

Effect of the compound used as a nitrogen source on Collard Greens (*Brassica oleracea*) plant growth

Plant the Moon Challenge

Fall 2022

Undergraduate Division

Minnesota West Bluejays

Team # 11077

Minnesota West Community & Technical College

Worthington, MN

Dalton Vorwald

Advised by Heidi Tarus, Dr. Tyler Wadzinski, and Dr. Paul Seifert

Grant Funded by NASA's Minnesota Space Grant Consortium

Introduction

As humans on Earth look towards future colonization of celestial bodies, we must address the basic needs of these colonizing humans including access to shelter, nutritious food, and clean water. This project focuses on addressing the human need of access to nutritious food that can be grown on the Moon to reduce the amount of food brought on spacecrafts. 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 nitrogen and the best source of nitrogen that will 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 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 space ship 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.

The team decided to plant Collard Greens (*Brassica oleracea*) because of the fast germination time of four to seven days, its ability to grow at lower temperatures between 4-18 degree Celsius (40-65 degrees Fahrenheit), its ability to continuously provide highly nutritious leafy greens over a long period of time once mature (Palomo 2021). *B. oleracea* is a dicot plant that contains high nutrient content in the leaves. According to the U.S. Department of Agriculture (2019), one cup of chopped raw collard greens (36g) contains 11 calories, 1g of protein, 1.44g of fiber, 2g of carbohydrate, and less than 1g of fat and sugar. In addition, *B.*

oleracea are an excellent source of many essential vitamins and minerals for humans including Vitamin A, Vitamin C, Vitamin K, calcium, potassium, folate, and antioxidant (Baby, n.d). The leaves can be eaten raw or cooked allowing for the plant to provide versatility and diversity in the types of food individuals can create using the plant.

We hypothesized that ammonium nitrate (NH_4NO_3) would produce the largest leaves and most leaf surface area in the *B. oleracea* plants 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 initial 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 4 replicates (Figure 1). Each pot (7cm x 7cm x 7.7cm) contained the same soil composition of 25% top soil, 25% sorghum peat moss mix, 50% lunar regolith by volume. Next, 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). After mixing was done, each pot was planted with 3-4 collard greens, *Brassica oleracea*, seeds and watered with 50ml of tap water. Over the course of the eight-week grow period, the set-up was kept indoors where the temperature was controlled 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 (Image 1). Initially the grow

light was located 48 cm from the top of the pots, however, the seedlings were becoming leggy once germinated. After conducting some research, it was determined the light intensity was not high enough (Ly, 2022) and thus the light was moved down to 12cm on October 6, 2022 from the top of the pots to reduce leggy seedlings.

Figure 1. Diagram of experimental set-up showing the four treatment groups (control, calcium nitrate tetrahydrate, ammonium nitrate, and urea) each with four replicate pots for a total of 16 pots.

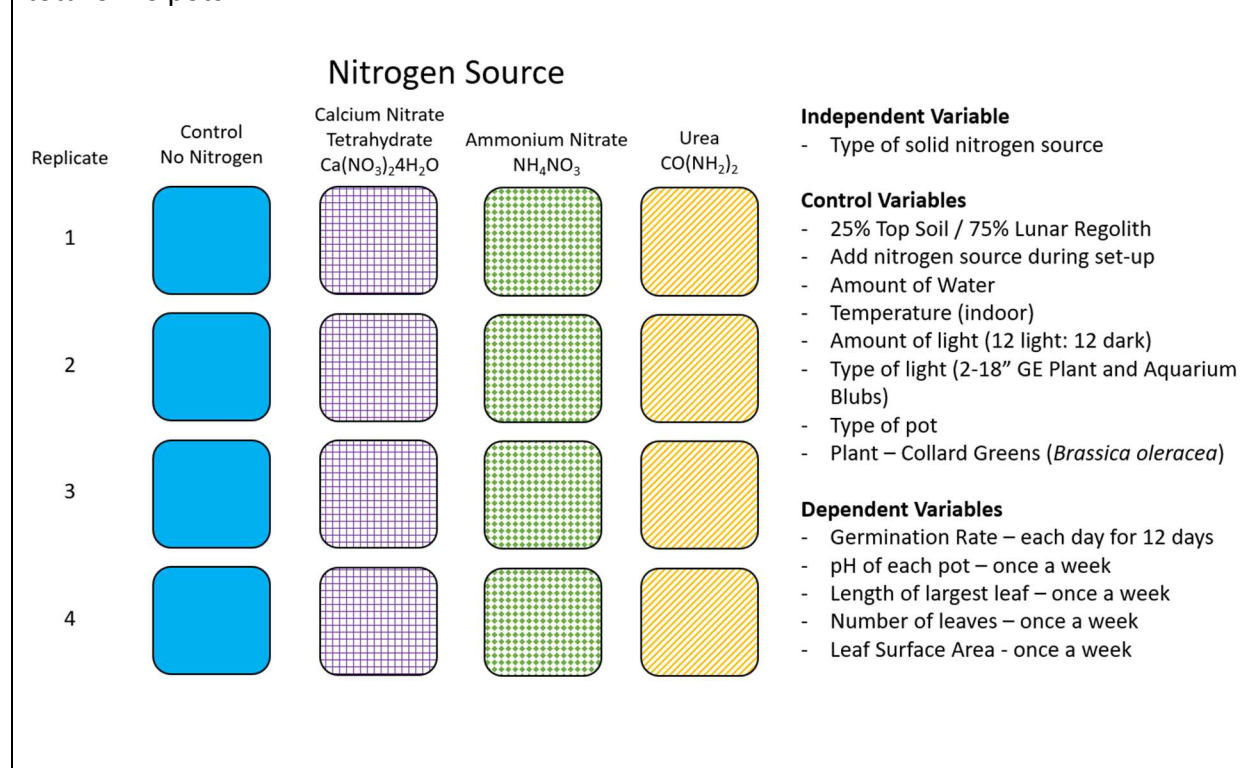


Table 1. Plant the Moon Fall 2022 Fertilizer Application Calculations

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 September 30, 2022.



