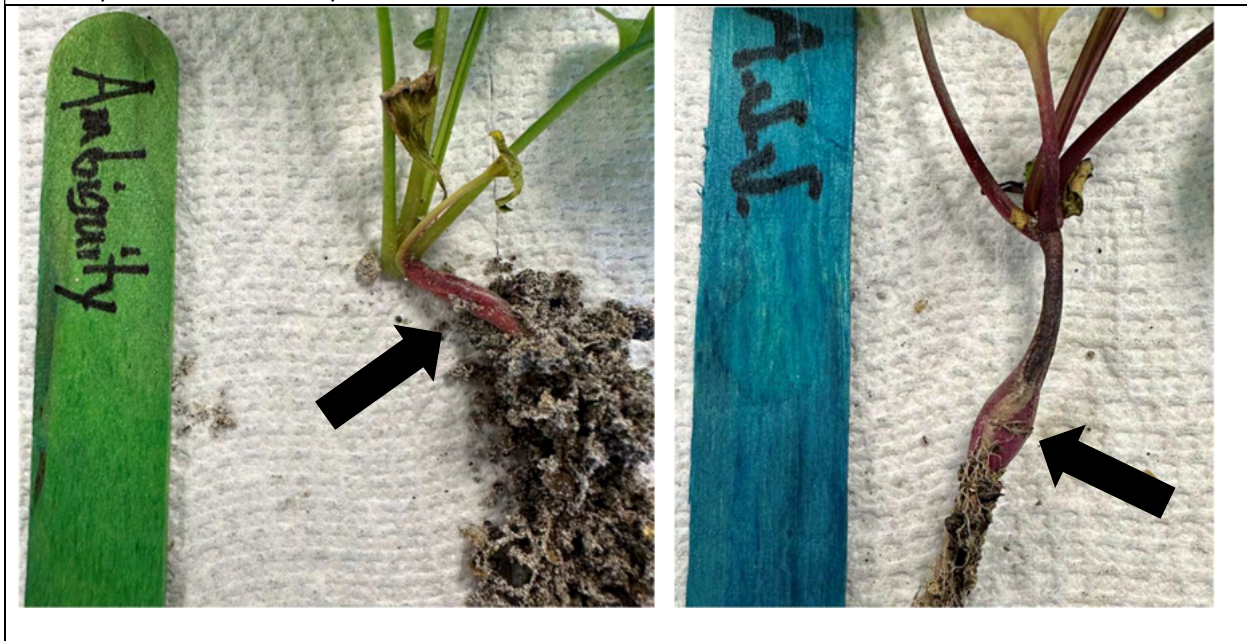
A Shapiro-Wilk tests showed a significance departure from the normality for the urea group, $W(7) = .692$, $p = .007$, but did not showed a significance departure from the normality for the control group, $W(5) = .909$, $p = .576$, the calcium nitrate tetrahydrate group, $W(5) = .899$, $p = .502$, or the ammonium nitrate group, $W(7) = .865$, $p = .187$. Because the data did not show normality for all four groups, a Kruskal-Wallis H test was used to analyze the between group data. The Kruskal-Wallis H test indicated that there is a significant difference in the final leaf surface area between the different groups, $\chi^2(3) = 12.63$, $p = .005$, with a mean rank score of 3.6 for the control group, 11.8 for the calcium nitrate tetrahydrate group, 13.71 for the

ammonium nitrate group, 18.14 for the urea group. The Post-Hoc Dunn's test using a Bonferroni corrected alpha of 0.0083 indicated that the mean rank of the between the control group and the urea group is significantly different.

Final Radish Bulb Mass

As discussed in the measurement methods section radish bulbs did not develop in the soil (Appendix B: Photos) so no quantitative data was collected on radish bulb size. Images were taken of the plants to document the qualitative results. After viewing the images, it was noticed that two plants in the ammonium nitrate group show slight swelling in the area where the radish bulbs are supposed to develop (Image Set 3); regrettably, the plants were disposed of prior to this discovery and thus quantitative data was not collected.

