I

The Introduction of Elements in an Ecosystem as a Means of Testing Phenotype Expression

Research Question

What effects do different nitrogen concentrations in soil have on the expression of phenotypes and growth rate in Wisconsin fast plants (*Brassica rapa*) grown?

Subject: Biology

Word Count: 3454

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Introduction

Within the stages of a plant's development and growth, many elements are utilized in said processes. Two of these elements are integral to not just plants, but to the entire ecosystem. Nitrogen has integral cycles that both the plants and ecosystems depend on. With growth of any organism, the physical expression of the genotype (phenotype) occurs as well. In this essay, I would like to explore if there is any correlation between the presence of these nutrients and what genes the plants end up expressing

The expression of a gene does not directly lead to the physical representation of a phenotype. Rather, it induces the synthesis of a protein (Medlineplus Genetics). The synthesis of these proteins leads to the eventual usage by the organisms in any way. This suage is often seen as physical changes such as growth or reproduction. I have always known that fertilizer is "good" for the plants, but I never truly understood why. Did it help the plants grow faster? Did it help the plants grow bigger? Hence why I decided to structure my experiment like so. Within doing this experiment I also wanted to test an alternative method of cultivation as a means to control my water usage.

Background Information

As our biosphere slowly degrades due to global warming and other human induced activities, the demand to feed a growing population of almost 8 billion becomes a daunting task. More resources are needed per day to feed this growing population, and not just by small values. Estimated by the FAO (Food and Agriculture Organization), we will need 60% more food to feed the population in just thirty years (*Silva*) This means our farmers and other providers must

turn to more efficient and safer methods of growing. For over 50 years, the use of nitrogen-based fertilizers has been used for the best growth yields on crops. Getting the best out of crop yields means that certain physical attributes are preferred over others; such is the nature of artificial selection. The main reason why fertilizer is used, is for that high output on the physical attributes expressed such as plant size, growth rate, and crop yield.

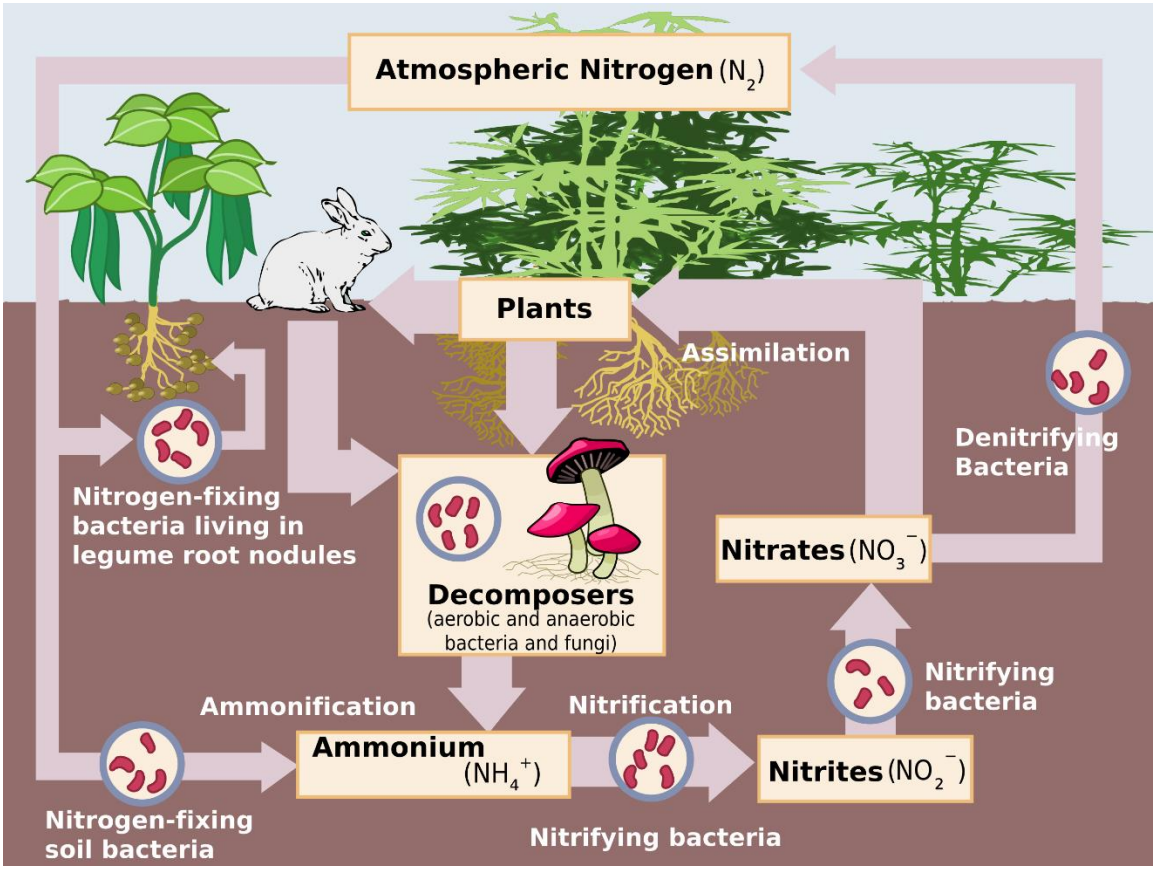
Generally considered a harmful substance, the ecological effects of nitrogen-based fertilizers are devastating. Runoff produced by fertilized cropland while benefiting conversely affects the river and/or marine life bordering the land. This negative effect is known as eutrophication, a biological phenomenon where algae in neighboring waters are overstimulated where excess amounts of oxygen are used as they decompose. This in turn creates areas of eutrophication or "dead zones", full of decaying algae and areas lacking oxygen and by result killing organisms in the affected areas. The eutrophic areas are highly dangerous and can shatter the entire flow of an aquatic ecosystem by means of staggering algae or producer growth and death cycles.

