Empirical study of leaf temperature dependence on various intrinsic and extrinsic factors.

Introduction

With an increase in average global temperatures over the last 200 years (at least 1.1°C since 1880, with the majority of the warming occurring since 1975 at a rate of roughly 0.15 to 0.20°C per decade; NASA's Goddard Institute for Space Studies (GISS)), it has become imperative to understand the impact of this warming on plants, particularly food crops. An important aspect of understanding this impact is studying the variation of leaf temperature and its relationship with a variety of environmental and genetic factors. Studying the thermal dynamics of leaves, which are the food factories of plants, may allow us to devise methods to control leaf temperature, thus enabling the plant to photosynthesize at its optimum.

Any property of an organism (which, in our study, is leaf temperature) is controlled by two aspects—intrinsic or genetic aspects and extrinsic or environmental aspects. The intrinsic factors affecting leaf temperature may include leaf area, width, length, presence of appendages, leaf surface texture, and leaf shape. The extrinsic factors affecting leaf temperature may include wind speed, solar radiation, humidity, and water availability. However, there is also a possibility of some factors being influenced by both genes and the environment.

Several past studies have shown that the rate of transpiration and the associated cooling effect may play a vital role in regulating leaf temperature.Additionally, some studies suggest that variation in leaf width, area, and shape (marginal complexity/dissection) affects its thermal dynamics (A. Leigh, 2017).

We designed an experiment that involved both intrinsic parameters (e.g., width, area, length) and an extrinsic parameter (effect of transpiration) as variables and attempted to find causal links between leaf temperature and these variables. Alongside this, we also aimed to identify a pattern of leaf temperature variation across four different species.

Methodology:

Part 1 - Experimental Procedure

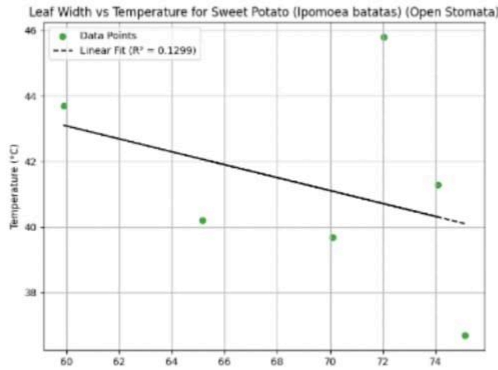
1. **Sample selection** – Among a cohort of 10 individual saplings, 6 saplings were selected. The criteria for selection was the presence of at least two leaves with similar spatial dimensions (one of which would function as the control while the other would be subjected to treatment).
2. **Application of Vaseline (the treatment)** – One leaf from each sapling was chosen as a subject. Vaseline was applied/smeared via earbuds on the underside of the leaf. The rationale for applying Vaseline only on the lower side was based on the assumption that the majority of leaf stomatal density lies on the underside of the leaf. Furthermore, care was taken to ensure no accidental smearing of Vaseline on the upper side of the leaf. Along with this, it was ensured that leaves were not subjected to any mechanical stress during the treatment procedure (which may lead to the secretion of chemicals and thus act as a confounder). Note that although the above precautions were attempted to be followed, some incorrect treatment did occur.
3. **Exposure to Sun along with positioning of leaves** – All the saplings were placed on the ground with approximately equal distances between them. We tried to ensure that both the control leaf and the subject leaf were completely exposed to the Sun, with none of their parts under shade, by using sticks and artificially orienting the leaves towards the Sun. Unfortunately, we were not able to ensure that the distance of the leaves from the ground was large enough to prevent radiation from the ground from affecting leaf temperature.
   Saplings were exposed to the Sun for approximately 20 minutes (the exposure time for saplings of each species was different due to logistical limitations).

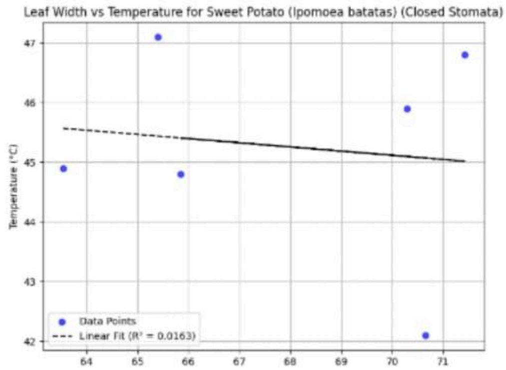
4. **Measurement of temperature and spatial dimensions of the leaf** –
   A special infrared camera (exact specifications unknown), called a thermal imaging camera, was used to measure leaf temperature.

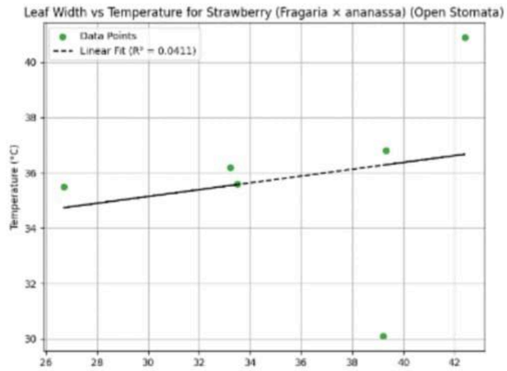
The leaf temperature was measured from approximately a distance of 30–40 cm (although the exact distance varied for each measurement).
Images of each leaf, alongside a reference scale against a white background (for contrast), were captured for further analysis.