Image Set 3. Pictures of team Ambiguity and Agricultural Space Scientists (A.S.S.) plants from the ammonium nitrate treatment showing swelling (black arrows) in the area where radish bulb development should take place.



Discussion & Conclusion

We hypothesized that (1) the nitrogen source will not influence the germination (primordial leaf emergence) rate of the *R. sativus* plants, (2) the *R. sativus* plants grown in pots

containing a nitrogen source will produce a higher leaf height growth rate, a larger leaf surface area, and the largest bulb compared to the control group and (3) out of the three nitrogen sources being tested, ammonium nitrate (NH_4NO_3) will produce the highest leaf height growth rate, the largest leaf surface area, and the largest bulbs due to the ability of ammonium nitrate to provide both fast acting short-term nitrate and slower acting long-term nitrate that goes through fixation.

Data from the experiment supported the first hypothesis. There was no significant difference between the treatment groups and the primordial leaf germination rate. The main external factors that influence *R. sativus* germination rate are soil moisture, oxygen, temperature, seed viability, seed depth, pH, salinity and seed vitality (Anja & Felipe, 2021). The soil moisture, oxygen, and temperature were controlled in the experiment. The seed viability and seed vitality may have played a factor; however, all seeds were taken from the same seed packet. The pH of the pots stayed consistent through the experiment; however, salinity was not measured over the course of the experiment. The plants could have been planted at different seed depths since the experiment was done in sub-groups and seed depth was not mentioned during planting.

Data from the experiment rejected the second and third hypothesis. Although all three the nitrogen sources produced a significantly higher height growth rate, only urea showed a significantly higher leaf surface area compared to the control. However, the urea group was not significantly different from the calcium nitrate tetrahydrate and ammonium nitrate groups which were not significantly different from the control group. The lack of significance could come from the small sample size in the experiment because the graph shows that the plants in

the nitrogen compound treatment groups did have larger leaf surface areas compared to the control groups (Figure 4). Leaf surface area served as a proxy for nitrogen uptake since nitrogen plays an important role in the synthesis of chlorophyll and other enzymes important for photosynthesis which is the metabolic process that drives plant growth and yield (Koch Agronomic Services, n.d.). As mentioned in the results, radish bulbs did not develop except for slight swelling on two of the plants in the ammonium nitrate treatment. Reasons why radish bulbs may not have developed was because the soil was too compact even with the addition of vermiculite and the possibility of adding too much nitrogen to the soil which promotes the growth of the leafy tops (Grant, 2021). The team suggest adding a different amendment that would reduce compaction of the soil to see if a different amendment would promote radish bulb formation. The team recommends adding peat moss, compost, manure, or earthworms to loosen the soil (Iannotti, 2021).

The overall goal of the project was to find a nitrogen source that produced the best plant growth while reducing the payload on the spacecraft. Although radish bulbs did not develop, based on the data the team recommends the use of urea as a nitrogen source when growing *R. sativus* as urea is the most weight efficient nitrogen source tested in this experiment. In addition, the requirement to bring urea from Earth to the Moon may not be needed as urea can potentially be extracted from the urine of humans living on the Moon. Humans typically produce 12 to 20 g of urea in urine over the course of 24-hours (Jacob, 2021). Depending on the number of humans and the nitrogen requirements of the plants being grown, this amount of urea could be sufficient to avoid bringing urea to the Moon. Investigations into the method and efficiency of urea extraction from human urine is needed.

Future Research

In addition, to future research discussed above the following areas are suggested for future research. The compounds used as a nitrogen source were added during the initial set-up, so experiments looking at the reapplication of nitrogen fertilizer and its influence *Raphanus sativus* plant growth should be investigated. This is especially significant for the calcium nitrate tetrahydrate and urea since these two compounds are susceptible to leeching quickly from the soil. Future experiments could involve comparing the nutritional content and quality of the *R. sativus* plants for each of the treatments which would allow the team to give a more informed recommendation as there could potentially be a tradeoff between payload the nutritional value and quality of the plants (Yousaf, 2021). In addition, nitrogen is important in building the enzyme ribulose-1-5-bisphosphate carboxylase-oxygenase (RuBisCO) which is the enzyme used during carbon fixation in the light-independent reaction during photosynthesis. A future study could measure the photosynthetic rate of the plants as a measure for nitrogen uptake in the plants. Finally, the team suggests replicating the set-up used during this experiment using a bigger sample size and using other plant species to see which nitrogen source would be best for growing other nutrient rich plants that could be used as a food source for humans on the Moon.

