Investigating how light intensity can affect stomatal density of tomato leaves.

Background Information:

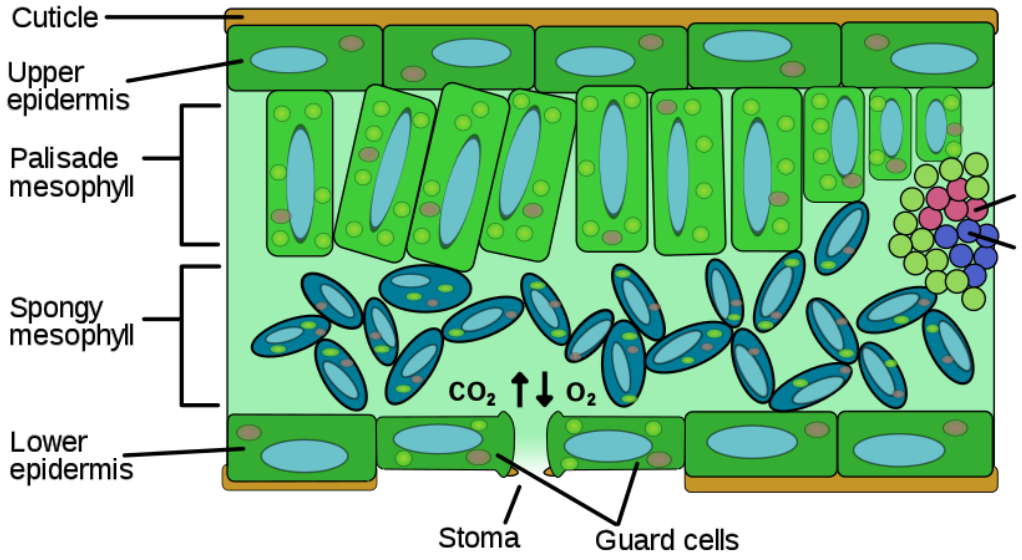
The stomata are openings bounded by two guard cells in the epidermis, occurring in vascular plants. They appear on green aerial parts of plants and stems, but more commonly on the leaves. By changing their shape, the guard cells control the size of the stomatal aperture to conduct an exchange between plants and the atmosphere.

According to the photosynthesis formula, atmospheric CO_2 is diffused and assimilated through leaf stomata, with the presence of sunlight, phosphate, nitrate, and other nutrients, converting into organic compounds. The equation of photosynthesis is formulated as:

$6CO_2 + 6H_2O + (energy) \rightarrow C_6H_{12}O_6 + 6O_2$ (Carbon dioxide + water + energy from light produces glucose and oxygen) **("Stomatal Anatomy and Stomatal Resistance")**

The frequency and size of leaf stomata determine how much gases plants have exchanged, how much energy, including sunlight, has been absorbed by plants, and how much energy is produced by plants.

Palisade mesophyll is located on the upper layer of the mesophyll, which is an area between the upper and lower epidermis of the leaf that contains many chloroplasts and allows CO_2 to circulate freely. When intense sunlight enters the space between the epidermises through the stomata, it causes the volume of the palisade mesophyll cells to increase, which enables more efficient light absorption. Leaves growing under a more intense light are better at absorbing CO_2 due to an increase in palisade mesophyll volume.



(fig.1 labelled of leaf transverse section)

("Specialized Cells of The Leaf System")

The process of photosynthesis takes place in chloroplasts, which are contained in mesophylls. The palisade mesophylls (upper) exposed under high-intensity light have 3.5 times more chloroplasts to capture light energy than the ones under low-intensity light. Furthermore, the spongy mesophylls (lower) under high-intensity light have 2.7 times more chloroplasts than the ones under low-intensity light. **("The Effect of Different Light Intensities on the Frequency and Size of Stomata, the Size of Cells, the Number, Size and Chlorophyll Content of Chloroplasts in the Mesophyll and the Guard Cells during the Ontogeny of Primary Leaves of Sinapis alba")**

It is proven theoretically and statistically through the previous studies that stronger light intensity and more efficient energy absorption have structured a positive correlation. However, will the positive correlation turn negative at a certain point? If so, what is the optimum of stomata frequency to maintain the most efficient energy absorption and how intense will the sunlight be? In order to answer these questions, I will be observing stomatal densities in tomato leaves under differing light intensities.