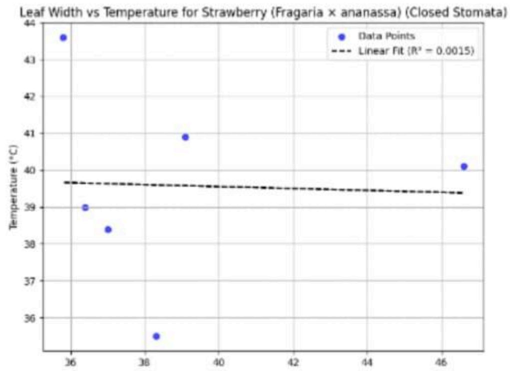
Part 2 - Analysis Tools

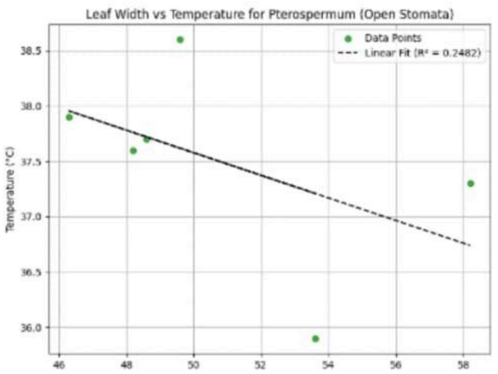
- To identify the spatial dimensions of the image, we used the open-access ImageJ software (ImageJ is a Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation (LOCI), University of Wisconsin).
  We obtained the width, length, and area of the leaf from this procedure.
- Numerical data analysis was performed using Microsoft Excel, while graph plotting was performed using Google Colab (programming language: Python).

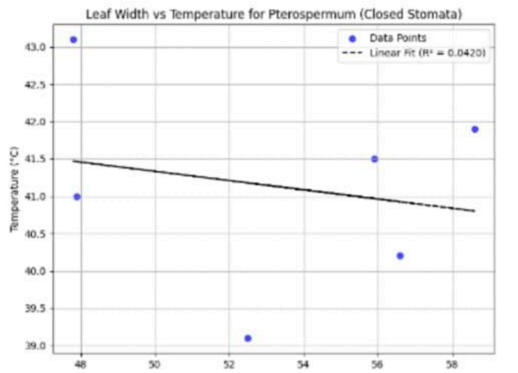

Leaf Width vs Temperature for Sweet Potato (Ipomoea batatas) (Open Stomata)
Data Points
Linear Fit (R² = 0.1299)
Temperature (°C)

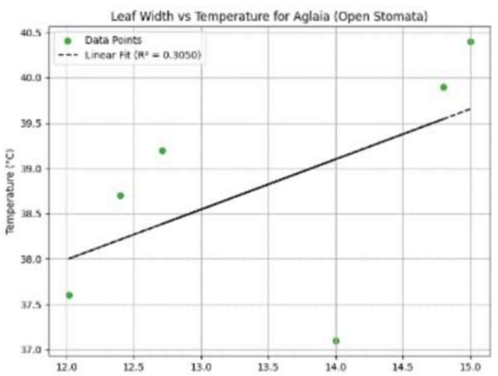

Leaf Width vs Temperature for Sweet Potato (Ipomoea batatas) (Closed Stomata)
Data Points
Linear Fit (R² = 0.0163)
Temperature (°C)

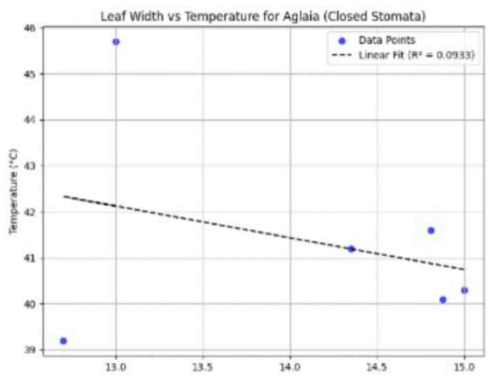

Leaf Width vs Temperature for Strawberry (Fragaria × ananassa) (Open Stomata)
Data Points
Linear Fit (R² = 0.0411)
Temperature (°C)

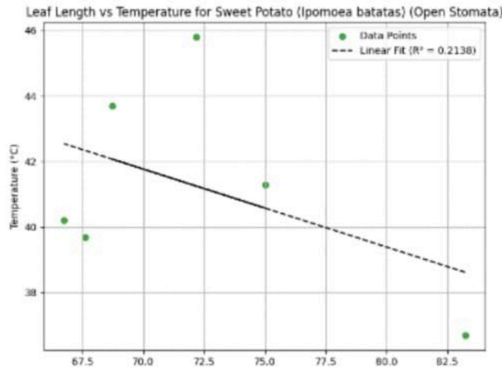

Leaf Width vs Temperature for Strawberry (Fragaria × ananassa) (Closed Stomata)
Data Points
Linear Fit (R² = 0.0015)
Temperature (°C)