What I aimed to test, was with the right conditions in place, does the stimulation of certain phenotypes truly by the introduction truly have a relationship and does it affect the growth rate. The backlash of implementing hundreds of tons of nitrogen throughout the years has contributed heavily to global warming and health crises. Some of these health crises entail health maladies from drinking water such as cholera which has an increasing cost to combat every year (*Kratz*). While the answer might be truly obvious for whether or not fertilizer is essential to plant growth, I grew plant samples in individual and isolated plates as to not cause competition. This allowed me to fully outline the effects of naturally growing plants as opposed to nitrogenous soil.

Water is a constant that remains in the soil through the transfer in a cotton wick, but plants must intake nutrients and materials through uptake in the roots. Nitrogen is not an element that can be

derived from the air in plants, and through the nitrogen cycle, it is taken from the soil after it has been fixed by various processes. (*Figure 1*). Other than carbon dioxide and water, nitrogen is an essential element to plant growth, since nitrogen is the main component behind nucleic acids proper protein synthesis will not take place thus negatively affecting plant growth. Conversely, an excess of nitrogen is harmful to plant growth as well. The excess nitrogen leads to oversized stems and leaves while the roots are left lacking in structure. The key is the small trace amounts nitrogen is used in plant development. (*Azcel*). Keeping light intensity, temperature, and water amount constant is essential for the validity of this experiment, as in regular plant growth sunlight and water, are essential. By drastically changing any one of these variables, the growth of the plant is severely negatively affected.

Figure 1 – *Diagram outlining the Nitrogen Cycle within a standard plant system (1)*



No secondary elements are tested in my experiment, since the fertilizer has the bulk of nitrogen in it. Understanding why nitrogen is used in fertilizer is essential, as it provides justification for the rapid growth and harvest of crops. Macromolecules such as nucleic acids and amino acids both contain significant amounts of nitrogen atoms. These macromolecules are molecules such as DNA, RNA, arginine, glycine, serine, and more (*protein power*). These molecules are in fact related, as with proper DNA and RNA present, there cannot be proper gene expression which entails fulfilling the function of a gene (to start the synthesis of a protein). Once again, even if there is a sufficient amount of nitrogen for genetic material, the amino acids that compose the protein are lacking. These proteins are essential for embryo and vegetative cells in the plant cells. Considering that an organism's main function is to reproduce and survive, the lack of not being able to synthesize proteins goes against this main function.

Hypothesis

With knowledge from my biology classes and accumulated personal knowledge, I predict that the nitrogen will induce more phenotypic expression such as flower bloom, leaf count, and stem length in Brassica rapa because of the increased protein concentration. The increased protein rates will aid in the development of the plant like higher leaf count, stem length, flower count, etc.