Tomato leaves will be collected for the investigation. Tomato plants are chosen because of the availability as a common plant. Additionally, tomato leaves are amphistomatous leaves, which means that they have stomata on both surfaces. This is important because the stomatal densities of amphistomatous are larger, which will help observational differences be clearer.

According to the photosynthesis formula, sunlight energy is an essential component with sufficient carbon dioxide and water. By absorbing stronger sunlight, tomato leaves will likely obtain energy more efficiently which leads to the results of more products (oxygen and glucose) transformation. The glucose will be produced, stored and transported through the plants for cellular respiration, while the oxygen will be released and become a necessity for human respiration. Therefore, the significance of my investigation is to maximise both products' conversion of glucose and oxygen through exploring if the maximums of reactants, specifically sunlight, will result in more fruitful tomato harvestings and more sufficient oxygen sources. **("Cellular Respiration and Photosynthesis")**

I have lived in six different houses/apartments before for at least one year each, and the houses have either had a garden or a playground where different plants grow, so I am interested in investigating plants due to this personal attachment. Consequently, I always pay additional attention to botanical topics as a connection to my life. Now I have tomatoes, one of my favourite vegetables (or fruits), planted in the backyard as they are beneficial for humans to reduce the risk of heart diseases and cancer by providing essential vitamins and potassium. The flavours and benefits of tomatoes make me invested in this topic.

Research question:

How does light intensity (56.7 lux - very weak, 138.9 lux - weak, 378.3 lux - moderate, 730.8 lux - strong, and 770.0 lux - very strong) affect stomatal density of tomato leaves?

Independent variable:

Five different light intensities from five different locations with different light exposures: very weak, weak, moderate, strong, and very strong (56.7 lux, 138.9 lux, 378.3 lux, 730.8 lux, and 770.0 lux) (± 0.1lux).

Dependent variable:

Stomatal densities of tomato leaves observed under microscope.

Controlled variables:

Variable	**Reason for control**	**How it will be controlled**
Leaves from the same tomato species	Collecting from the same tomato species maintains the constant of amphistomatous leaves.	Collecting the leaves from the same tomato plants in the garden
Quantity of leaves being collected	The same amount of leaves under five different light conditions is constant for collecting reliable data.	Collecting three leaves under five different light conditions
Clear nail polish	Same clear nail polish without impurities or other chemicals provides a clearer observation on leaves.	Collecting from the same nail polish bottle
Microscope clarity	Different clarities of the microscope can affect the counting of stomata; therefore, maintaining the same resolution is needed.	Using the same microscope and the same objective lens (400x)
Time when the leaves are collected and the light intensity is detected	Light intensity can only be detected precisely during the same period to obtain the most precise data correlation.	Collecting tomato leaves and detecting light intensities during the same period of the day
Light intensity probe and labquest screen	Different probes and screens have varied functions, resulting in varied data that will in turn result in imprecise results.	Having one light intensity probe and screen without switching

Exploration:

Materials:

Compound microscope with magnification up to 400x

One microscope slide

One pair of forceps

One marker pen

Clear nail polish

Tomato leaves x15 (x3 under each light intensity)

TI-84+ calculator

Light intensity probe 0 to 770 lux (± 0.1lux)

Labquest screen

Risk Assessment:

Concerns	**Hazard**	**Precaution**
Chemicals in clear nail varnish	Low hazard.	Work in a well-ventilated space because it can cause headaches

Method:

1. Find the suitable tomato plants under five different light intensities to investigate and collect three leaves from each location:

Detected light intensity (lux)	Light Intensity Sources
50-60	The light intensity was detected from the most shaded side of the patio, where the tomato leaves were all shaded with no direct light illumination.
130-140	The light intensity was detected from the back of the patio, where light could not be directly received by tomato leaves.
370-380	The light intensity was detected from the middle of the patio, where light could only be directly received by tomato leaves during a certain period of time from 3:00 PM to 6:30 PM in early September for three and a half hours.
730-740	The light intensity was detected from the front of the patio, where light could be received by tomato leaves from 8:30 AM to 2:00 PM in early September for five and a half hours.
760-770	The light intensity was detected from the middle of the garden, where light could be directly received by tomato leaves from 8:00 AM to 7:00 PM in early September for eleven hours.