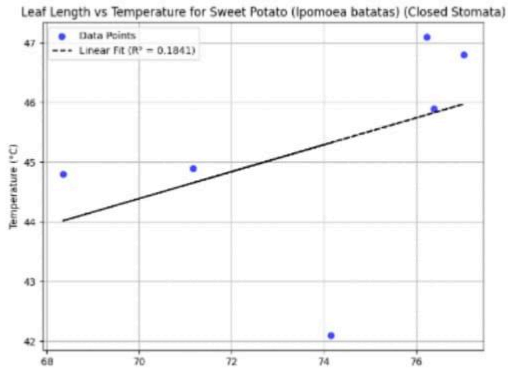

Leaf Width vs Temperature for Pterospermum (Open Stomata)
Data Points
Linear Fit (R² = 0.2482)
Temperature (°C)

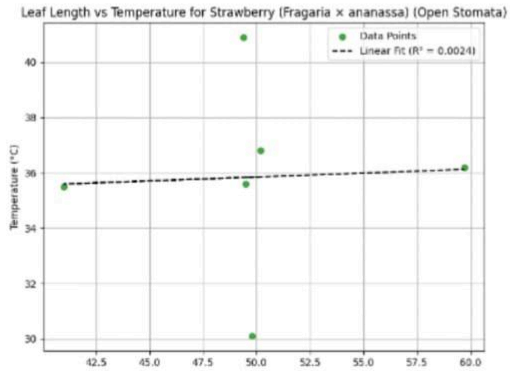

Leaf Width vs Temperature for Pterospermum (Closed Stomata)
Data Points
Linear Fit (R² = 0.0420)
Temperature (°C)

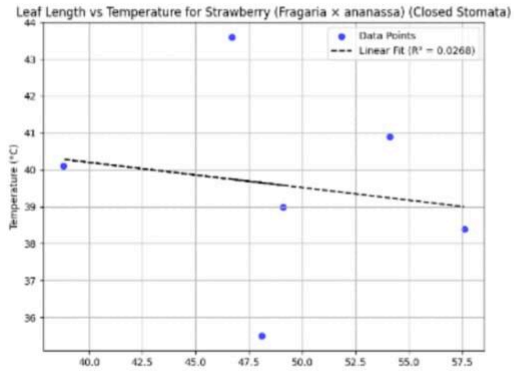

Leaf Width vs Temperature for Aglaia (Open Stomata)
Data Points
Linear Fit (R² = 0.3050)
Temperature (°C)

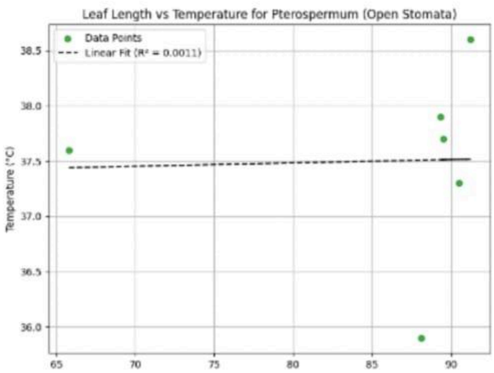

Leaf Width vs Temperature for Aglaia (Closed Stomata)
Data Points
Linear Fit (R² = 0.0933)
Temperature (°C)

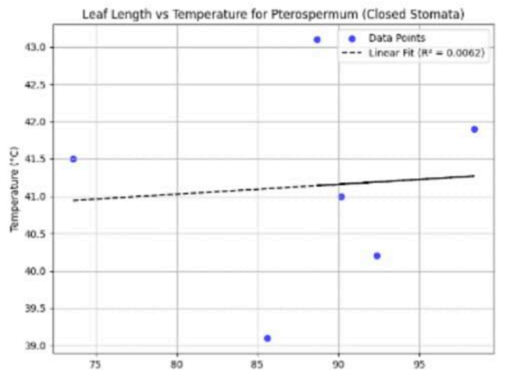

Leaf Length vs Temperature for Sweet Potato (Ipomoea batatas) (Open Stomata)
Data Points
Linear Fit (R² = 0.2138)
Temperature (°C)

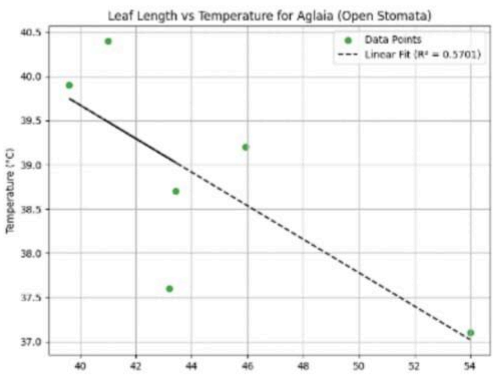

Leaf Length vs Temperature for Sweet Potato (Ipomoea batatas) (Closed Stomata)
Data Points
Linear Fit (R² = 0.1841)
Temperature (°C)