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Appendix A: Raw Data

Table 2. Germination rate (primordial leaf emergence) 14 days after planting on March 2nd. Data shows the percent of the 3 seeds that germinated in the pot.

Team Name	Control	Calcium Nitrate Tetrahydrate	Ammonium Nitrate	Urea
Tarus	0%	0%	0%	33%
DIL	100%	0%	66%	66%
DO	0%	66%	100%	100%
ATT	100%	100%	33%	66%
ND	0%	100%	33%	33%
A.S.S.	33%	33%	66%	33%
I.M.	100%	33%	100%	100%
Ambiguity	66%	66%	66%	66%
Average	50%	50%	58%	62%
SE (+/-)	16%	13%	11%	9%

Ammonium Nitrate	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr	Growth Rate (cm/day)	Linear regression statistics
Tarus	----	----	----	----	----	----	----	----	
DIL	0.2	1.5	2.7	3.9	4	3.7	5	0.1036	X predicted Y, $R^2 = .87$, $F(1,5) = 32.61$, $p = .002$.
DO	3.6	4.7	4.8	6	6	6.5	6.5	0.06962	X predicted Y, $R^2 = .89$, $F(1,5) = 42.1$, $p = .001$.
ATT	4.5	6.2	7.5	9.1	9.5	9.5	9.6	0.1231	X predicted Y, $R^2 = .84$, $F(1,5) = 26.22$, $p = .004$.
ND	1.6	1.5	1.6	8	7.5	7.9	11	0.2437	X predicted Y, $R^2 = .85$, $F(1,5) = 29.06$, $p = .003$.
A.S.S.	1.5	2.7	4.1	7.4	8	6.5	6.5	0.1366	X predicted Y, $R^2 = .67$, $F(1,5) = 10.03$, $p = .025$.
I.M.	2	4.5	6.5	6.8	8	8	8	0.1362	X predicted Y, $R^2 = .8$, $F(1,5) = 20.59$, $p = .006$.
Ambiguity	1	3.7	5	5	5.4	5.5	8.5	0.1365	X predicted Y, $R^2 = .82$, $F(1,5) = 22.76$, $p = .005$.
	2.06	3.54	4.60	6.60	6.91	6.80	7.87	0.1356	X predicted Y, $R^2 = .91$, $F(1,5) = 48.13$, $p < .001$.
Urea	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr	Growth Rate (cm/day)	Linear regression statistics
Tarus	3.1	5.5	6.6	9	9	9	9	0.1394	X predicted Y, $R^2 = .79$, $F(1,5) = 18.26$, $p = .008$.
DIL	3.4	4	7	8.2	8.5	8.2	8.3	0.1268	X predicted Y, $R^2 = .75$, $F(1,5) = 15.1$, $p = .012$.
DO	2.7	5.5	7.6	7.8	8.5	8.5	8.5	0.1246	X predicted Y, $R^2 = .72$, $F(1,5) = 13.18$, $p = .015$.
ATT	1.5	2.8	4.5	5.4	6.5	6.5	5.1	0.1039	X predicted Y, $R^2 = .69$, $F(1,5) = 10.89$, $p = .021$.
ND	0	0	0	3.5	3.5	3.9	4.2	0.1241	X predicted Y, $R^2 = .83$, $F(1,5) = 23.91$, $p = .005$.
A.S.S.	1.4	3	3.5	4.5	5	5.5	6	0.1047	X predicted Y, $R^2 = .95$, $F(1,5) = 91.35$, $p < .001$.
I.M.	2	3.8	5.6	7.5	8.5	9.4	9.4	0.1875	X predicted Y, $R^2 = .93$, $F(1,5) = 70.33$, $p < .001$.
Ambiguity	1.6	3.5	5.5	8	9	8.3	9	0.1821	X predicted Y, $R^2 = .85$, $F(1,5) = 28.13$, $p = .003$.
	1.96	3.51	5.04	6.74	7.31	7.41	7.44	0.1366	X predicted Y, $R^2 = .86$, $F(1,5) = 31.5$, $p = .002$.