Method

Independent Variables

- Time used for full life cycle
- The species of plants being used

Manipulated Variables

- Fertilizer Type (Osmocote Smart release & Osmocote Flower food)
- Type of Brassica rapa used (standard vs Purple Flower Bud)

Controlled Variables

- Volume of potting soil per plant (7 oz)
- Number of seeds per growth container
- Volume of water added per week
- Intensity of light given
- The type of growth containers (deli containers)
- Temperature kept at 68 F

Responding Variables

- Time for flower bloom
- Leaf count
- Stem Length
- Presence of successful reproduction

Method Development

Before starting my real experiment, I first ran some preliminary trials. This allowed for me to not only gauge what I had to do, but also let me understand some limitations I would potentially come across. My preliminary trials were designed not to gather data, rather they were run to help me design my real experiment. From the beginning, I understood that I needed to have a control group. The control group needs to have no factors that can influence the results of its growth which in terms of this experiment meant no usage fertilizer.

After growing my control group, I next had to tackle the goal of growing the plants in a sustainable and low maintenance fashion. My main issue with the preliminary generation was that I spent too much time watering the plants and could not deduce how much water to add for each plant. With inspiration from the curriculum-based Wisconsin Fast Plants growth plate, instead of using the water transfer string, I used a cotton wick.

Figure 2 – *UW Madison growth method and my modification*

After testing to see if water successfully transfers to the soil by checking its moisture, I started to get my experiment ready my materials (See Appendix A). Knowing what I wanted to test was difficult, but from my research into the background and processes of nitrogen in the plant cycle, I now had a good idea on what to test (See Responding Variables).

As any science experiment goes, there were things I had to keep constant in order to only get data that I wanted. Considering that I wanted to run this experiment in the fasted amount of time as possible, I opted to keep my plant lights which had a 6000 Kelvin light color range, suitable for indoor plants such as Brassica rapa. I also kept the composition of my water constant, only using distilled water canisters to fill the water portion of the growth containers. Along with this, the experiment groups and control group would use the same type of potting soil to ensure even more control. As for temperature, I kept the plants in small room in my house where I could somewhat control ventilation and temperature (68 degrees Fahrenheit). The amount of dirt I used was 7 ounces per container. As to viably measure my plants, I opted for 4 seeds per container to control competition and possible unintended cross pollination. As for fertilizer, considering that they have different nitrogen compositions, I decided that adding different amounts of them in their corresponding experimental groups would benefit the findings of this experiment. I put 8 pellets of the fertilizers in the “less” experimental group and 12 pellets in the “more” group. And finally, the control group, where I added no fertilizer.

I had successfully set up my experiment and until my plants had flowered, I had to wait. I used two types of Brassica rapa: a standard pure bred and Purple Flower Bud mutants. Once all 6 flowers appear, I would pollinate to test whether the plants could successfully reproduce after being exposed to fertilizer and if the fertilizer had any effect on progeny yield. I would pollinate the fertilized groups with the control group, but not with each other. To ensure no cross

pollination, I used a different bee stick each time I pollinated. Once this was done, I waited for the seed containers to sprout. Once the seed containers were fully mature and the flowers started to wilt, I stopped adding water to the plants. Once the plants had died and the seed pods dropped, the experiment was concluded.

Finally, I tested for any correlation. When testing for any type of correlation, I learned through my biology classes that the Chi Squared test would be best for testing the correlation between two things. For each measure I would take (See Responding Variables) I would run the chi squared test with the control group. Finally, by analyzing all the chi squared values, I can come to a conclusion on whether or not fertilizer and phenotypic expression has any correlation.

Results

In general, I noticed that the fertilized plants were bigger and were more "green," possibly indicating the presence of more chlorophyll. I also noticed that the flowers all sprouted within 2-3 days of each other, regardless of fertilized or not. Water consumption was also relatively constant, with me having to fill about the same amount of water peer week into the water containers of the growth plate

Raw Data

All Measured data points are curated in the tables below. I also added graphs to provide a means of visual representation.