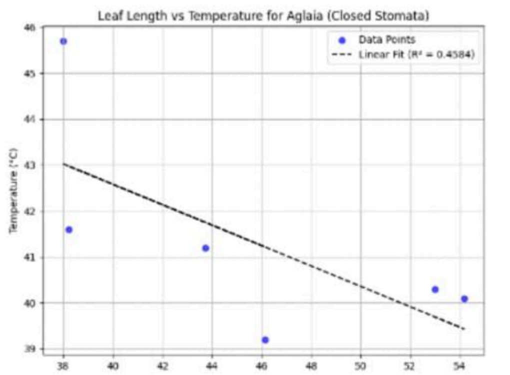

Leaf Length vs Temperature for Strawberry (Fragaria × ananassa) (Open Stomata)
Data Points
Linear Fit (R² = 0.0024)
Temperature (°C)

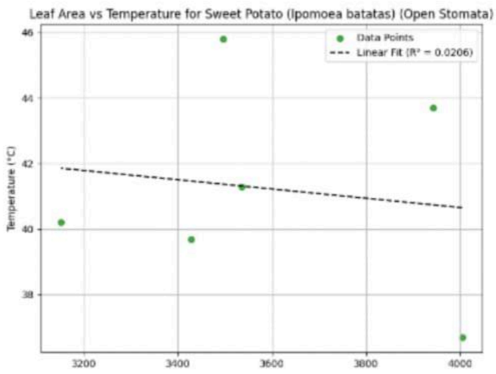

Leaf Length vs Temperature for Strawberry (Fragaria × ananassa) (Closed Stomata)
Data Points
Linear Fit (R² = 0.0268)
Temperature (°C)

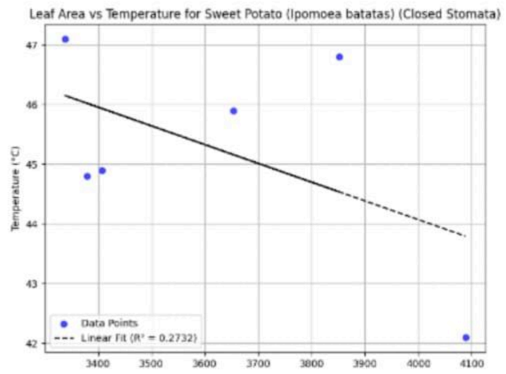

Leaf Length vs Temperature for Pterospermum (Open Stomata)
Data Points
Linear Fit (R² = 0.0011)
Temperature (°C)

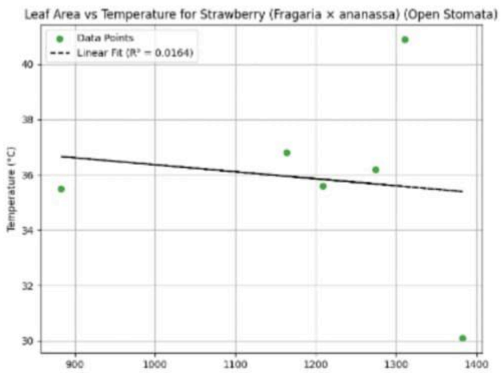

Leaf Length vs Temperature for Pterospermum (Closed Stomata)
Data Points
Linear Fit (R² = 0.0062)
Temperature (°C)

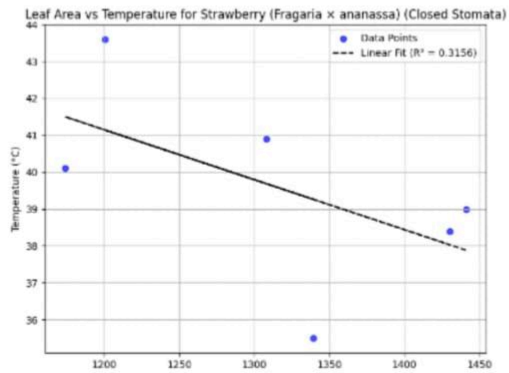

Leaf Length vs Temperature for Aglaia (Open Stomata)
Data Points
Linear Fit (R² = 0.5701)
Temperature (°C)

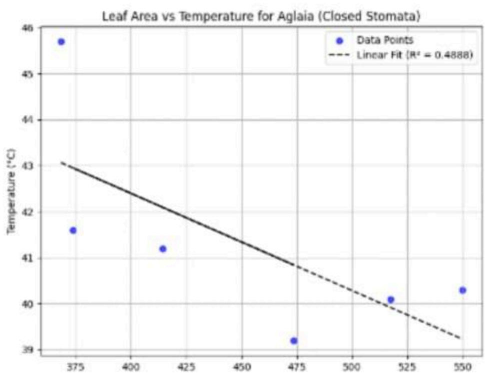

Leaf Length vs Temperature for Aglaia (Closed Stomata)
Data Points
Linear Fit (R² = 0.4584)
Temperature (°C)

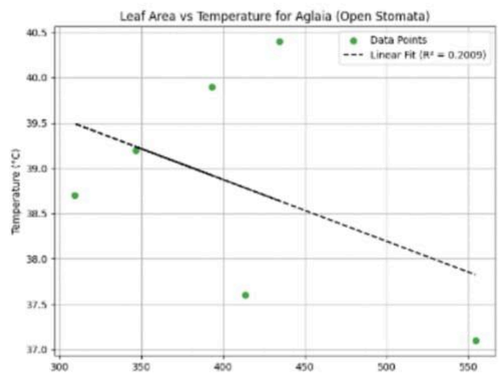

Leaf Area vs Temperature for Sweet Potato (Ipomoea batatas) (Open Stomata)
Data Points
Linear Fit (R² = 0.0206)
Temperature (°C)