2. Coat a layer of clear nail polish on both sides of the leaf surface. Leave it to dry.
3. Carefully peel off the thin layers of clear nail polish from both sides of the leaf by using forceps.
4. Place the thin layer flatly on a piece of tape and tape it on the microscope slide.
5. Place the slide under the 400x magnification of the microscope.
6. Count and record the number of stomata in the field of view.
7. Repeat steps 2-6 two more times using a different leaf from the same location.
8. Repeat steps 2-7 with leaves from the same plant from the four other different light intensity conditions.
9. Calculate the average number of stomata for each light condition.

Raw Quantitative Data:

Table 1: number of stomata (to the enearest whole number) under different light intensities (± 0.1lux).

Light Intensity (lux)	Number of Stomata		
	Trial 1	Trial 2	Trial 3
56.7 lux - Very Weak	37	34	31
138.9 lux - Weak	32	31	32
378.3 lux - Moderate	43	33	51
730.8 lux - Strong	57	76	84
770.0 lux - Very Strong	50	57	45

Processed Data:

Table 2: Average number of stomata (to the nearest whole number) under different light intensities (± 0.1lux).

Light Intensity (lux)	Average Number of Stomata
56.7 lux - Very Weak	34
138.9 lux - Weak	32
378.3 lux - Moderate	42
730.8 lux - Strong	72
770.0 lux - Very Strong	51

The average number of stomata was calculated for each light intensity by using this formula:

Average number of stomata = (trial 1 + trial 2 + trial 3)/3

For example, at weak light intensity at 138.9 lux:

