

A study in the effect of lauric acid on *brassica rapa*

I. Personal Engagement

Growing up in Vietnam, my mother's family were farmers. I grew up near rural Vietnam and had significant exposure to countryside life. My spirituality is also deeply connected to nature, because of Taoist influences on Vietnamese Buddhism. I am not much of a deeply religious person though; I consider myself a secular Buddhist, concerned with using the teachings of my people to care about life on earth, and it has been reinforced by my mother's green thumb. She has grown many gardens across the homes we have lived in and I have developed a deeper appreciation for the life we can foster.

I am quite concerned about the preservation of the environment, because of my upbringing. Climate change and overpopulation are the long-term issues that I believe we have no way of tackling with the current infrastructure and science we have now. I want to take the small-scale planting I do in my backyard and be able to do that in places that have been heavily deforested and where the plant biodiversity has been devastated.

I also want to get a biochemistry degree and work with genetic engineering techniques to modify how plants and animals can be humanely modified to benefit the environment, such as creating a wheat strain that uses less water and space.

II. Exploration

- How do varying increments of lauric acid affect the growth of the Wisconsin Fast Plant's roots and shoots (*brassica rapa*)?
- **BACKGROUND**

Brassica Rapa

Brassica rapa refers to a member of the cabbage family, though the plant can take on many morphs, such as turnips, Chinese cabbage, and canola (Wisconsin Fast Plant). One morph is the Wisconsin Fast Plant, which has been selectively bred to be a model organism with its short germination and flowering times and its responsiveness to

the environment. The plants also flower at roughly 14 days and have a rapid life cycle, allowing for it to be grown quickly and easily without using a lot of resources or space (Wisconsin Fast Plant).

Plant Hormones

6-Benzylaminopurine molecules are very stable and “exert a prolonged inhibitory effect on rooting” (Podwyszynska, 1). Shoots that develop under exposure to high BAP levels tend to be smaller and have smaller leaves, while the roots have a strong positive correlation with shoot growth and development.

BAP is a type of plant hormone that is known as a cytokinin. Cytokinins are hormones that stimulate plant development and cellular division (an increased rate of cytokinesis) (Britannica). Therefore, BAP stimulates the setting of blossoms and fruit richness through increased amounts of cell production, which has a higher sugar content being required to support more mass.

Cytokinins also create change and react alongside auxins to impede the process of senescence, though in the early stages of a plant’s life cycle, it is a phase of metabolism and not just decay (Britannica). This therefore prevents the yellowing of leaves by preventing the breakdown of proteins and chlorophyll and stabilizing the molecules in the leaf and the chloroplasts. High auxin and low cytokinin concentrations give rise to root development,” whereas the inverse will promote the development of the shoot (Britannica). Lauric acid is a type of saturated fatty acid that can act as a substitute for BAP and have similar effects.

Lauric acid is a solid at room temperature but is very soluble in boiling water. “The molecule is a saturated medium-chain fatty acid with a 12-carbon backbone” and can be found in plant and animal oils, mainly coconut and palm kernel oil (NCI). Like BAP, lauric acid can react alongside auxins to impede early stage senescence. It also boosts meristematic tissue growth and promotes development.

- **HYPOTHESIS**
 - The solutions with the higher amounts of lauric acid will result in faster germination and development.
- **VARIABLES**
 - Independent Variable: The variable that is being adjusted and tested is the concentration of lauric acid being put into the soil.
 - Dependent Variable: The length of the plant specimen from apical root to apical shoot (in centimeters) will reflect the effects of the independent variable.
 - Control Variables:

- The amount of water in each molar solution should be equal to one cup.
- Each cup should have one teaspoon of lauric acid mixed and dissolved in it.
- Each 25.4 x 17.78 x 17.78 cm has to be filled with 1 pt of soil.
- One plant per small pot.
- All of the plants should be exposed to the same light source and temperature.