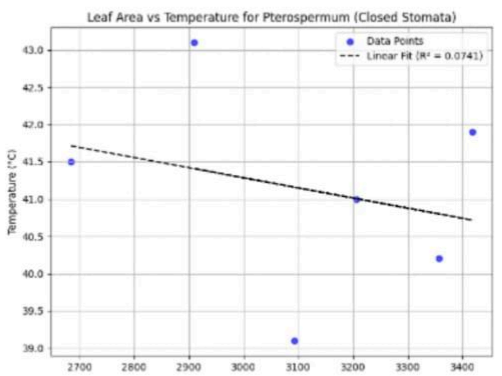

Leaf Area vs Temperature for Sweet Potato (Ipomoea batatas) (Closed Stomata)
Data Points
Linear Fit (R² = 0.2732)
Temperature (°C)

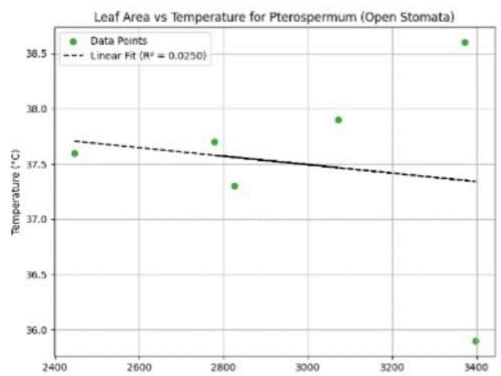

Leaf Area vs Temperature for Strawberry (Fragaria × ananassa) (Open Stomata)
Data Points
Linear Fit (R² = 0.0164)
Temperature (°C)