Table 4. Leaf surface area (cm²) for each pot during the last week of the growing period. Data was collected on April 4, 2023 using the LeafByte app. The table shows leaf surface area by leaf, total leaf surface area by plant, average leaf surface area by treatment, and standard error by treatment. Team ATT's urea treatment plant leaf surface area was not measured due to lack of water causing the leaves to shrivel up - the plant was excluded from the data analysis. Data boxes with "----" indicate that no plant germinated and thus data could not be collected.

Control						Calcium Nitrate Tetrahydrate					
Team	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Total Surface Area	Team	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Total Surface Area
Tarus	----	----	----	----	----	Tarus	----	----	----	----	----
DIL	3.235	2.374	0.5	0.5	6.609	DIL	----	----	----	----	----
DO	----	----	----	----	----	DO	----	----	----	----	----
ATT	3.238	3.778	----	----	7.016	ATT	9.583	11.2	8.526	1.5	30.809
ND	----	----	----	----	----	ND	10.384	9.84	12.843	1.5	34.567
A.S.S.	2.281	1	1.5		4.781	A.S.S.	4.45	1.857	2.13	6.287	14.724
I.M.	2.768	1.633	3.119	0.25	7.77	I.M.	6.792	5.202	3.188	1	16.182
Ambiguity	2.399	----	----	----	2.399	Ambiguity	14.956	10.686	15.519	----	41.161
Average					5.715	Average					27.4886
SE (+/-)					0.86	SE (+/-)					4.64

Ammonium Nitrate						Urea					
Team	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Total Surface Area	Team	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Total Surface Area
Tarus	----	----	----	----	----	Tarus	16.703	21.718	5	3	46.421
DIL	9.254	6.786	----	----	16.04	DIL	14.335	11.656	7.312	15.291	48.594
DO	12.582	7.52	0.5	----	20.602	DO	16.864	14.255	15.041	2	48.16
ATT	15.521	10.687	7.412	3.461	37.081	ATT	----	----	----	----	----
ND	11.155	11.195	15.452	0.5	38.302	ND	3.518	2.718	----	----	6.236
A.S.S.	10.465	6.39	8.611	13.442	38.908	A.S.S.	13.831	11.565	9.192	----	34.588
I.M.	11.241	13.204	3.517	0.5	28.462	I.M.	7.961	10.239	30	----	48.2
Ambiguity	9.789	14.675	14.445	2.5	41.409	Ambiguity	6.902	11.175	13.607	10.678	42.362
Average					31.543	Average					39.223
SE (+/-)					3.49	SE (+/-)					5.38

Table 5. Soil pH Raw Data								
Control								
Team	16-Feb	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr
Tarus	8	8	8	8	8	8	8	8
DIL	8	8	8	8	8	8	8	8
DO	8	8	8	8	8	8	8	8
ATT	8	8	8	8	8	8	8	8
ND	8	8	8	8	8	8	8	8
A.S.S.	8	8	8	8	8	8	8	8
I.M.	8	8	8	8	8	8	8	8
Ambiguity	8	8	8	8	8	8	8	8
Calcium Nitrate Tetrahydrate								
Team	16-Feb	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr
Tarus	8	8	8	8	8	8	8	8
DIL	8	8	8	8	8	8	8	8
DO	8	8	8	8	8	8	8	8
ATT	8	8	8	8	8	8	8	8
ND	8	8	8	8	8	8	8	8
A.S.S.	8	8	8	8	8	8	8	8
I.M.	8	8	8	8	8	8	8	8
Ambiguity	8	8	8	8	8	8	8	8
Ammonium Nitrate								
Team	16-Feb	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr
Tarus	8	8	8	8	8	8	8	8
DIL	8	8	8	8	8	8	8	8