Plant Care

Each pot was watered with 25ml of distilled water on Tuesday and Friday each week for the first five weeks after planting and then with 30ml of water on Tuesday and Friday during the last two weeks of the grow period. The increase in water during the last two weeks was done because the plants were showing water stress by having limp folded up leaves as the plants started to grow bigger. On October 11, 2022 (Day 11) a toothpick was inserted into each pot to mark one seedling to complete measurement on and keep during the thinning process which occurred on October 25, 2022 (Day 25). Thinning is a common practice in gardening and was done to give one seedling in each pot the best chance to grow. The height of the grow lamp was kept at to 12cm from the top of the pots to from October 6, 2022 to the last day of the

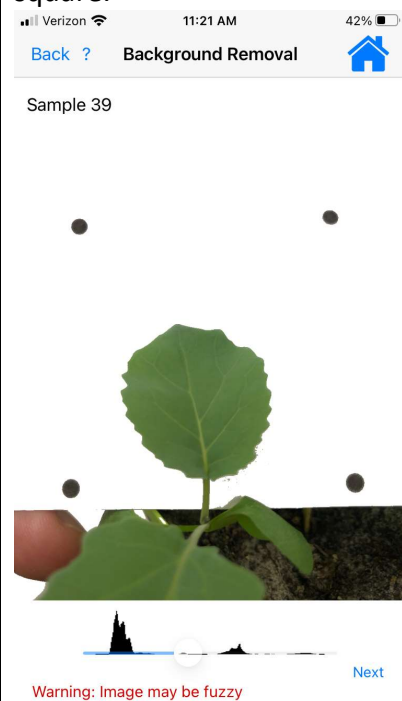
grow period on November 18, 2022 and was kept on a 12-hour light: 12-hour dark cycle by using a GE 24-hour heavy duty indoor plug-in mechanical timer. The plants were kept in the same room through the duration of the experiment and were moved around to take measurements and then replaced back in generally the same location after each measuring event.

Measurements

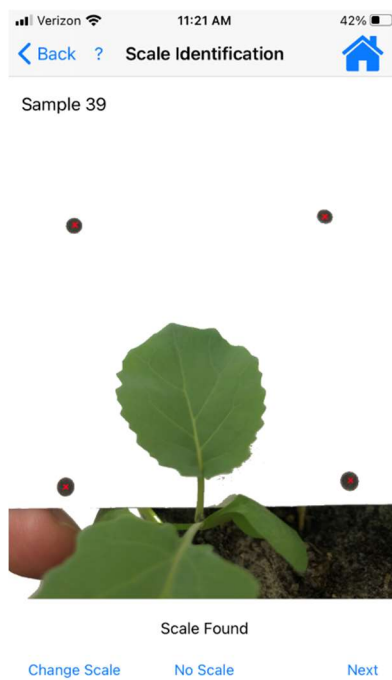
Germination rate was collected by visual observation of the number of germinated seeds in each pot each day, including weekends, for 12 days after planting. At that point, all of the seeds were germinated and the data collection for germination rate and time was complete. The soil pH was collected each Friday and the data collected did not show a significant change and was thus not included in this report. Once true leaves emerged the length of largest leaf, the number of leaves, and leaf surface area was collected on Tuesday and Friday of each week. The number of leaves were visually counted and recorded. The length of the largest leaf was measured in centimeters from the leaf base where the leaf attaches to the petiole to the tip of the leaf. Leaf surface area was collected 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.

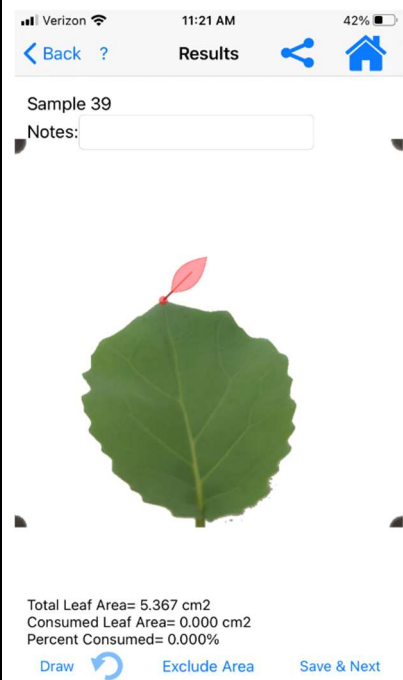
Step 1. A picture is taken with a known scale made up of four black dots arranged in a square.



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



Step 3. The app calculates the surface area:



In addition, a picture of the entire set-up was taken at least once a week (See 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 was stored, analyzed with linear fit models to calculate rates, and graphed using Microsoft Excel. ANOVA statistical tests were done using a free online website, Statistics Kingdom - <https://www.statskingdom.com/>.

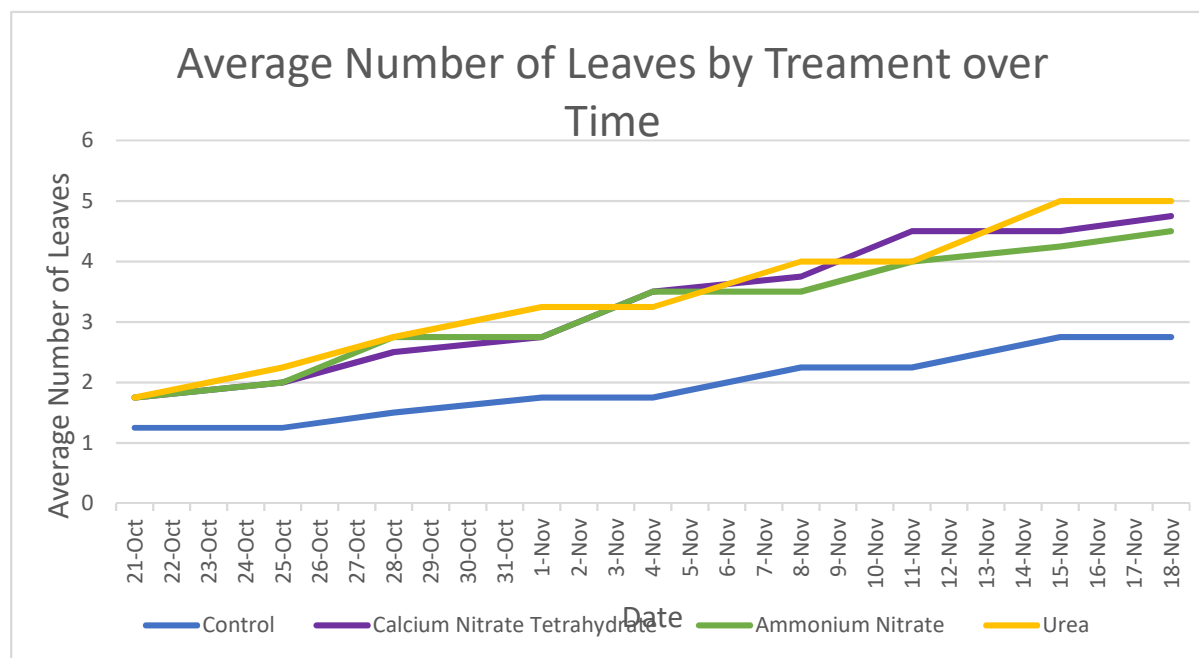
Soil pH & Germination Rate

The soil pH of each pot did not change over the course of the eight weeks and stayed around a neutral pH of 6-7. Because the pH did not change over time the raw data and results are not included in this report. The germination data shows that the seeds in the control, calcium nitrate tetrahydrate, and ammonium nitrate pots germinated about 1 day before the seeds planted in the urea supplemented pots (Appendix A Table 2).