Average number of stomata = (32 + 31 + 32)/3≈31.67 = 32

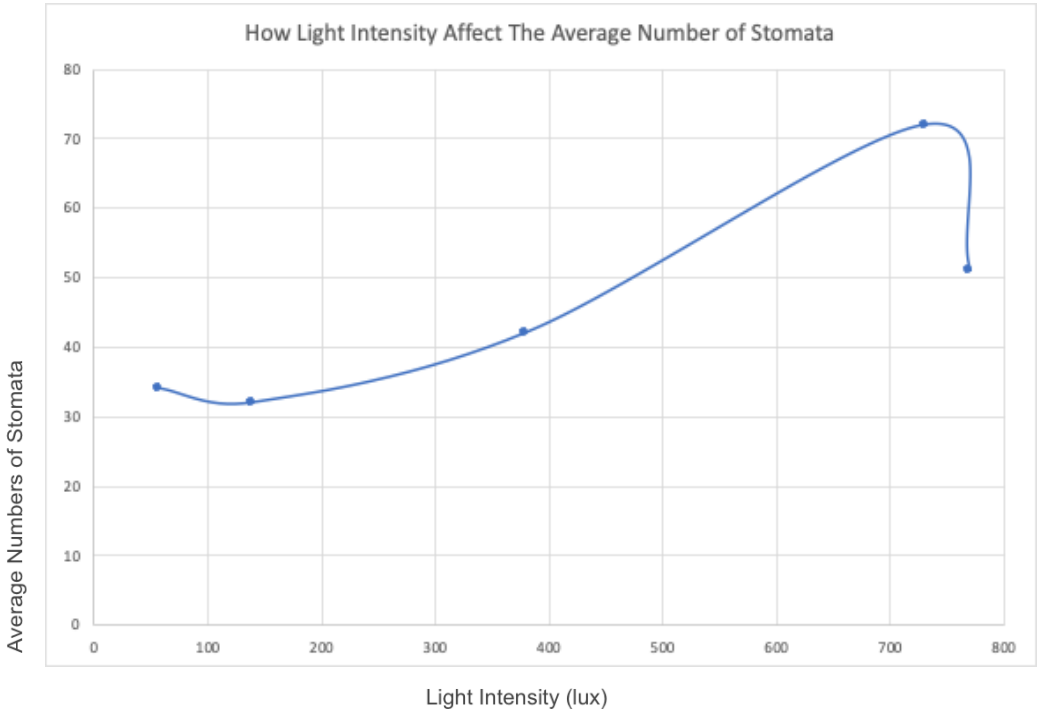


Figure 1 - the exact average number of stomata in tomato leaves (to the nearest whole number) under different light intensities - Excel

Visually, Figure 1 is closely linked to a cubic function. In order to examine the relationship between the number of leaf stomata and light intensity, regression analysis is utilized. Additionally, the optimum of stomata frequency, which is the local maximum, is demonstrated in the graph with the corresponding light intensity. In this case, this cubic regression predicts the most suitable light intensity for tomato plants to absorb energy most efficiently. The interpolation line is demonstrated for a purpose of comparison between the exact number and predicted the number of stomata under certain ranges of light intensity.

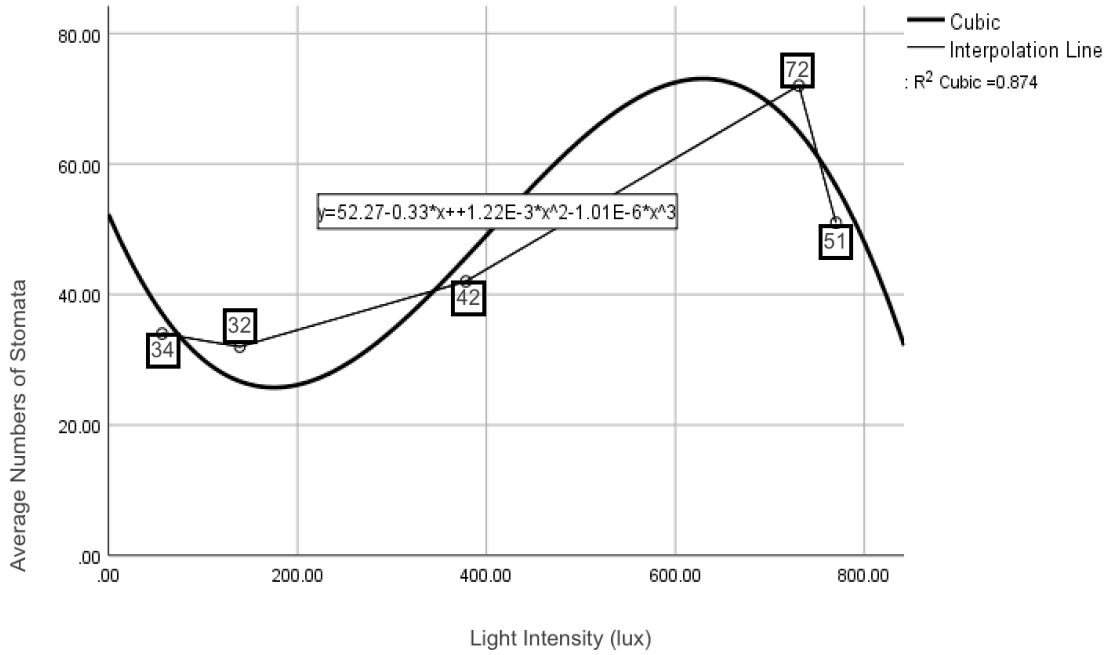


Figure 2 - cubic regression and interpolation line (linear) of the average number of stomata in tomato leaves under different light intensities - SPSS

In order to ensure the accuracy of predicted cubic regression, the R Square of the correlation coefficient method measures the variance between the exact and predicted values. It proves the reliability of the predicted optimum of stomata numbers under light intensity. Through exploring further the correlation between light intensity and stomatal density, the validity of predicted regression is revealed:

Model Summary and Parameter Estimates

Dependent Variable: averagenumberofstomata

	Model Summary					Parameter Estimates			
Equation	R Square	F	df1	df2	Sig.	Constant	b1	b2	b3
Cubic	.874	2.316	3	1	.442	52.266	-.334	.001	-1.010E-6

The independent variable is lightintensity.