<u>Tables</u>

Average Leaf Count per Plate

Weeks	Control Group 1	Control Group 2	Smart release "less"	Smart release "more"	Flower food "less"	Flower food "more"
1	0	0	0	0	0	0
2	2	2	3	3	3	4
3	6	7	7	7	11	12
4	17	17	21	24	28	33
5	23	25	27	31	35	38
6	24	25	27	31	35	38
7	24	25	27	31	36	38
8	24	25	27	31	36	38
9	24	25	27	31	32	38
10	24	21	23	26	29	30
11	24	21	15	26	24	28
12	18	19	12	17	19	22
13	12	15	9	14	16	15

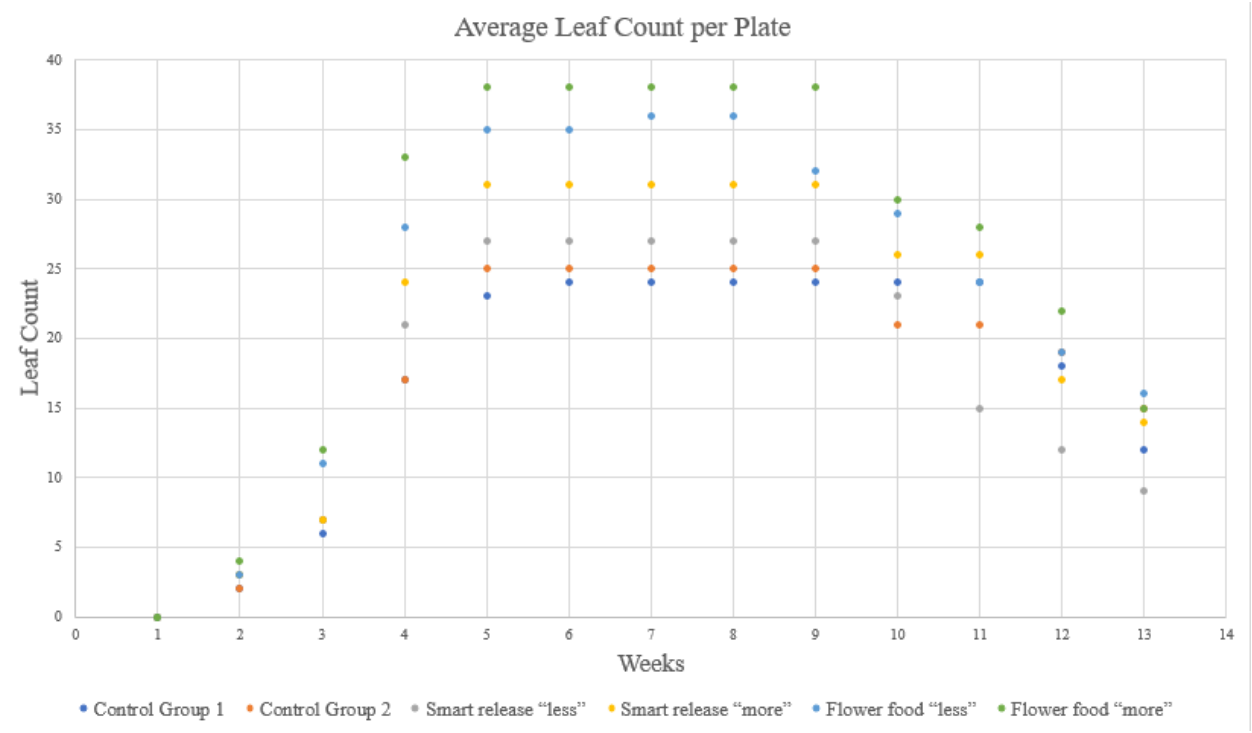
Average Stem Length per Plate (cm)

Weeks	Control Group 1	Control Group 2	Smart release "less"	Smart release "more"	Flower food "less"	Flower food "more"
1	0	0	0	0	0	0
2	3.5	3.7	4.2	4.4	5.3	5.6
3	5.8	6.1	7.2	7.6	8.3	8.4
4	7.4	7.2	8.6	9.2	9.9	10.5
5	10.6	10.9	12.2	12.6	13.4	14.1
6	12.8	13.2	14.6	15.0	16.7	17.1
7	14.4	14.8	15.1	16.3	17.2	18.0
8	14.6	14.8	15.1	16.3	17.2	18.0
9	14.6	14.8	15.1	16.3	17.2	18.0
10	14.6	14.8	15.1	16.3	17.2	18.2
11	14.0	14.8	14.7	16.3	17.2	18.0
12	14.0	14.2	14.7	15.9	17.2	17.4
13	14.0	14.2	14.3	15.4	17.2	16.8