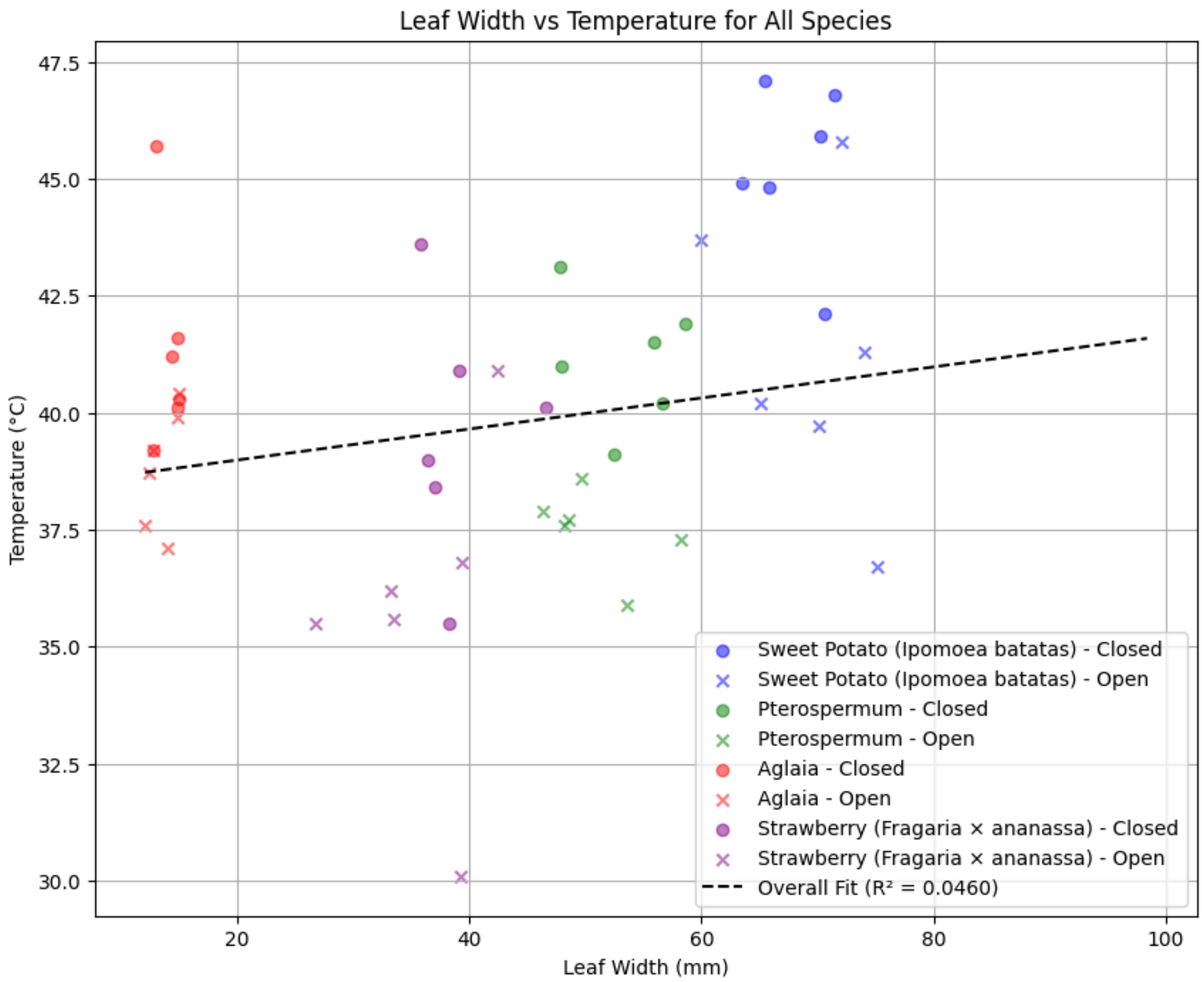

Leaf Area vs Temperature for Strawberry (Fragaria × ananassa) (Closed Stomata)
Data Points
Linear Fit (R² = 0.3156)
Temperature (°C)

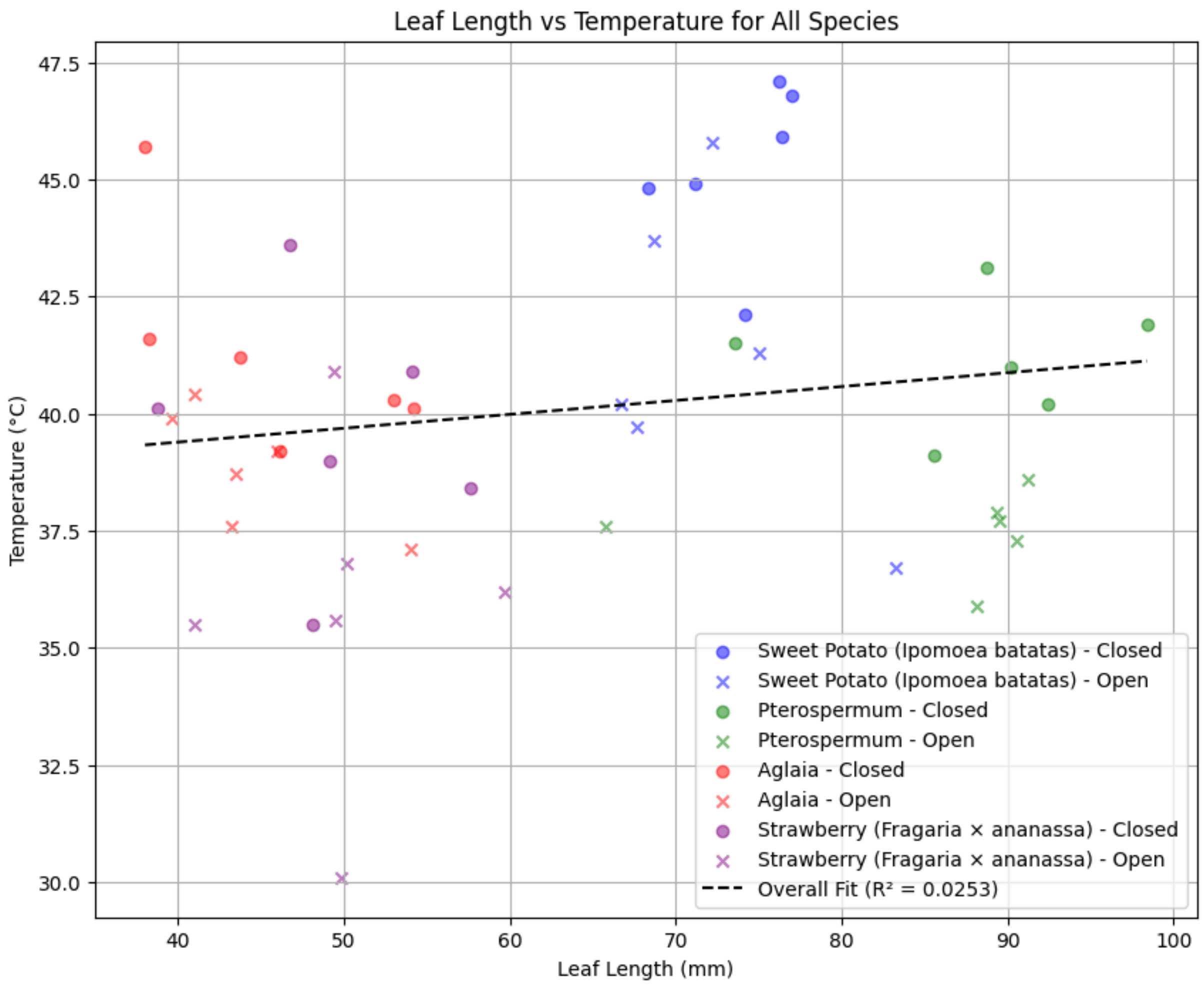

Leaf Area vs Temperature for Aglaia (Closed Stomata)
Data Points
Linear Fit (R² = 0.4888)
Temperature (°C)


Leaf Area vs Temperature for Aglaia (Open Stomata)
Data Points
Linear Fit (R² = 0.2009)
Temperature (°C)