○ **MATERIALS**

- 1000ml Sterile Water
- 3000ml water
- Murashige and Skoog Medium 5L
- pH strips to adjust the agar solution
- 1M HCl to adjust the pH of the medium (minimum sizes)
- 1 1000 ml beaker
- 1 Pressure cooker at 17 psi
- 1 pair of gloves
- 30 pots 1 pt of soil
- 30 grams of lauric acid
- 30 seeds of *brassica rapa*
- 1 balance

○ **METHOD**

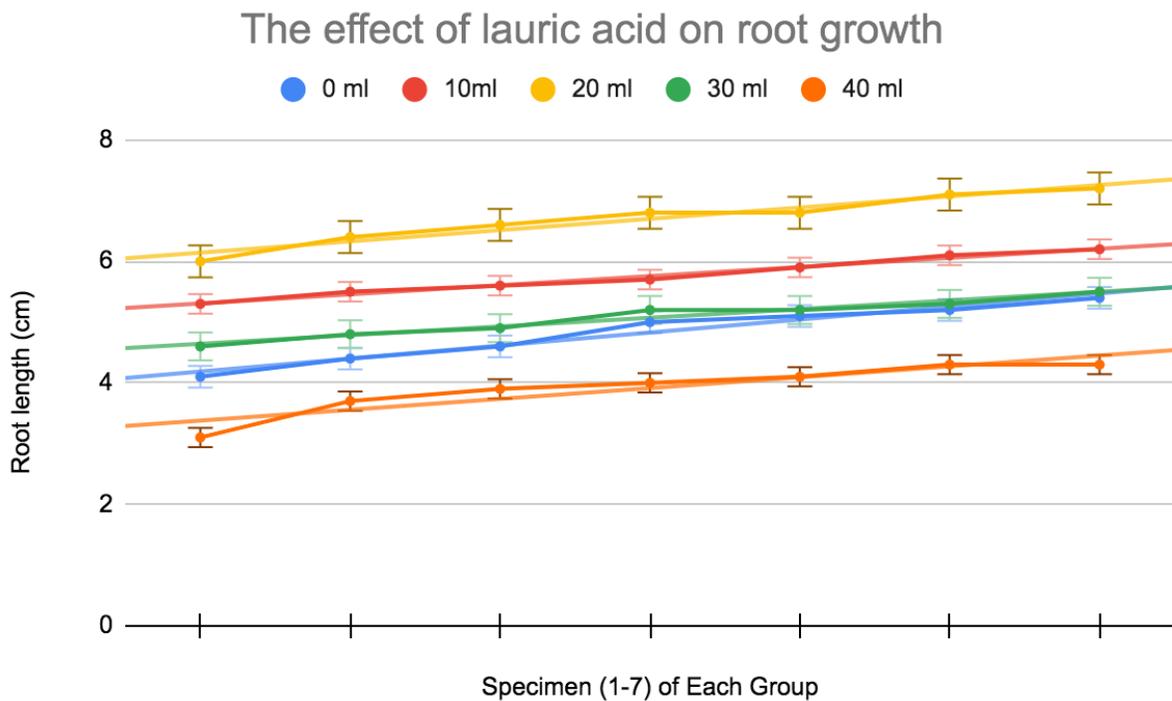
- 1. Gather materials and put on gloves. Tie up hair and make sure to wear closed toe shoes.
- 2. Label each row of pots with the numbers 1-5, corresponding to each row that the pots are on. Each row should have 7 pots and 5 pots per column. Masking tape and/or a permanent marker can be used. Place them in a wide area that has access to light.
- 3. Take 600 ml of distilled water and add 4.54 grams of dehydrated Murashige and Skoog medium at room temperature (15-30 C).
- Rinse the media vial with a small quantity of distilled water to remove trace residues of powder.
- 4. Add the desired heat stable supplements prior to pressure cooking. Continue stirring until the powder has completely dissolved.