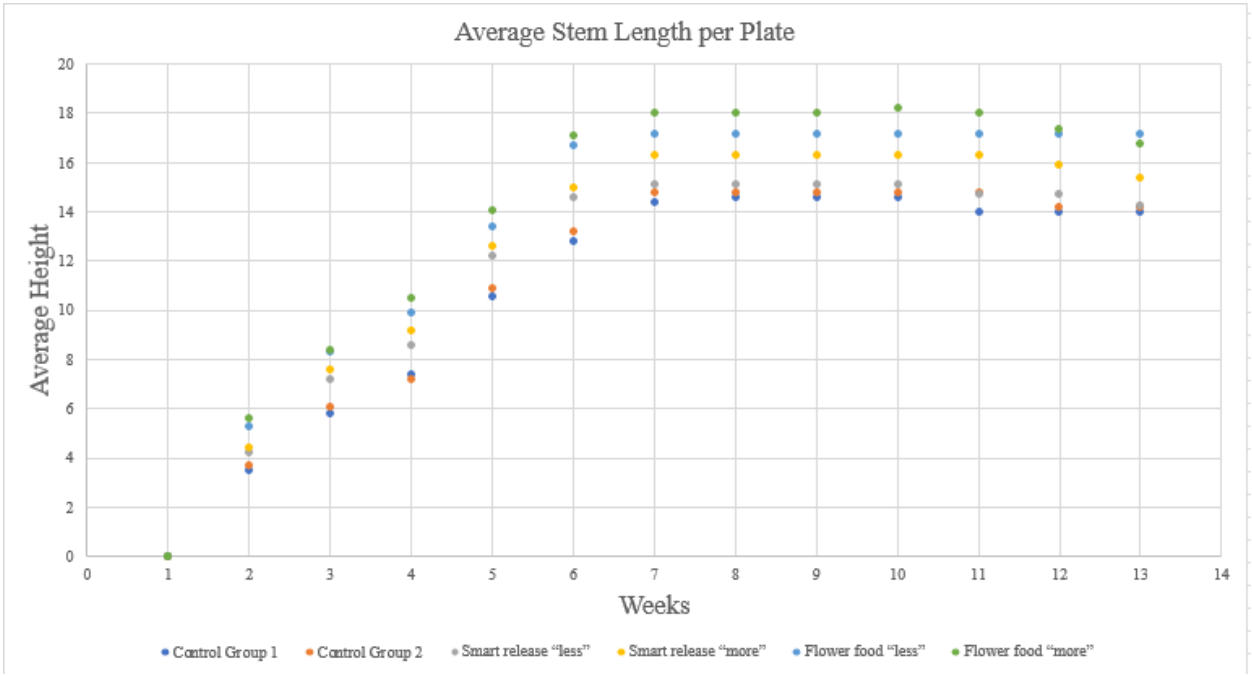
Flower Bloom Presence

Weeks	Control Group 1	Control Group 2	Smart release "less"	Smart release "more"	Flower food "less"	Flower food "more"
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	12	14	13	12	14	12
7	12	14	13	12	14	12
8	12	14	13	12	14	12
9	12	14	13	10	14	12
10	12	14	12	10	14	12
11	0	10	12	0	9	8
12	0	0	0	0	0	4
13	0	0	0	0	0	0

Leaf Count



Stem Length



Risk Assessment:

Using nitrogenous material while in small quantities is not dangerous, can lead to sicknesses in large quantities and should in general be kept out of touch. While nitrogen along is not the main factor in this chemical there are other chemicals which can cause harm especially if ingested. This is why when working with these grains, I wore face masks and gloves to avoid contact. When working with the soil, I also wore gloves. This is to reduce the risk of contamination as skin oils and cells can have unprecedented effects in the soil composition and vitality. By making sure all parts of my experiment are sterile, this will ensure my results are valid. When dealing with the flowers, I made sure to not damage any petals or pollen sacs. If in the case that these are broken, it will reduce not only the validity but the cross-pollination attempts. At times there might have been an excess amount of carbon dioxide or oxygen in the room. The lack of a truly controlled environment can mean that my results are not truly accurate, and there is a measure of uncertainty that must be considered when analyzing the data and its results/findings.