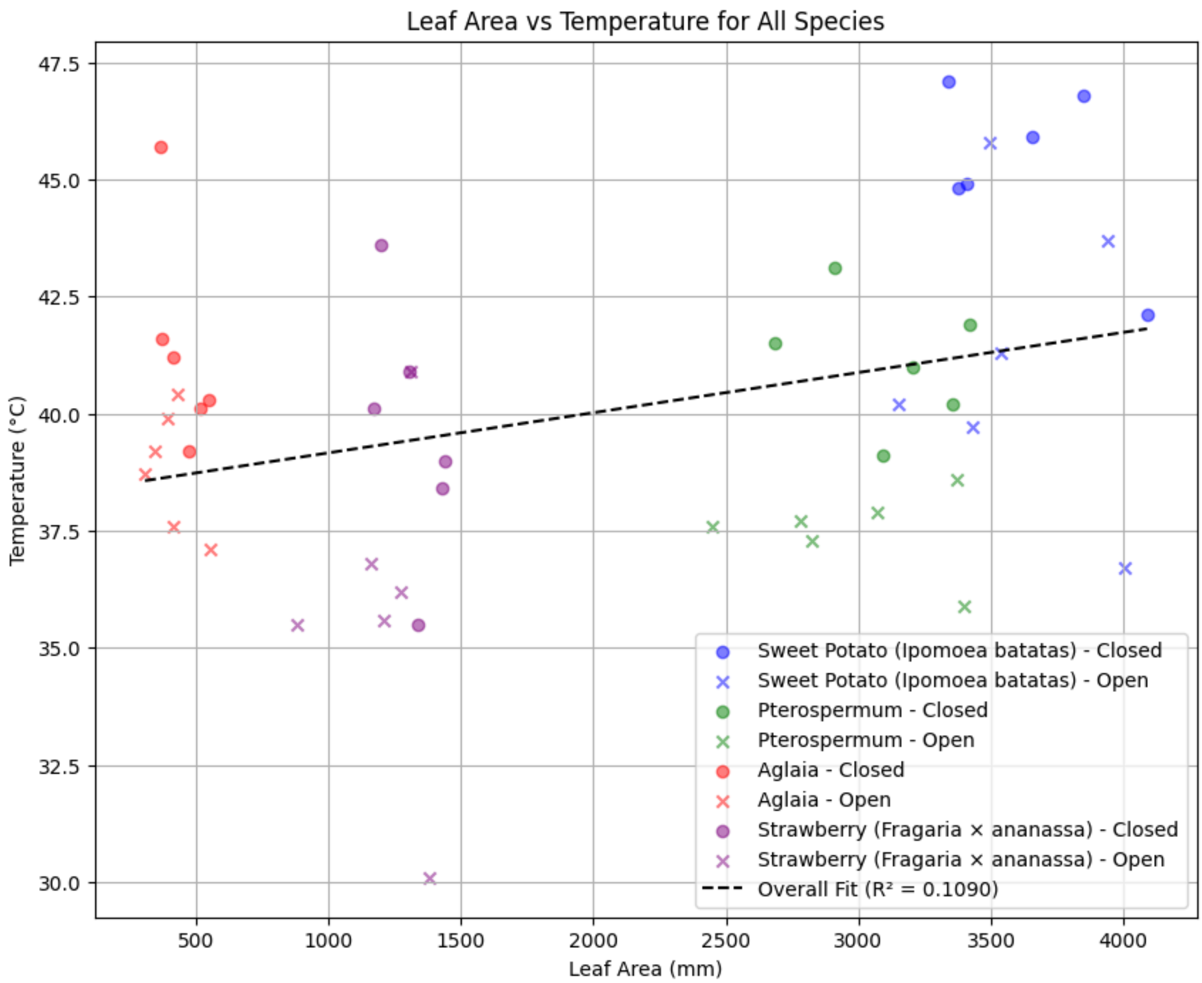

Leaf Area vs Temperature for Pterospermum (Closed Stomata)
Data Points
Linear Fit (R² = 0.0741)
Temperature (°C)


Leaf Area vs Temperature for Pterospermum (Open Stomata)
Data Points
Linear Fit (R² = 0.0250)
Temperature (°C)