Number of Leaves

The raw data for the number of leaves is available in Appendix A Table 3. A linear fit calculated the average rate of leaf production during the measurement period was 0.06 leaves/day for the control plants, 0.12 leaves/day for the calcium nitrate tetrahydrate plants, 0.10 leaves/day for the ammonium nitrate plants, and 0.12/day for the urea plants (Figure 2 and Appendix A Table 3).

Figure 2. Effect of compound used as a nitrogen source on the number of leaves. Each treatment contained four plants where the number of leaves was measured and averaged.



A one-way ANOVA was performed to compare the effect of the compound used as a nitrogen source on the rate of leaf production. The test revealed that there is a statistically significant difference in the leaf growth rate between at least two groups ($F(3,12) = 9.1469$, $p = 0.002$). Tukey's HSD Test for multiple comparisons found that the mean value of leaf growth rate was significantly different between the control and the calcium nitrate tetrahydrate group ($p = 0.00372$, 95% C.I. = [0.019, 0.09448]), the control and the ammonium nitrate group ($p = 0.03663$, 95% C.I. = [0.00228, 0.07775]), and the control and the urea group ($p = 0.002946$, 95% C.I. = [0.02076, 0.09623]). There was no statistically significant difference in the leaf growth rate between the calcium nitrate tetrahydrate and ammonium nitrate group ($p = 0.5709$), the calcium nitrate tetrahydrate and urea group ($p = 0.999$) or between the ammonium nitrate and urea group ($p = 0.4926$).

ANOVA for Number of Leaves Growth Rate

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	3	0.008867	0.002956	9.1469	0.002*
Within Groups	12	0.003878	0.0003231		
Total	15	0.01274	0.0008496		

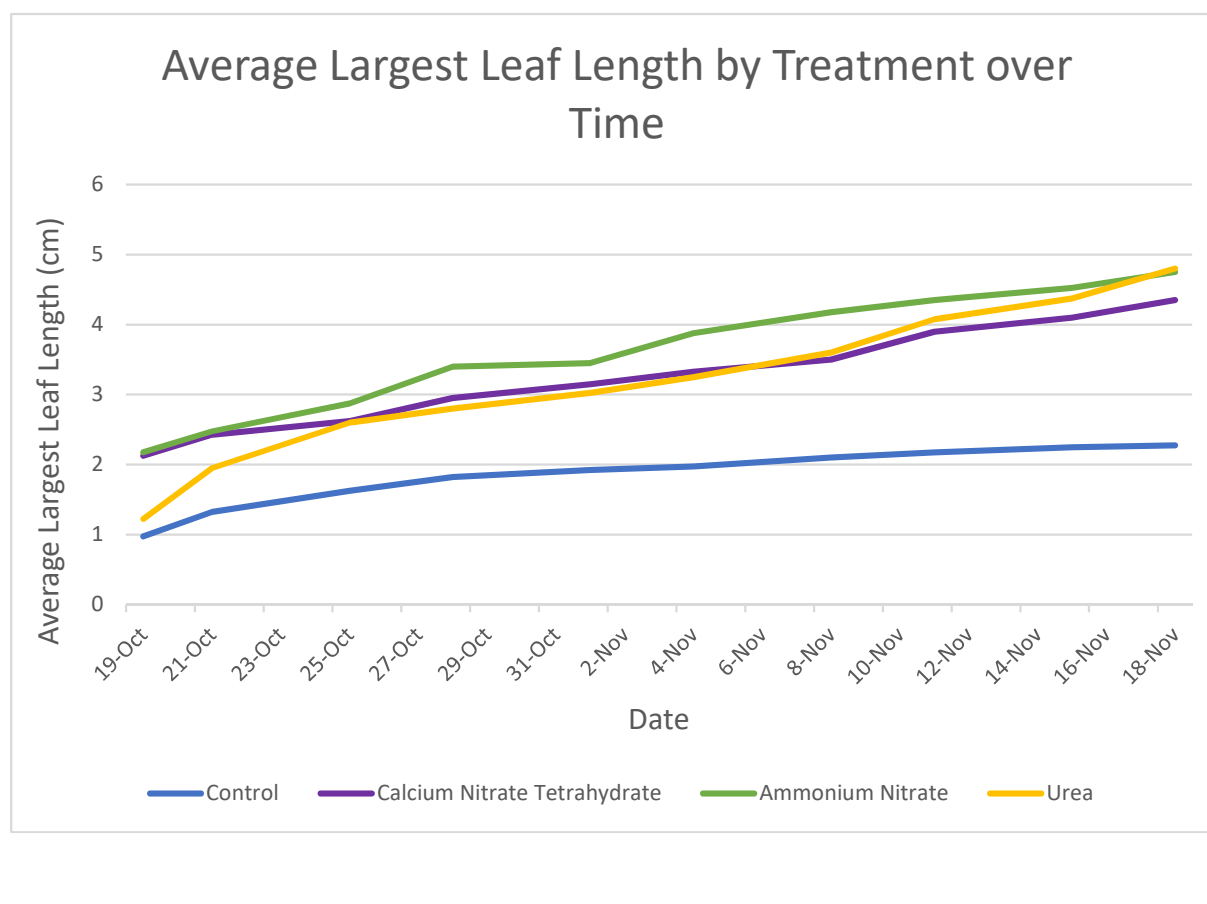
Multiple Comparisons - Tukey HSD

		Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	calcium nitrate tetrahydrate	0.05674	0.008988	0.00372*	0.019	0.09448
	ammonium nitrate	0.04002	0.008988	0.03663*	0.00228	0.07775
	urea	0.05849	0.008988	0.002946*	0.02076	0.09623
calcium nitrate tetrahydrate	control	0.05674	0.008988	0.00372*	0.019	0.09448
	ammonium nitrate	0.01672	0.008988	0.5709	-0.02101	0.05446
	urea	0.001753	0.008988	0.999	-0.03598	0.03949
ammonium nitrate	control	0.04002	0.008988	0.03663*	0.00228	0.07775
	calcium nitrate tetrahydrate	0.01672	0.008988	0.5709	-0.02101	0.05446
	urea	0.01848	0.008988	0.4926	-0.01926	0.05621
urea	control	0.05849	0.008988	0.002946*	0.02076	0.09623
	calcium nitrate tetrahydrate	0.001753	0.008988	0.999	-0.03598	0.03949
	ammonium nitrate	0.01848	0.008988	0.4926	-0.01926	0.05621