Data Analysis

Just by looking at this raw data, the two out of the three variables had a significant change with the presence of fertilizer. Both stem length and leaf count had a distinct difference between the control group and experimental groups. On the surface level, I could say that there is a correlation between fertilizer and phenotype expression, however by conducting the Chi-Squared test, I can make a more educated stance.

Statistics

Chi Squared Test

$$\chi_c^2 = \sum \frac{(O_i - E_i)^z}{E_i}$$

where:

c = degrees of freedom

O = observed value(s)

E = expected value(s)

Leaf Count

Average Control Group	Average Smart Release	Average Flower Food	Totals
0	0	0	0
2	3	3.5	8.5
6.5	7	11.5	25
17	22.5	30.5	70
24	29	36.5	89.5
24.5	29	36.5	90
24.5	29	37	90.5
24.5	29	37	90.5
24.5	29	35	88.5
22.5	24.5	29.5	76.5
22.5	20.5	26	69
18.5	14.5	20.5	53.5

13.5	11.5	15.5	40.5
224.5	**248.5**	**319**	**792**

Null Hypothesis

There is no correlation between the presence of fertilizer and plant phenotype expression in leaf count.

Alternate Hypothesis

There is a correlation between the presence of fertilizer and plant phenotype expression in leaf count.

Average Control Group & Average Smart Release/ Flower Food Osmocote

Average Control Group	Average Smart Release	Average Flower Food	Totals
0	0	0	0
2.409406566	2.666982323	3.423611111	8.5
7.086489899	7.844065657	10.06944444	25
19.84217172	21.96338384	28.19444444	70
25.36963384	28.08175505	36.04861111	89.5
25.51136364	28.23863636	36.25	90
25.65309343	28.39551768	36.45138889	90.5
25.65309343	28.39551768	36.45138889	90.5
25.08617424	27.76799242	35.64583333	88.5
21.68465909	24.00284091	30.8125	76.5

19.55871212	21.64962121	27.79166667	69
15.16508838	16.78630051	21.54861111	53.5
11.48011364	12.70738636	16.3125	40.5
224.5	248.5	319	792

Average Control Group	Average Smart Release	Average Flower Food
0	0	0
0.069566398	0.041582868	0.001704417
0.048538897	0.09082622	0.203237548
0.407109675	0.013110771	0.188533114
0.073942606	0.03002568	0.005652144
0.040094149	0.020527712	0.001724138
0.05183096	0.012868189	0.008256864
0.05183096	0.012868189	37.55686797
0.013696797	0.054661592	0.011701247
0.030656733	0.010297413	0.055907708
0.442318202	0.061046284	0.115504748
0.733370964	0.311394997	0.051028127
0.355392033	0.11471925	0.040469349

X^2		41.33286492
p		1.0584E-09

Stem Length

Null Hypothesis

There is no correlation between the presence of fertilizer and plant phenotype expression in stem length

Alternate Hypothesis

There is a correlation between the presence of fertilizer and plant phenotype expression in stem length.

Average Control Group & Average Smart Release/Flower Food Osmocote

Average Control Group	Average Smart Release	Average Flower Food	Totals
0	0	0	0
3.6	4.3	5.45	13.35
5.95	7.4	8.35	21.7
7.3	8.9	10.2	26.4
10.75	12.4	13.75	36.9
13	14.8	16.9	44.7
14.6	15.7	17.6	47.9
14.7	15.7	17.6	48
14.7	15.7	17.6	48
14.7	15.7	17.7	48.1
14.4	15.5	17.6	47.5

14.1	15.3	17.3	46.7
14.1	14.85	17	45.95
141.9	**156.25**	**177.05**	**475.2**

Average Control Group	Average Smart Release	Average Flower Food	Totals
0	0	0	0
3.986458333	4.389599116	4.973942551	13.35
6.479861111	7.13515362	8.084985269	21.7
7.883333333	8.680555556	9.836111111	26.4
11.01875	12.13304924	13.74820076	36.9
13.34791667	14.69775884	16.65432449	44.7
14.30347222	15.74994739	17.84658039	47.9
14.33333333	15.78282828	17.88383838	48
14.33333333	15.78282828	17.88383838	48
14.36319444	15.81570918	17.92109638	48.1
14.18402778	15.61842382	17.6975484	47.5
13.94513889	15.35537668	17.39948443	46.7
13.72118056	15.10876999	17.12004945	45.95
141.9	**156.25**	**177.05**	**475.2**