DO	8	8	8	8	8	8	8	8
ATT	8	8	8	8	8	8	8	8
ND	8	8	8	8	8	8	8	8
A.S.S.	8	8	8	8	8	8	8	8
I.M.	8	8	8	8	8	8	8	8
Ambiguity	8	8	8	8	8	8	8	8
Urea								
Team	16-Feb	24-Feb	2-Mar	9-Mar	16-Mar	23-Mar	30-Mar	6-Apr
Tarus	8	8	8	8	8	8	8	8
DIL	8	8	8	8	8	8	8	8
DO	8	8	8	8	8	8	8	8
ATT	8	8	8	8	8	8	8	8
ND	8	8	8	8	8	8	8	8
A.S.S.	8	8	8	8	8	8	8	8
I.M.	8	8	8	8	8	8	8	8
Ambiguity	8	8	8	8	8	8	8	8

Appendix B: Experiment Photos

The provided photos document the growth of the plants over the eight-week grow period.



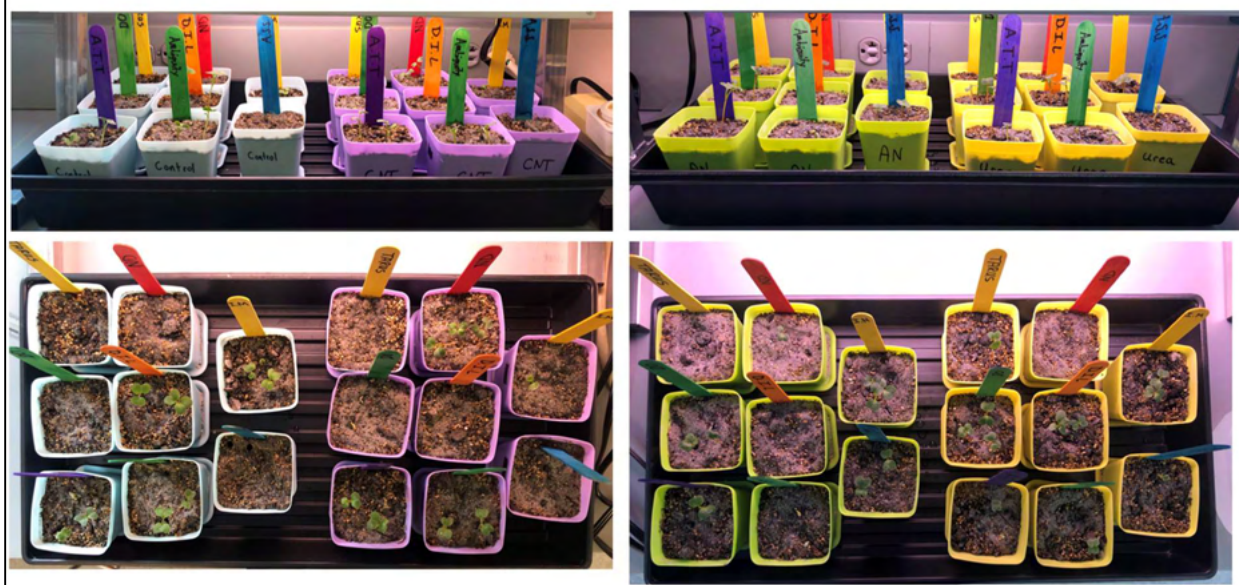
Day 0 - Initial Set-Up
Feb. 16, 2023

Minnesota West Bluejays
Team #12926
Spring 2023

Day 5 – Feb. 21, 2023