Table 1 - cubic regression's summary and estimates table for the correlation of light intensity and average numbers of stomata - SPSS

R Square (R^2) = 1 - $\frac{sum\ squared\ regression\ (SSR)}{total\ sum\ squares\ (SST)}$ = 1 - $\frac{\Sigma(y_i - \widehat{y_i})^2}{\Sigma(y_i - \overline{y_i})^2}$

y_i= actual average numbers of stomata on the interpolation line (± 5 units, measurement uncertainty by naked-eye observing)

$\widehat{y_i}$= predicted average numbers of stomata on the cubic regression (± 5 units since it is based on the actual average)

$\overline{y_i}$= the mean of all the average numbers of stomata (± 5 units, also impacted by former statistics collecting)

For example, the numerator of this fraction is the difference between actual average numbers of stomata (interpolation line) and predicted average numbers of stomata (cubic regression) that are corresponding, squared, and summed up.

The denominator is the differences between the actual average numbers of stomata and the mean of all the average numbers of stomata that are corresponding, squared, and summed up.

Eventually, R Square is the result to measure the goodness of fit of the model. The R Square for the average number of stomata in tomato leaves under different light intensities was calculated by SPSS to three decimal places: 0.874. It means 87.4% of the variation in y values (average numbers of stomata) is accounted for by the x values (light intensities).

Uncertainty calculations remain because of naked-eye observation when counting. The imprecise counting slightly impacts on the absolute accuracy of the exact and predicted trend. However, the imprecision is slight because of the subtraction. For example, $y_i - \widehat{y_i}$= $(accurate\ average + 5) - (predicted\ average\ based\ on\ accurate\ average + 5)$. In this case, the existing uncertainties are eliminated by subtracting each other. Similar as: $y_i - \widehat{y_i}$= $(accurate\ average - 5) - (predicted\ average\ based\ on\ accurate\ average - 5)$.

Conclusion:

Figure 1 and Figure 2 have mostly supported my research question: stronger light intensity and stomatal densities have structured a positive correlation because there is a strong positive correlation from when light intensity x is 130 lux to 730 lux approximately according to the actual values. By conducting the data analysis in SPSS, the created cubic regression figure has also shown a positive correlation of light intensity and numbers of stomata, excluding the decreases y values (average numbers of stomata) in the beginning and the end. According to the interpolation line, a slight negative correlation in numbers of stomata has demonstrated from 56.7 lux to 138.9 lux in a range of very weak light intensity. However, a quite steep negative correlation in numbers of stomata was demonstrated in a range of very strong light intensity from 730.8 lux to 770.0 lux.

Figure 2 has presented the local maximum of the cubic regression, which is possibly the optimum of most efficient light energy absorption through leaf stomata. In the strong light intensity range of 730.0 lux to 740.0 lux, the average numbers of tomato stomata have achieved the optimum. In this mean, the products' conversion can be maximised to release glucose and oxygen at an optimum rate, thus boosting the cellular respiration cycle of plants' growth and human respiration sources.

By analysing the R Square in Table 3 through SPSS, the reliability of the predicted cubic regression is much more guaranteed. Because $\sqrt{R^2} = \sqrt{0.874} \approx 0.935$, in which reveals a very strong correlation, the predicted cubic regression is reliable.

Overall, the processed data answer the research question: how does light intensity affect stomatal density of tomato leaves. Before the optimization, light intensity affects stomatal density of tomato leaves positively; the correlation is positive. After the optimization, light intensity affects stomatal density of tomato leaves negatively; the correlation is negative. So the optimal amount of stomata under the optimal light intensity on the predicted regression can have the most efficient light absorption and photosynthesis functions.