X^2	0.307475869
P	0.857496712

Post Data Analysis

When conducting a chi squared test, the final numerical p-value I receive lets me know whether to fail to reject the null hypothesis or to reject it. In the Chi-squared test, I put up against the critical value of the appropriate degrees of freedom and 5% chance. In my case, the critical value I will be using is 2.920. If my p-values from any of the 2 tests are less than this critical value, it means that my data is statistically significant and there is correlation (Critical Values of the Student's t Distribution). In the case of Stem length and Leaf Count we can reject the null hypothesis, supporting the stance that there is correlation between fertilizer, that the data is statistically significant, and that there is phenotypic expression as a result of fertilizer.

Evaluation

My method of creating a controlled environment on which I can test 3 different types of scenarios was a large success due to the data I was able to create. Whether or not this data is accurate is still up for questioning, however. But the notion that viable and usable data was created allows me to call it a success. When it came to collecting the data, one of the blurred lines in my collection came when I counted my leaves. Considering something a leaf vs was not one challenge I arrived upon, but after careful observation of what makes a leaf, I was able to come to a solid distinction. The same was of flowers as well; I decided to not count budding flowers as part of the flower count, only considering a pollen producing flower a bloomed flower. Having a set quota on how to distinguish data points contributed to the reliability of my results.

Conclusion

This experiment aimed to answer the question: What effects do different nitrogen concentrations in soil have on the expression of phenotypes and growth rate in Wisconsin fast plants (Brassica rapa) grown?

By conducting the experiment and analyzing the data I received, Nitrogen concentrations induce phenotypic traits such as stem length and leaf count. Flower count is a genetic trait that cannot be changed with the introduction of nitrogen as supported by this source: “Cold Spring Harbor Laboratory. "Gene network controls how many flowers and fruits plants will make in critical growth window: Flower production is directly related to how much our crops yield." (ScienceDaily. ScienceDaily, 8 November 2016.). Within the experiment, the most growth seen was done by the highest concentration of Osmocote Flower Food. Based on the information provided by the canister, each pellet is composed of usable 13% nitrogen, as compared to the 8% in the Osmocote Smart Release. This percentage is also supported by the data and Chi squared test, which aimed to prove or disprove any correlation between the fertilizers and phenotype presence. The test supported the alternative hypothesis and my personal one as well

As provided by context in the Background section, Macromolecules such as nucleic acids and amino acids both contain significant amounts of nitrogen atoms. These macromolecules are molecules such as DNA, RNA, arginine, glycine, serine, and more (*protein power*). These molecules are in fact related, as with proper DNA and RNA present, there cannot be proper gene expression which entails fulfilling the function of a gene (to start the synthesis of a protein). Once again, even if there is a sufficient amount of nitrogen for genetic material, the amino acids that compose the protein are lacking. These proteins are essential for embryo and vegetative cells

in the plant cells. Considering that an organism's main function is to reproduce and survive, the lack of not being able to synthesize proteins goes against this main function.

All in all, this test supported the notion that nitrogen-based fertilizers help the production of "better plants" due to the differences in phenotype expression observed in the control group as opposed to experimental.

Appendix

Appendix A: Materials List

Equipment/Materials	**Use in Experiment**	**Quantity**
Deli container (8 oz)	Soil is put inside here with opening for the cotton wick	6
Deli Container (16 oz)	This is for the water; the tail of the cotton wick will transfer water	6
Distilled Water (7.5 oz)	There is a constant amount of water per container. This amount will	Distribute 6 times
Growth Lamp 50W	This light is used to simulate the sunlight and constant growth	1
Wisconsin Fast Plant Seeds	This one of the groups of plants used in the experiment	12
Purple Flower Mutant Wisconsin Fast Plant Seeds	This the second of the groups of the plants used in the experiment	12
Osmocote Plus Outdoor & Indoor	This the first fertilizer used in the experiment which has a lower nitrogen concentration	8 in less group, 14 in more group
Miracle Gro Shake ‘n Feed	This is the second fertilizer used in the experiment which has a higher nitrogen concentration	8 in less group, 14 in more group
Miracle Gro Potting Mix	This the main soil that is used for the plants. It is modified with the introduction of fertilizer	7 ounces per container
Cotton Wick	This the way that water will be transferred to the soil within the whole timeframe of the experiment	6, one per each container

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