- 5. If the powder does not completely dissolve then use HCL and mix, heat, and rotate until the solution becomes clear. Adjust the final volume up to 1000 ml with distilled water.
- 6. Dispense the mediums into suitable closed containers and pressure cook at 121-125 C for about 15-20 minutes, never over 20 psi.
- 7. Evenly pour 10ml of the solution into each pot of soil, in the center of the pot.
- 8. Take the bottle of lauric acid, and pour 25 grams into 5 into 1200 ml of water heated to 37 C. Mix until the lauric acid is saturated in the water.
- 9. Pour 10 ml each into the second row of pots. Then pour 20 ml of the solution into the third row, 30ml each pot for the fourth row, and 40ml each pot for the fifth row. Stir the dirt.
- 10. Plant 1 seed per pot.
- 11. Every two days, water the plants with 300 ml for each plant. Repeat the process of watering and observe for 45 days.
- 12. Observe how many new seedlings are in each pot and then pull out the specimen. Measure the length of the roots and stem separately.
- 13. Safely discard and clean up lab materials, then clean off any surrounding surfaces to prevent chemical poisoning or the buildup of toxic residues on lab equipment. Once this process is completed, take off the gloves and discard them safely.
- **RISK ASSESSMENT**
 - Gloves should be worn because the agar, Murashige and Skoog medium, and the lauric acid are all chemical grade materials. The chemical residues can result in some skin irritation and burning. There is low risk of exposure to the eyes, but it is advised that glasses are worn when handling the agar, medium, and lauric acid because all of them are in powder form.
 - While aprons are not necessary, it can prevent the solution from soaking into one's clothes and body and causing sickness. The heating of the pressure cooker could potentially create a hot solution that can result in burns if dropped.
 - The plants after they complete their life cycle should be appropriately discarded or composted to avoid introducing this plant into a non-native ecosystem.
 - Forceps were used to minimize even further the amount of contact made with the solutions, one of the reasons being to avoid any of the fluid getting on the arm, especially after retrieving the agar from the pressure cooker.

III. Analysis

- **RAW DATA:** The top row from 0-40ml is the amount of lauric acid each plant in the column received. The integers horizontally descending is the specimen number for the column. The data represents the length of the roots. The tape measure used has an error margin of $\pm .159$ centimeters. Graphs for both dependent variables can be used as a visual aid to see the relationship.

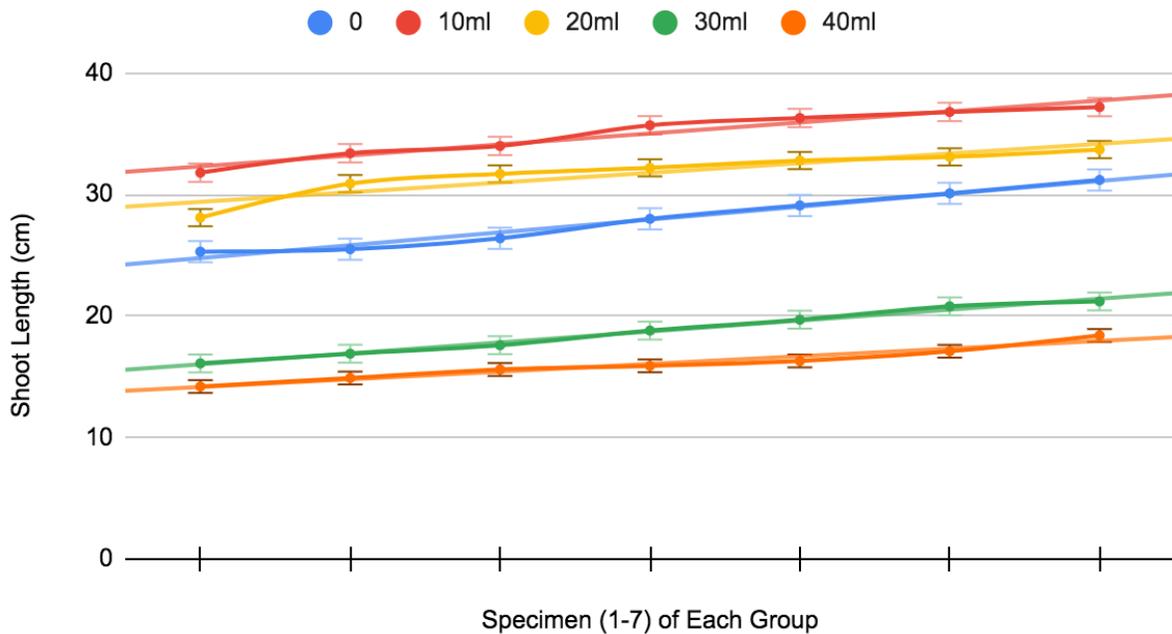
	0 (control)	10ml	20ml	30ml	40ml
1	4.1 \pm .159	5.3 \pm .159	6 \pm .159	4.6 \pm .159	3.1 \pm .159
2	4.4 \pm .159	5.5 \pm .159	6.4 \pm .159	4.8 \pm .159	3.7 \pm .159
3	4.6 \pm .159	5.6 \pm .159	6.6 \pm .159	4.9 \pm .159	3.9 \pm .159
4	5 \pm .159	5.7 \pm .159	6.8 \pm .159	5.2 \pm .159	4 \pm .159
5	5.1 \pm .159	5.9 \pm .159	6.8 \pm .159	5.2 \pm .159	4.1 \pm .159
6	5.2 \pm .159	6.1 \pm .159	7.1 \pm .159	5.3 \pm .159	4.3 \pm .159
7	5.4 \pm .159	6.2 \pm .159	7.2 \pm .159	5.5 \pm .159	4.3 \pm .159