Length of Largest Leaf

The raw data for length of the largest leaf is available in Appendix A Table 4. A linear fit calculated the average rate of leaf length growth during the measurement period was 0.04 cm/day for the control plants, 0.07 cm/day for the calcium nitrate tetrahydrate plants, 0.08 cm/day for the ammonium nitrate plants, and 0.10 cm/day for the urea plants.

Figure 3. Effect of compound used as a nitrogen source on the length of the largest leaf. Each treatment contained four plants where the length of the largest leaf was measured and averaged.



A one-way ANOVA was performed to compare the effect of the compound used as a nitrogen source on the leaf length growth rate. The test revealed that there is a statistically significant difference in the leaf length growth rate between at least two groups ($F(3,12) = [14.16985]$, $p = 0.00030064$). Tukey's HSD Test for multiple comparisons found that the mean value of leaf length growth rate was significantly different between the control and the calcium nitrate tetrahydrate group ($p = 0.040488$, 95% C.I. = $[0.0012335, 0.061367]$), the control and the ammonium nitrate group ($p = 0.0045422$, 95% C.I. = $[0.013956, 0.074089]$), and the control and the urea group ($p = 0.00018563$, 95% C.I. = $[0.034256, 0.094389]$). In addition, there was a

significant difference between the calcium nitrate tetrahydrate and the urea group ($p = 0.030098$, 95% C.I. = [0.050367, 0.030067]. There was no statistically significant difference in leaf length growth rate between the calcium nitrate tetrahydrate and ammonium nitrate group ($p=0.6055$) or between the ammonium nitrate and urea group ($p=0.23956$).

ANOVA for Number of Leaves Growth Rate

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	3	0.0087195	0.0029065	14.16985	0.00030064*
Within Groups	12	0.0024614	0.00020512		
Total	15	0.011181	0.00074539		

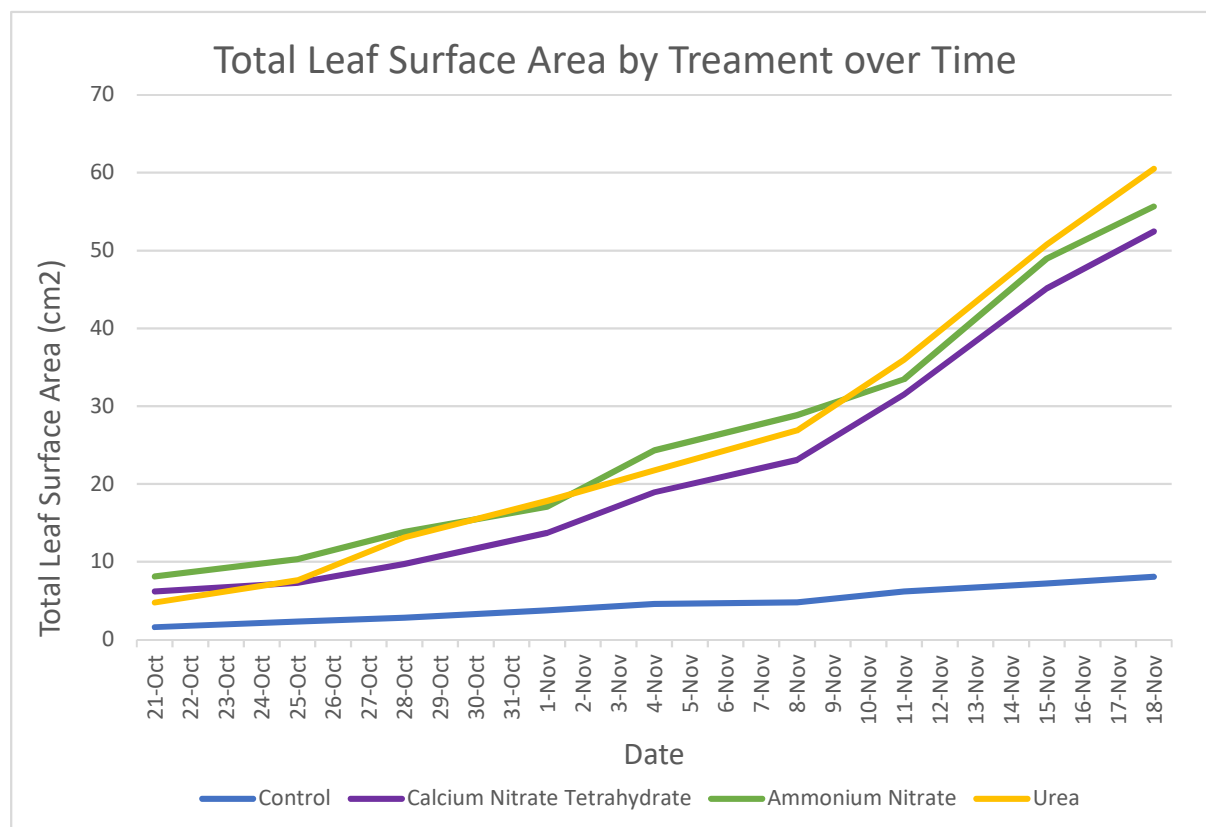
Multiple Comparisons - Tukey HSD

		Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	calcium nitrate tetrahydrate	0.0313	0.007161	0.040488*	0.0012335	0.061367
	ammonium nitrate	0.044022	0.007161	0.0045422*	0.013956	0.074089
	urea	0.064323	0.007161	0.00018563*	0.034256	0.094389
calcium nitrate tetrahydrate	control	0.0313	0.007161	0.040488*	0.0012335	0.061367
	ammonium nitrate	0.012722	0.007161	0.6055	-0.017344	0.042789
	urea	0.033022	0.007161	0.030098*	0.002956	0.063089
ammonium nitrate	control	0.044022	0.007161	0.0045422*	0.013956	0.074089
	calcium nitrate tetrahydrate	0.012722	0.007161	0.6055	-0.017344	0.042789
	urea	0.0203	0.007161	0.23956	-0.009767	0.050367
urea	control	0.064323	0.007161	0.00018563*	0.034256	0.094389
	calcium nitrate tetrahydrate	0.033022	0.007161	0.030098*	0.002956	0.063089
	ammonium nitrate	0.0203	0.007161	0.23956	-0.009767	0.050367

Leaf Surface Area

The raw data for leaf surface area is available in Appendix A Table 5. A linear fit calculated the average growth rate of the leaf surface area during the measurement period was $0.23 \text{ cm}^2/\text{day}$ for the control plants, $1.67 \text{ cm}^2/\text{day}$ for the calcium nitrate tetrahydrate plants, $1.7 \text{ cm}^2/\text{day}$ for the ammonium nitrate plants, and $1.94 \text{ cm}^2/\text{day}$ for the urea plants (Figure 4).