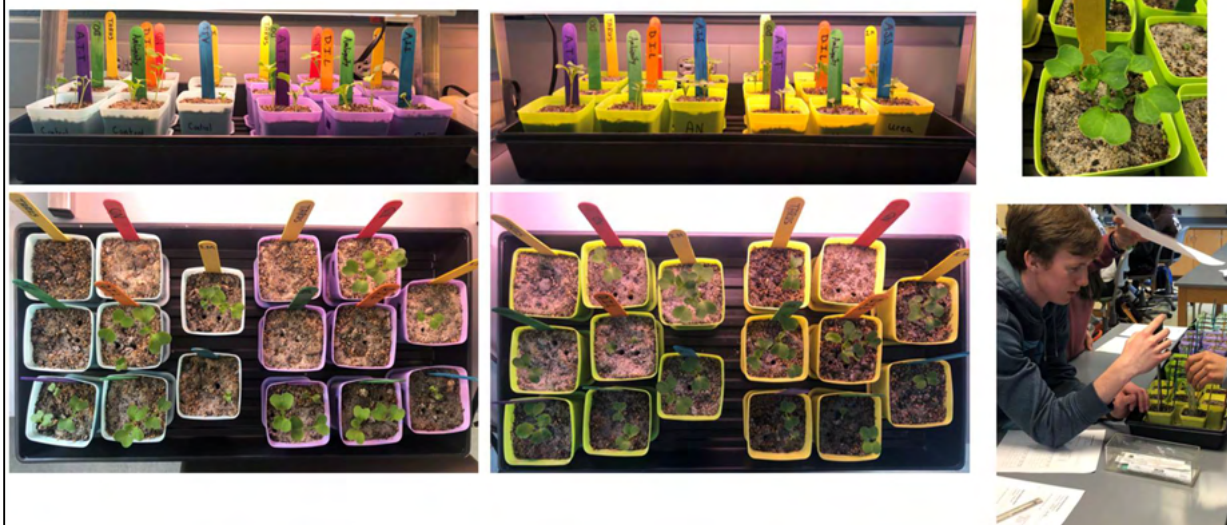
Day 8 – Feb. 24, 2023



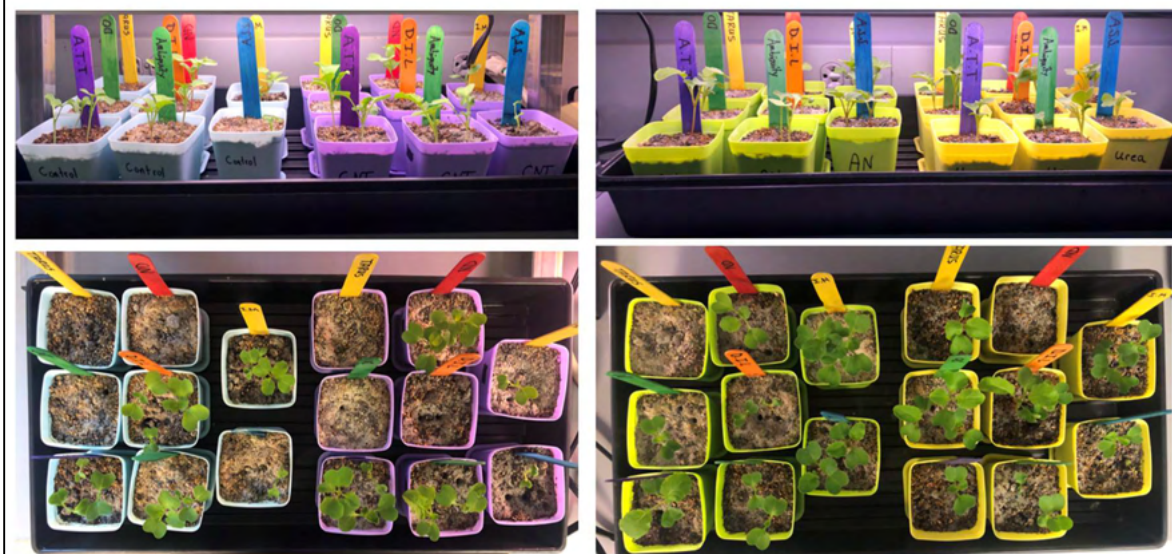
Day 12 – Feb. 28, 2023



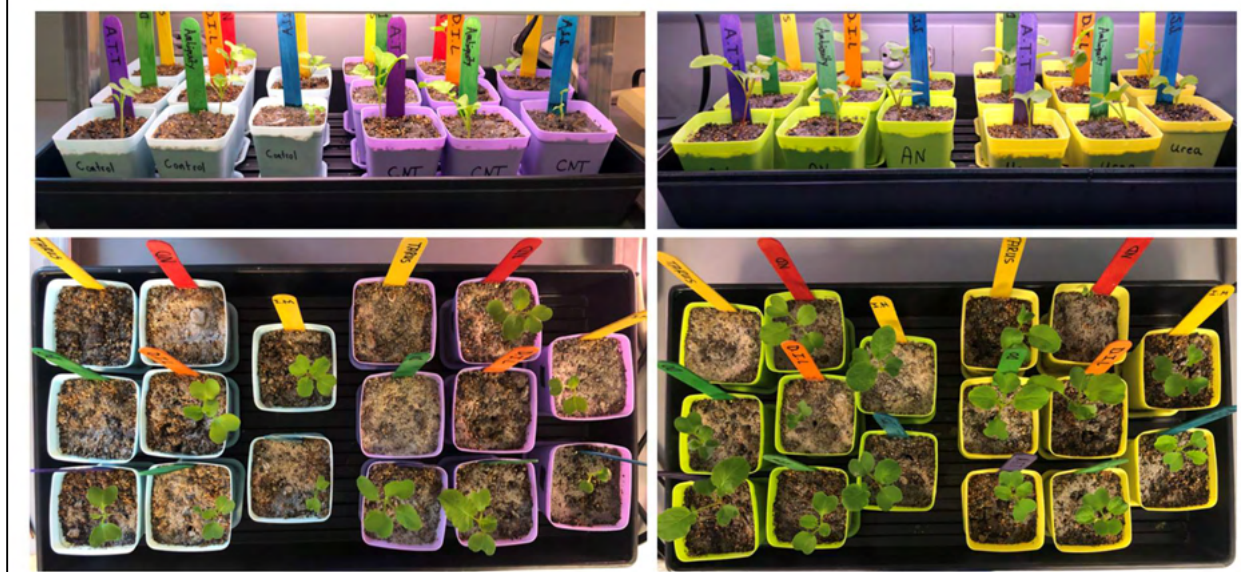
Day 14 –March 2, 2023



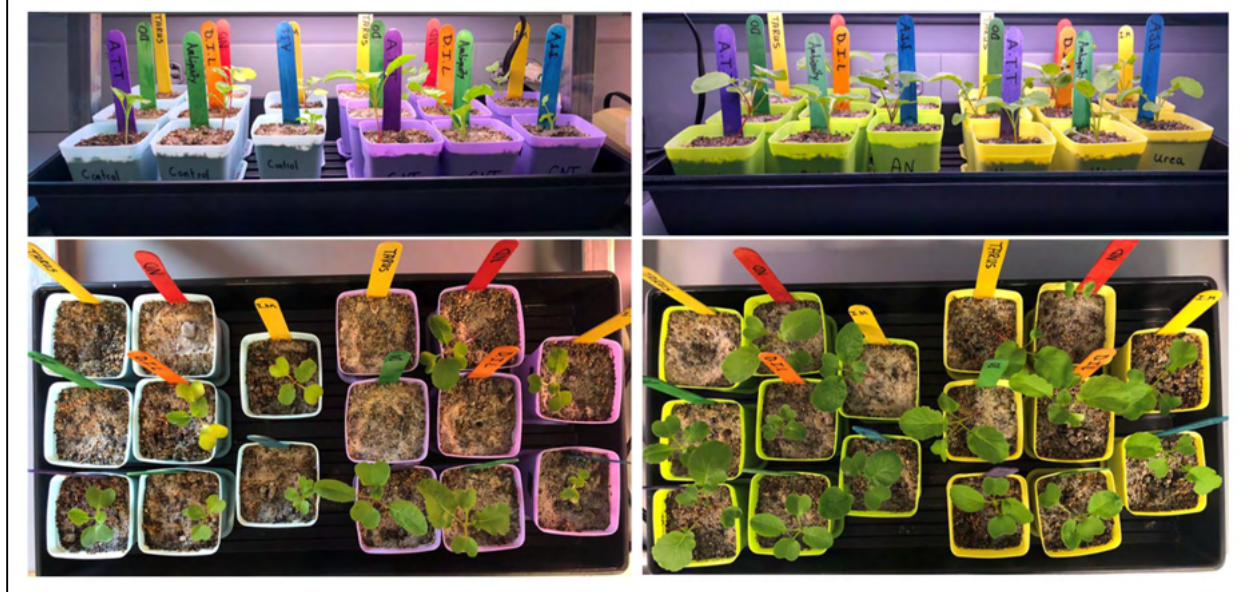
Day 19 –March 7, 2023



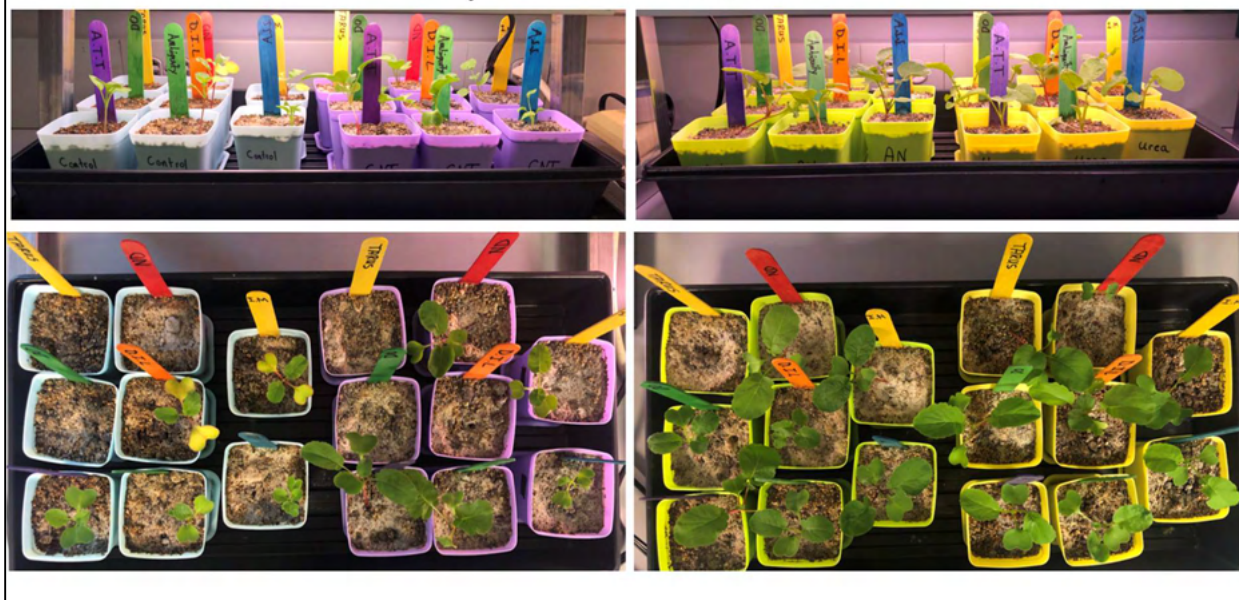
Day 21 – March 9, 2023
Thinned – Kept Tallest Plant



Day 26 – March 14, 2023



Day 28 –March 16, 2023



Day 33 –March 21, 2023



Day 35 – March 23, 2023



Day 40 – March 28, 2023



Day 42 –March 30, 2023



Day 47 –April 4, 2023



Day 49 – April 6, 2023



Day 49 – April 6, 2023



The following photos documents the final plant growth above and below ground.