III. The top row from 0-40ml is the amount of lauric acid each plant in the column received. The integers horizontally descending is the specimen number for the column. The data represents the length of the shoot.

	0 (control)	10ml	20ml	30ml	40ml
1	25.3± .159	31.8± .159	28.1± .159	16.1± .159	14.2± .159
2	25.5± .159	33.4± .159	30.9± .159	16.9± .159	14.9± .159
3	26.4± .159	34± .159	31.7± .159	17.6± .159	15.6± .159
4	28± .159	35.7± .159	32.2± .159	18.8± .159	15.9± .159
5	29.1± .159	36.3± .159	32.8± .159	19.7± .159	16.3± .159
6	30.1± .159	36.8± .159	33.1± .159	20.8± .159	17.1± .159
7	31.2± .159	37.2± .159	33.7± .159	21.2± .159	18.4± .159

The effect of lauric acid on shoot growth



o **PROCESSED DATA**

IV. The raw data has error bars based on the standard error of the mean (SEM).

SEM or $s_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$ where σ represents a sample's standard deviation and n is the amount of data points. Taking the data of the 40ml shoot length, the SEM can be calculated.

The first step is finding the value of the standard deviation. $\sigma = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$.

The mean can be calculated as $\bar{x} = \frac{(3.1 + 3.7 + 3.9 + 4 + 4.1 + 4.3 + 4.3)}{7} = 3.9142857142857$

$\sigma = \sqrt{\frac{1}{7-1} \sum_{i=1}^7 (x_i - 3.9142857142857)^2}$. N is equal to the number of specimens in each group, 7.

Squaring both sides of the equation simplifies the equation. $\sigma^2 = \frac{\sum (xi - \bar{x})^2}{N-1}$. The sum of the mean subtracted from all of the data points is equal to the top portion of the fraction.

$$\frac{\sum (xi - \bar{x})^2}{N-1} = \frac{(3.1-3.9\dots)^2 + (3.7-3.9\dots)^2 + (3.9-3.9\dots)^2 + (4-3.9\dots)^2 + (4.1-3.9\dots)^2 + (4.3-3.9\dots)^2 + (4.3-3.9\dots)^2}{7-1}$$

Completing all of the operations on the top and bottom, the fraction becomes . The standard deviation squared or the variance is 0.1747619047619. Square rooting both sides means that $\sigma = 0.4180453381655$.

Now the value for the standard deviation can be plugged into the equation $s_x = \frac{\sigma}{\sqrt{n}}$. N

is 7, so the equation becomes $s_x = \frac{0.4180453381655}{\sqrt{7}} = 0.15800628593369$. This

means that the standard amount of error for that group sample is 15%. This can seem high but the presence of the rosette phenotype should be considered, which is significantly shorter than the other phenotypes. The other phenotypes are roughly the same height, so the presence of this dwarf stock greatly changes the SD, making it seem that it is not accurate (Wisconsin Fast Plant).