Figure 4. Effect of compound used as a nitrogen source on leaf surface area growth rate. Each treatment contained four plants where leaf surface area was measured and averaged.



A one-way ANOVA was performed to compare the effect of the compound used as a nitrogen source on the leaf surface area growth rate. The test revealed that there is a statistically significant difference in the leaf surface area growth rate between at least two groups ($F(3,12) = [44.6334]$, $p = 8.752e-7$). Tukey's HSD Test for multiple comparisons found that the mean value of leaf surface area growth rate was significantly different between the control and the calcium nitrate tetrahydrate group ($p = 7.781e-6$, 95% C.I. = [0.9515, 1.9296]), the control and the ammonium nitrate group ($p = 6.392e-6$, 95% C.I. = [0.9789, 1.9569]), and the control and the urea group ($p = 1.291e-6$, 95% C.I. = [1.2165, 2.1946]). There was no statistically

significant difference in leaf growth rate between the calcium nitrate tetrahydrate and ammonium nitrate group ($p=0.9983$), the calcium nitrate tetrahydrate and urea group ($p=0.41$) or between the ammonium nitrate and urea group ($p=0.4986$).

ANOVA for Leaf Surface Area Growth Rate

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	3	7.2663	2.4221	44.6334	8.752e-7*
Within Groups	12	0.6512	0.05427		
Total	15	7.9175	0.5278		

Multiple Comparisons - Tukey HSD

		Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	calcium nitrate tetrahydrate	1.4405	0.1165	7.781e-6*	0.9515	1.9296
	ammonium nitrate	1.4679	0.1165	6.392e-6*	0.9789	1.9569
	urea	1.7055	0.1165	1.291e-6*	1.2165	2.1946
calcium nitrate tetrahydrate	control	1.4405	0.1165	7.781e-6*	0.9515	1.9296
	ammonium nitrate	0.02735	0.1165	0.9983	-0.4617	0.5164
	urea	0.265	0.1165	0.41	-0.224	0.754
ammonium nitrate	control	1.4679	0.1165	6.392e-6*	0.9789	1.9569
	calcium nitrate tetrahydrate	0.02735	0.1165	0.9983	-0.4617	0.5164
	urea	0.2376	0.1165	0.4986	-0.2514	0.7267
urea	control	1.7055	0.1165	1.291e-6*	1.2165	2.1946
	calcium nitrate tetrahydrate	0.265	0.1165	0.41	-0.224	0.754
	ammonium nitrate	0.2376	0.1165	0.4986	-0.2514	0.7267

Discussion & Conclusion

The overall results show that *B. oleracea* grew significantly more in terms of leaf length, number of leaves, and leaf surface area in the presence of a compound used as a nitrogen source compared to when no nitrogen source was provided. Based on the data collected the

Minnesota West Bluejays team recommends adding one of the three tested nitrogen compounds using a fertilizing loading rate of 0.5 lb N per 1000 ft² to a 25% top soil, 25% sorghum peat moss mix, 50% lunar regolith (by volume) soil mixture when growing *B. oleracea*. These results partially support the team's hypothesis because the plants provided with a nitrogen source grew better compared to the control plants; however, the team also hypothesized that ammonium nitrate would show the best growth.

The data did not support the second part of the hypothesis since there was no major significant difference in plant growth between the three different nitrogen sources. The overall goal of the project was to find the nitrogen source that produced plant growth while reducing the payload on the spacecraft; therefore, the team recommends the use of urea as a nitrogen source when growing *B. oleracea*. Urea only requires 2.1 g of compound per gram of nitrogen delivered making it 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 would be needed.

Although not specifically analyzed, the *B. oleracea* plants provided with urea took approximately one day longer to germinate (Appendix A Table 2) and appeared to have slower growth for leaf length and surface area at the beginning; however, the urea plants quickly caught up with the calcium nitrate tetrahydrate and the ammonium nitrate plants and started

to show more growth at the end of the experiment (Figure 3 and Figure 4). The slower start to the urea plants could be accounted for the fact that 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). It would be interesting to have the experiment run longer to see if the high growth rate trend for the urea supplemented *B. oleracea* plants would continue and become significant.

Future experiments could involve comparing the nutritional content of the *B. oleracea* plants for each of the treatments would allow the team to give a more informed recommendation as there could potentially be a tradeoff between payload the nutritional value of the plants. The compounds used as a nitrogen source were added during the initial set-up, so it would be interesting to look how reapplication of fertilizer would influence *B. oleracea* plant growth. In addition, replicating the set-up used during this experiment using other plant species is recommended to see if urea is a good nitrogen sources for other nutrient rich plants that could be used as a food source for human on the Moon.