Evaluation:

Figure 1 and Figure 2 have not only demonstrated a positive correlation between light intensity and numbers of stomata, but also investigated the optimum of light intensity to allow the maximum of tomato stomata. However, it is unclear exactly why there are two sections of negative correlation in the earliest and latest stages. In order to resolve the concerns, this experiment needs to be repeated to minimize the occasional errors. As research has further shown, extreme high-intensity of light can damage the light absorbing function of chlorophyll molecules. With the damage inside the leaves, stomata in the epidermises are likely to stay closed to protect the inner respiration system of leaves.

Besides, the light probe and labquest can only measure up to 770.0 lux. Even under the top of the tomato plants with the less strong light intensity, the equipment soared swiftly to its maximum 770.0 lux. That is to say, the top of the tomato plants have the strongest

light intensity which is higher than 770.0 and beyond the measurement of labquest and light probe.

There are a number of ways in which the experiment could be improved to give more reliable data and so as to allow a more accurate conclusion to be drawn:

Limitation	**Effect of limitation on the raw data**	**How to overcome**
Number of tomato leaves that have been collected	Limited data (fifteen leaves in total and three under each light intensity) collected to demonstrate the correlation between light intensity and numbers of stomata. More values of leaves collected will allow a more precise figure and a more foreseen or smoother prediction graph.	More tomato leaves can be collected for further investigation.
Locations of different light intensity	The data (five different light intensities) restricts to explore the most efficient light absorption through stomata. Without more light intensities locations between “moderate” and “very strong”, the optimum rate of stomata frequency will never be precise enough.	Collect tomato leaves from more locations in the garden for further investigation.
Fluctuation in measurement	The numbers on the labquest fluctuated although the environment was quite stable. They change by small amounts ($\pm$0.1 lux - $\pm$3.0 lux), the differences from the actual data would not allow a precisely statistical analysis.	Use light intensity stabilizer, which automatically adjusts then stabilizes, to monitor.
The maximum reading of light probe	The highest light intensity of the equipment can be detected is 770.0 lux, although the top of tomato plants has a relatively stronger light intensity. It prevents me from obtaining the most precise data with a significance of finding the optimum.	See if there is a probe with a higher maximum reading.

Possible extension for the investigation:

Investigation of the connection between wavelengths of light and size of stomata opening is a potential extension. In particular, how light wavelength ranges affect stomatal opening will be researched. This is because the scientific articles have demonstrated that blue light and red light stimulate stomatal openings as photoreceptors.

Blue light is from one range of the light wavelength at 380nm - 500nm, which includes violet, indigo, and blue in visible light. Since blue light demonstrates a greater quantum efficiency, the ratio of solar energy collected, than red light, the blue light will be the main variable in this extension. **("The Multisensory Guard Cell. Stomatal Responses to Blue Light and Abscisic Acid")**

Since larger opening stomatal sizes result in the most suitable light wavelength, an extension of this investigation can involve determining the optimum of light wavelength in a blue light scale for the tomato stomatal opening sizes reaching its optimum. In this case, there will be another path to maxmise the harvesting crops and improve living conditions through photosynthetic functions.

In order to further investigate, spectrometers will be used primarily to determine a certain area has blue light within, where tomato leaves will be collected. Next, the tomato leaves will repeat as step 2-6 in the **Method** above, so microscope, forceps, nail polish, marker pen, and microscope slides will be applied in the experiment. To calculate the stomatal opening sizes, a digital camera will be required to record the stomata amplification with the same magnification; therefore, measurements of radiuses will be applicable through viewing the constant images. Eventually, the data of stomatal opening sizes will be gathered and corresponded to the blue light wavelength. Again, by substituting the data in SPSS for interpretation, the regression analysis will be executed to predict the optimum of light wavelength when tomato stomatal opening sizes are optimal. Limitation and evaluation should also be included in the extension experiment.

By combining the former investigation and the potential extension, not only the number of leaf stomata is investigated, but also the size of leaf stomata affected by light wavelength will be further investigated for the significance of respiration in plants and humans.

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