Since there are two dependent variables and one independent variable, the MANOVA test will be used to examine the relationship between these variables. The first step will be calculating the sum of squares of the model, which is represented by the equation

$$SS_m = \sum_{i=1}^n (\bar{x}_k - \bar{x})^2$$

The mean of the different factors, or group intervals, must be calculated. \bar{x}_{kDVN} represents the mean of the different factors where DV1 represents the root growth and DV2 represents the shoot growth.

Factor 1's (the control group) mean of the first dependent variable is equal to $\frac{(4.1 + 4.4 + 4.6 + 5 + 5.1 + 5.2 + 5.4)}{7} = 4.82857143$. The same steps can be repeated to give $1F_2 = 5.75714286$, $DV1F_3 = 6.7$, $DV1F_4 = 5.07142857$, and $DV1F_5 = 3.91428571$. These steps can be repeated with the second dependent variable to give $DV2F_1 = 27.942857142857$, $DV2F_2 = 35.028571428571$, $DV2F_3 = 31.785714285714$, $DV2F_4 = 18.728571428571$, and $DV2F_5 = 16.057142857143$.

The total means for both dependent variables are needed. For DV1, this is 5.2542857142857, and for DV2 it is 25.908571428571. Proceed to subtract the total means from each of the factor's means and then square it. Then add all of the values together to get the sum. The first dependent variable's $SS_m = \sum_{i=1}^n (\bar{x}_k - \bar{x})^2 =$

$$(4.82857143 - 5.75714286)^2 + (5.2542857142857 - 5.75714286)^2 + (6.7 - 5.75714286)^2 + (5.07142857 - 5.75714286)^2 + (3.91428571 - 5.75714286)^2 = 5.870416359.$$

The second independent variable's $SS_m = 270.4565714$.

Then the squares of the error must be calculated, using the equation $SS_E = \sum_{i=1}^n s_k^2 (n_k - 1)$.

This essentially means that the sum of the squares of all of the factors together is needed. In order to get this, the mean of the different factors (F_k) must be subtracted from the data points of their respective factors squared and added together. This will be demonstrated with the control group of the first dependent variable.

$$(4.1 - 4.82857143)^2 + (4.4 - 4.82857143)^2 + (4.6 - 4.82857143)^2 + (5 - 4.82857143)^2 + (5.1 - 4.82857143)^2 + (5.2 - 4.82857143)^2 + (5.4 - 4.82857143)^2 = 1.334285714.$$

This should be repeated for the other 4 groups of the first dependent variable, and then all of the values are to be added together. Therefore, $SS_E = 1.334285714 + 0.6371428571 + 1.02 + 0.5942857143 + 1.048571429 = 4.634285714$

The squares of the error for the second dependent variable is calculated the same way and equals = 111.4914286. Now that the sum of the squares and the squares of the error have been calculated, there is enough information to look at the covariance, -- how the dependent variables are related both for the model and for the error.

In order to study the covariance, the cross product for the model must be calculated.

The equation for the cross product is $CP_m = \sum_{(0-40ml)}^n n_k(\bar{x}_{group} DV1 - \bar{x}DV1)(\bar{x}_{group} DV2 - \bar{x}DV2)$.

The first step is to do this for the first factor. This can be derived by subtracting the DV1's total mean from the group mean of the factors and then multiplying by DV2's total mean from the group means of the factor. Since the k represents the amount of terms, which is 7, the product should be multiplied by 7.

$$7(4.82857143 - 5.2542857142857)(27.942857142857 - 25.908571428571) = -6.062171408$$

The process should be repeated for the next four groups and all total values should be added together.

$$7(4.82857143 - 5.2542857142857)(27.942857142857 - 25.908571428571) + 7(5.75714286 - 5.2542857142857)(35.028571428571 - 25.908571428571) + 7(6.7 - 5.2542857142857)(31.785714285714 - 25.908571428571) + 7(5.07142857 - 5.2542857142857)(18.728571428571 - 25.908571428571) + 7(3.91428571 - 5.2542857142857)(16.057142857143 - 25.908571428571) = 187.1137149$$

$$CP_m = \sum_{(0-40ml)}^n n_k(\bar{x}_{group} DV1 - \bar{x}DV1)(\bar{x}_{group} DV2 - \bar{x}DV2) = 187.1137149$$