References

- Baby, D. P. (Ed.). (n.d.). *Collard greens: Health benefits, nutrients per serving, preparation information, and more*. Health Benefits of Collard Greens. Retrieved from <https://www.webmd.com/diet/health-benefits-collard-greens>
- Clark, M. A., Douglas, M., & Choi, J. (2018, March 28). *Biology 2e*. OpenStax. <https://openstax.org/details/books/biology-2e>.
- Isleib, J. (2017, February 1). *A quick look at the nitrogen cycle and nitrogen fertilizer sources – part 2*. MSU Extension. Retrieved from https://www.canr.msu.edu/news/nitrogen_cycle_and_nitrogen_fertilizer_sources_part_2
- Jacob, D. (2021, November 24). *Are urea and urine the same thing? abnormal levels, symptoms, Bun Test*. Are Urea and Urine the Same Thing? Retrieved from https://www.medicinenet.com/is_urea_and_urine_the_same_thing/article.htm

- Kokemuller, J. (2016, October 7). *How to fertilize your garden with nitrogen*. Home Guides | SF Gate. Retrieved from <https://homeguides.sfgate.com/fertilize-garden-nitrogen-38432.html>
- Ly, L. (2022, May 17). *Leggy seedlings: What causes them and how to fix them*. Garden Betty. Retrieved from <https://www.gardenbetty.com/leggy-seedlings-what-causes-them-and-how-to-correct-them/>
- Mengel, D. B. (n.d.). *Types and uses of nitrogen fertilizers for crop production*. Agronomy Guide. Retrieved from <https://www.extension.purdue.edu/extmedia/ay/ay-204.html>
- Palomo, E. (2021, December 17). *How long does it take to grow collard greens?* Home Guides | SF Gate. Retrieved from <https://homeguides.sfgate.com/long-grow-collard-greens-69867.html>
- Semeco, A., & Marengo, K. (2022, August 25). *High protein vegetables: The top 10 and how to eat them*. Medical News Today. Retrieved from <https://www.medicalnewstoday.com/articles/318600#collard-greens>
- Swift, C. E., & Hammond, E. (2020, August 14). *Vegetable gardening - nitrogen recommendations - 7.247*. Colorado State University Extension. Retrieved from <https://extension.colostate.edu/topic-areas/yard-garden/vegetable-gardening-nitrogen-recommendations-7-247/>
- U.S. Department of Agriculture. (2019, April 1). *Fooddata Central Search Results*. FoodData Central Collards, raw. Retrieved November 30, 2022, from <https://fdc.nal.usda.gov/fdc-app.html#/food-details/170406/nutrients>
- Wanekubj, G. S. W., Frissel, J.Y., Krijnen, W.H.J., Verwoert, M.R., Goedhart, P.W. (2014). Can plants grow on Mars and the Moon: A growth experiment on mars and moon soil simulants. *PLOS ONE* 9(8). <https://doi.org/10.1371/journal.pone.0103138>

Appendix A: Raw Data

Table 2. Germination Data for each pot from October 1 to October 12, 2022. Data shows the total number of seeds germinated on each day.

Date	1-Oct	2-Oct	3-Oct	4-Oct	5-Oct	6-Oct	7-Oct	8-Oct	9-Oct	10-Oct	11-Oct	12-Oct
Control 1	0	0	0	1	2	2	2	2	2	2	2	2
Control 2	0	0	1	1	3	3	3	3	3	3	3	3
Control 3	0	0	0	2	2	2	2	2	2	2	2	2
Control 4	0	0	1	1	2	2	3	3	3	3	3	3
Calcium Nitrate Tetrahydrate 1	0	0	0	1	2	2	2	2	3	3	3	3
Calcium Nitrate Tetrahydrate 2	0	0	1	1	2	3	3	3	3	3	3	3
Calcium Nitrate Tetrahydrate 3	0	0	1	1	3	3	3	3	3	3	3	3
Calcium Nitrate Tetrahydrate 4	0	0	0	2	3	3	3	3	3	3	3	3
Ammonium Nitrate 1	0	0	1	1	1	1	1	1	1	1	1	1
Ammonium Nitrate 2	0	0	0	2	2	2	2	2	2	2	2	2
Ammonium Nitrate 3	0	0	1	1	2	3	3	3	3	3	3	3
Ammonium Nitrate 4	0	0	1	2	2	2	2	2	3	3	3	3
Urea 1	0	0	0	0	1	2	2	2	2	2	2	2
Urea 2	0	0	0	1	2	2	2	2	2	3	3	3
Urea 3	0	0	0	1	3	3	3	3	3	3	3	3
Urea 4	0	0	0	1	2	2	2	2	2	2	2	2

Table 3. Number of Leaves for each pot from October 21 to November 18, 2022, the average number of leaves for each treatment, the rate of leaf production using a linear fit.

	21-Oct	25-Oct	28-Oct	1-Nov	4-Nov	8-Nov	11-Nov	15-Nov	18-Nov	Rate (leaves/day)
Control 1	2	2	2	3	3	3	3	4	4	0.08
Control 2	1	1	2	2	2	2	2	3	3	0.07
Control 3	1	1	1	1	1	2	2	2	2	0.05
Control 4	1	1	1	1	1	2	2	2	2	0.05
Average	1.25	1.25	1.5	1.75	1.75	2.25	2.25	2.75	2.75	0.06
Calcium Nitrate Tetrahydrate 1	1	2	2	2	3	3	4	4	4	0.11
Calcium Nitrate Tetrahydrate 2	2	2	3	3	4	4	5	5	5	0.12
Calcium Nitrate Tetrahydrate 3	2	2	3	3	4	4	5	5	6	0.14
Calcium Nitrate Tetrahydrate 4	2	2	2	3	3	4	4	4	4	0.09
Average	1.75	2	2.5	2.75	3.5	3.75	4.5	4.5	4.75	0.12
Ammonium Nitrate 1	2	3	4	4	4	4	5	5	6	0.11
Ammonium Nitrate 2	1	1	2	2	3	3	3	4	4	0.11
Ammonium Nitrate 3	2	2	3	3	4	4	4	4	4	0.08
Ammonium Nitrate 4	2	2	2	2	3	3	4	4	4	0.09
Average	1.75	2	2.75	2.75	3.5	3.5	4	4.25	4.5	0.10
Urea 1	2	2	3	3	3	4	4	5	5	0.11
Urea 2	2	3	3	4	4	5	5	6	6	0.14
Urea 3	2	2	3	3	3	4	4	5	5	0.11
Urea 4	1	2	2	2	3	3	3	4	4	0.10
Average	1.75	2.25	2.75	3	3.25	4	4	5	5	0.12

Table 4. Length of longest leaf (cm) for each pot from October 19 to November 18, 2022, the average leaf length (cm) for each treatment, and the leaf growth rate using a linear fit.

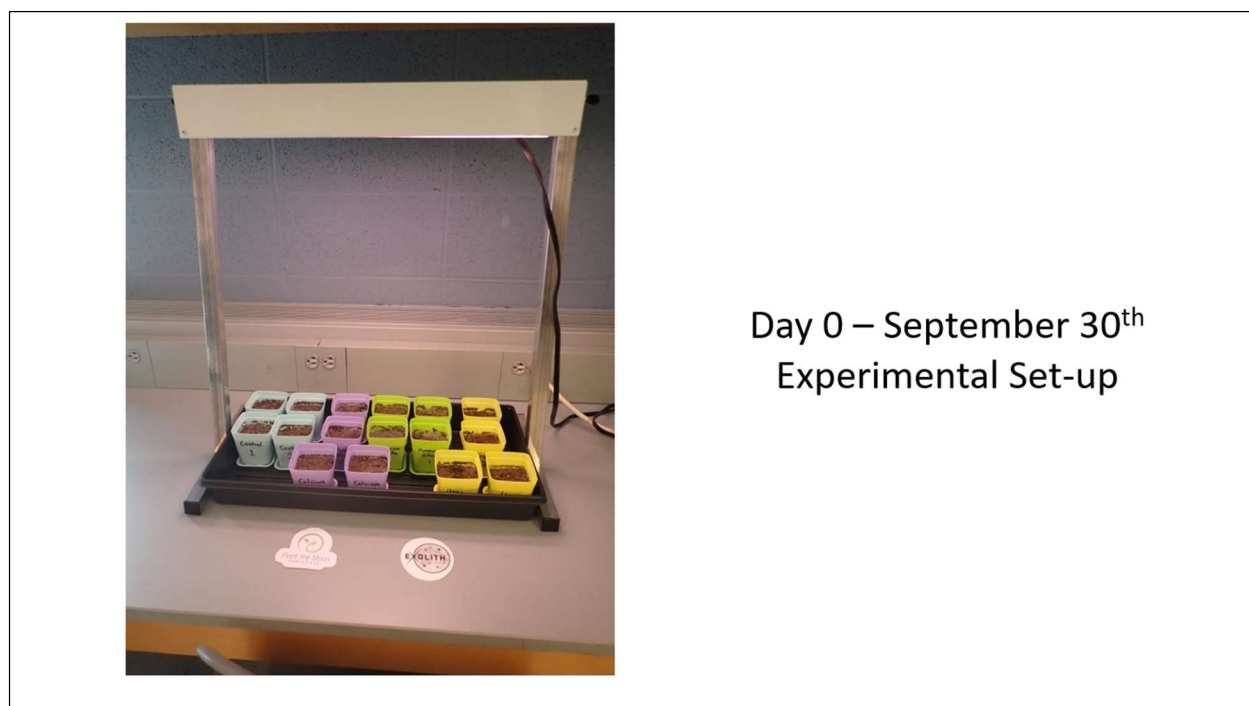
	19-Oct	21-Oct	25-Oct	28-Oct	1-Nov	4-Nov	8-Nov	11-Nov	15-Nov	18-Nov	Rate (cm/day)
Control 1	1.2	1.4	1.7	2.0	2.2	2.2	2.2	2.2	2.4	2.4	0.04
Control 2	1.2	1.6	2.1	2.4	2.5	2.5	2.7	2.7	2.8	2.8	0.04
Control 3	1.0	1.3	1.3	1.5	1.5	1.7	1.9	2.0	2.0	2.1	0.03
Control 4	0.5	1.0	1.4	1.4	1.5	1.5	1.6	1.8	1.8	1.8	0.03
Average	0.98	1.33	1.63	1.83	1.93	1.98	2.10	2.18	2.25	2.28	0.04
Calcium Nitrate Tetrahydrate 1	1.8	2.2	2.4	2.9	3.1	3.1	3.7	4.2	4.2	4.4	0.08
Calcium Nitrate Tetrahydrate 2	2.4	2.9	2.9	3.4	3.5	3.5	3.5	4.0	4.5	4.5	0.06
Calcium Nitrate Tetrahydrate 3	2.1	2.5	2.7	3.0	3.0	3.2	3.3	3.7	3.7	4.0	0.05
Calcium Nitrate Tetrahydrate 4	2.2	2.1	2.5	2.5	3.0	3.5	3.5	3.7	4.0	4.5	0.07
Average	2.13	2.43	2.63	2.95	3.15	3.33	3.50	3.90	4.10	4.35	0.07
Ammonium Nitrate 1	2.1	2.2	2.5	3.2	2.9	3.4	3.7	3.8	4.1	4.5	0.07
Ammonium Nitrate 2	1.7	2.2	3.1	3.7	4.1	4.5	4.6	4.7	5.1	5.1	0.10
Ammonium Nitrate 3	2.4	2.7	2.8	3.5	3.8	4.0	4.3	4.3	4.3	4.4	0.07
Ammonium Nitrate 4	2.5	2.8	3.1	3.2	3.0	3.6	4.1	4.6	4.6	5.0	0.08
Average	2.18	2.48	2.88	3.40	3.45	3.88	4.18	4.35	4.53	4.75	0.08
Urea 1	0.7	2.1	2.5	3.0	3.4	3.8	4.2	4.9	5.0	5.0	0.13
Urea 2	1.1	1.4	1.9	2.1	2.4	2.5	3.0	3.5	4.0	4.2	0.10
Urea 3	1.7	2.2	3.0	3.0	3.1	3.5	4.0	4.3	4.8	5.0	0.10
Urea 4	1.4	2.1	3.0	3.1	3.2	3.2	3.2	3.6	3.7	5.0	0.08
Average	1.23	1.95	2.60	2.80	3.03	3.25	3.60	4.08	4.38	4.80	0.10

Table 5. Leaf surface area (cm²) for each pot from October 21 to November 18, 2022. the average leaf surface area (cm²) for each treatment, and the rate of leaf surface area growth using a linear fit.