The next element that is needed is the cross product of the error. The cross product of

the error is $CP_E = \sum_{(0-40ml)}^n (x_i - \bar{x}DV1)(x_i - \bar{x}DV2)$. The first data points for the two

dependent variables (specimen 1 measurements of the control) must have the group means subtracted from them and then multiplied. The process should be repeated for all data points and groups. $(4.1 - 4.82857143)(25.3 - 27.942857142857) + (4.4 - 4.82857143)(25.6 - 27.942857142857) + (4.6 - 4.82857143)(26.4 - 27.942857142857) + \dots = 17.32775511$

With the sum of squares of the model, and the error for both dependent variables, the cross products for the model and for the error, the cross product matrices can be made.

The cross product matrix for the model is $H =$

$$(SS_{m1} \quad CP_m) = (5.870416359 \quad 187.1137149)$$

$$(CP_m \quad SS_{m2}) = (187.1137149 \quad 270.4565714)$$

The matrix of the error is $E =$

$$(SS_{E1} \ CP_E) = (4.634285714 \ 17.32775511)$$

$$(CP_E \ SS_{E2}) = (17.32775511 \ 111.4914286)$$

The processing will require a follow-up test. Using the two matrices that have been created, a follow-up test can be done using statistical software, since it is not possible to do by hand. The follow-up test that will be used is the Pillai's trace test, done in XLstat of Microsoft Excel. From the D values, F-value, and Lambda, the p-value is less than 0.0001. This means that the risk to reject the null hypothesis while it is true is lower than 0.01%. This indicates that the variable has a significant effect on the dependent variables at a 99.9% confidence level.

Lambda	1.852
F-value	90.409
D value of F1	8
D value of F2	58
Critical F Values	2.103
P-value	< 0.0001

CONCLUSION

- The effect of lauric acid on both root and shoot length show a strong relationship.

IV. Evaluation

○ STRENGTHS:

- The plant chosen was specifically bred to be a model organism because of its uniform and short flowering time, short lifespan, low resource cost, and high responsiveness to the environment. The seeds are inexpensive to purchase.
- Uniformity was established by using the same pots and seeds across a uniform amount of soil per pot (as per a scale).
- The materials were affordable and were widely available. Lauric acid is inexpensive, non-toxic, and is generally safe to handle.
- Furthermore, since the class has been taught about the plant cell structure, life cycle, and the role of hormones, the background knowledge was used to make the process much easier, especially with researching and formulating a hypothesis.

- The process was quick, and with gloves, the risk of contamination and error being made is lessened. Excess agar or lauric acid solution was safely disposed of through super-dilution.
- The pressure cooker was used with gloves and glasses. The process was short and ensured that the agar solution would be sterile and pure.

○ **WEAKNESS, LIMITATION, AND IMPROVEMENT**

Weaknesses and/or Limitation	Improvements
Lauric acid tends to solidify in the presence of water because of its non-polar nature and resistance to mixing with water. Some small clumps of lauric acid solidified on top of the soil instead of being absorbed by it initially after the hot lauric acid and water solution was poured on top of the soil.	Once the water and the lauric acid is mixed together, pour the solution in a hole in the center of the pot's soil. Then, cover it up and mix the soil to ensure that it does not solidify.
Better documentation of the plants should have been kept. Pictures were taken but were not high quality enough for annotation of the specimen. Some of the specimens were outliers, and the documentation does not reflect the recessive phenotypes and other outliers well.	Images should have been taken of an individual plant while it was still in the pot and after removal, so that the roots can be annotated.
Not all of the plants were of the same phenotype. Normally this would not affect the height too much, but there is a dwarf morph that is smaller than the typical phenotypes. However, the rosette phenotype only manifested twice in the total sample.	Exclusion of the rosette phenotype or ensuring all of the plants that were measured and used for data processing were all of the same stock.
Usually data processing of a MANOVA would require advanced statistical software that is hard to access. The mathematics here was done by hand, when usually MANOVAs and followup tests are done with the help of programs. The last part of the	There is some software that can replace the advanced statistical software that universities and research organizations use. XLstat can perform the followup MANOVA tests.

MANOVA test was done however with a program.	
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