	21-Oct	25-Oct	28-Oct	1-Nov	4-Nov	8-Nov	11-Nov	15-Nov	18-Nov	Rate (cm ² /day)
Control 1	3.187	4.255	5.166	7.778	9.218	8.726	11.662	12.032	14.076	0.38
Control 2	1.635	2.888	3.536	4.151	5.437	6.040	8.362	8.816	9.607	0.29
Control 3	1.079	1.384	1.472	1.861	2.132	2.689	3.016	5.084	5.123	0.15
Control 4	0.470	0.781	1.052	1.300	1.459	1.732	1.728	2.886	3.451	0.10
Average	1.59	2.33	2.81	3.77	4.56	4.80	6.19	7.20	8.06	0.23
Calcium Nitrate Tetrahydrate 1	3.751	4.428	7.098	11.707	17.453	21.873	29.399	45.067	49.399	1.71
Calcium Nitrate Tetrahydrate 2	8.508	9.161	13.688	17.455	26.148	26.907	40.824	56.491	59.231	1.94
Calcium Nitrate Tetrahydrate 3	6.316	8.421	9.345	12.176	16.817	25.593	32.333	44.805	56.440	1.75
Calcium Nitrate Tetrahydrate 4	6.173	7.220	8.691	13.450	15.298	18.029	23.421	34.116	44.739	1.28
Average	6.19	7.31	9.71	13.70	18.93	23.10	31.49	45.12	52.45	1.67
Ammonium Nitrate 1	10.794	12.899	19.073	20.928	30.534	30.249	39.565	55.744	63.399	1.71
Ammonium Nitrate 2	3.150	6.204	8.497	15.465	20.706	25.148	30.713	49.005	53.522	1.94
Ammonium Nitrate 3	10.585	12.492	14.730	19.594	27.390	34.027	32.472	43.040	49.872	1.75
Ammonium Nitrate 4	7.876	9.691	12.995	12.373	18.710	25.971	31.009	47.951	55.787	1.28
Average	8.10	10.32	13.82	17.09	24.34	28.85	33.44	48.94	55.65	1.70
Urea 1	5.417	9.104	17.876	21.632	29.915	29.729	40.486	59.738	69.078	2.18
Urea 2	3.888	5.242	10.150	11.708	17.952	25.759	37.933	46.671	57.937	1.95
Urea 3	6.093	10.202	15.329	21.929	24.884	32.836	42.843	59.385	61.943	2.08
Urea 4	3.618	5.976	9.230	16.044	14.092	19.109	22.607	37.332	53.105	1.53
Average	4.75	7.63	13.15	17.83	21.71	26.86	35.97	50.78	60.52	1.94

Appendix B: Photos

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





Day 3 – October 3rd

We have germination!



Day 4 – October 4th



Day 6 – October 6th
 Moved Grow light down to
 reduce leggy seedlings.



Day 10 – October 10th
We have true leaves!



Day 11 – October 11th
Marked seedling for keeping when we
thin next week.



Day 14 – October 14th



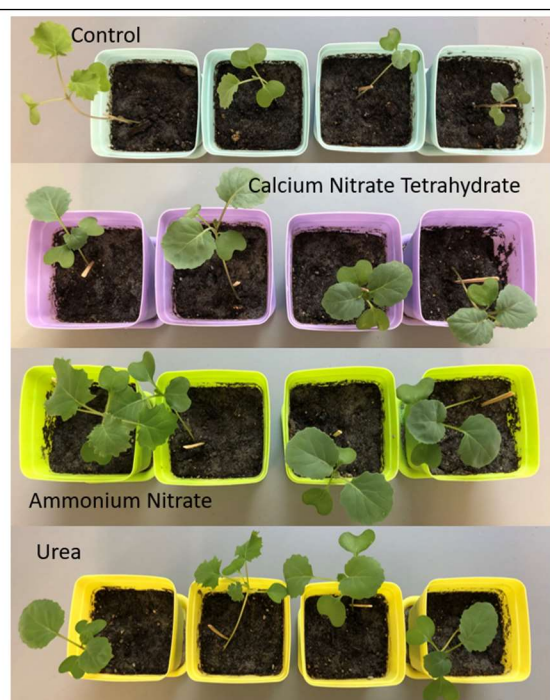
Day 19 – October 19th



Day 25 – October 25th
Thinned plants to one per pot

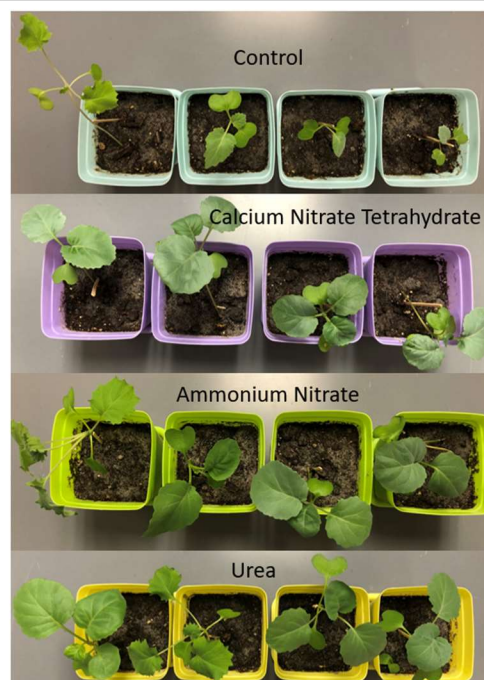


Day 28 –
October 28th





Day 35 –
November 4th

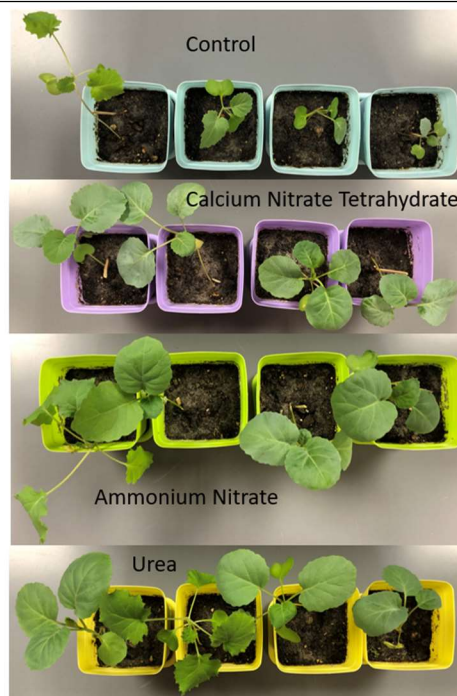


Day 39 –
November 8th

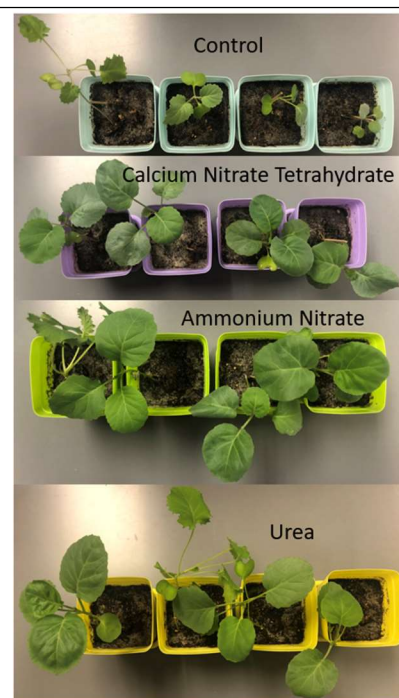




Day 42 –
November 11th



Day 46 –
November 15th





Day 49 –
November 18th