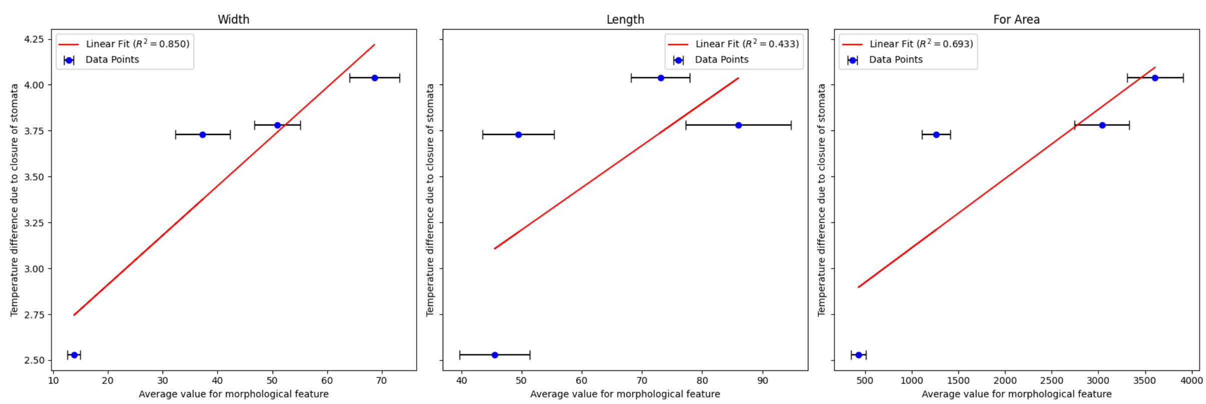

Leaf Width vs Temperature for All Species
Temperature (°C)
Leaf Width (mm)
47.5
45.0
42.5
40.0
37.5
35.0
32.5
30.0
20
40
60
80
100
Sweet Potato (Ipomoea batatas) - Closed
Sweet Potato (Ipomoea batatas) - Open
Pterospermum - Closed
Pterospermum - Open
Aglaia - Closed
Aglaia - Open
Strawberry (Fragaria × ananassa) - Closed
Strawberry (Fragaria × ananassa) - Open
Overall Fit (R² = 0.0460)



Leaf Length vs Temperature for All Species
Temperature (°C)
Leaf Length (mm)
47.5
45.0
42.5
40.0
37.5
35.0
32.5
30.0
40
50
60
70
80
90
100
Sweet Potato (Ipomoea batatas) - Closed
Sweet Potato (Ipomoea batatas) - Open
Pterospermum - Closed
Pterospermum - Open
Aglaia - Closed
Aglaia - Open
Strawberry (Fragaria × ananassa) - Closed
Strawberry (Fragaria × ananassa) - Open
Overall Fit (R² = 0.0253)



Leaf Area vs Temperature for All Species
Temperature (°C)
Leaf Area (mm)
Sweet Potato (Ipomoea batatas) - Closed
Sweet Potato (Ipomoea batatas) - Open
Pterospermum - Closed
Pterospermum - Open
Aglaia - Closed
Aglaia - Open
Strawberry (Fragaria × ananassa) - Closed
Strawberry (Fragaria × ananassa) - Open
Overall Fit (R² = 0.1090)



Width
Length
For Area
Linear Fit ($R^2 = 0.850$)
Linear Fit ($R^2 = 0.433$)
Linear Fit ($R^2 = 0.693$)
Data Points
Temperature difference due to closure of stomata
Average value for morphological feature

Analysis

The plot for Absolute Temperature vs. Leaf Area for Vaseline and non-Vaseline plants shows that the points are dispersed, and there is no linear relationship between these parameters, as the linear fit (R^2 value < 0.5).
The temperature of these leaves lies within a range of 35°C – 47.5°C.
In general, the temperature of Vaseline-treated leaves is higher than that of non-Vaseline-treated leaves.

While sweet potato and Pterospermum have a more rightward optimum temperature range compared to Aglaia and strawberry when plotted on an increasing temperature scale, their overall range is quite similar.
The specific heat capacity of sweet potato is significantly higher than that of other species, allowing it to retain heat for a longer duration.

If we consider one or two points in each of the six-point graphs as outliers, a parabolic relationship emerges. However, removing these outliers isn't advisable due to the small sample size.

There is a positive correlation between temperature difference due to closure and leaf width (as indicated by the linear fit, $R^2 = 0.850$), but no correlation with leaf length or leaf area.

The plot for Absolute Temperature vs. Leaf Width for Vaseline and non-Vaseline plants shows that the points are dispersed, and there is no linear relationship between these parameters, as the linear fit (R^2 value < 0.5).

The plot for Absolute Temperature vs. Leaf Length for Vaseline and non-Vaseline plants shows that the points are dispersed, and there is no linear relationship between these parameters, as the linear fit (R^2 value < 0.5).

Discussion:

Based on the analysis of each graph, we aim to provide a plausible explanation for the observed trend in the following section. At the end, some extra comments have been added.

1.Leaf Area vs. Temperature for Non-Vaseline

In general, as all species show no trend with temperature, we can say that although we expect to see an increase in temperature with area due to greater absorption of solar radiation, the equivalent factor of the availability of a larger surface area to cool (as stomata are not covered) balances out. Hence, no variation in temperature is observed.

2.Leaf Area vs. Temperature for Vaseline

Similar to non-Vaseline leaves, no trend was observed. This proves one thing: although leaf area, stomatal density, and rate of transpiration are correlated, surprisingly, there is no difference in the relationship between temperature and area, regardless of whether stomata are covered or not.

3.Leaf Area vs. Temperature Difference Between Vaseline and Non-Vaseline Leaves Across Species

A very poor correlation between leaf area and temperature difference does show that species with larger leaf area are affected more due to the closure of stomata than those with smaller leaf area. However, this contradicts the discussion in point 2. It is very difficult to reconcile these two results. Interestingly, if we exclude the strawberry data point, we obtain a linear fit with $R^2 = 1$—this is probably a coincidence, or it might also be possible that the degree of error in all measurements for strawberry is very high.

4.Leaf Width vs. Temperature for Non-Vaseline

Similar to leaf area, no trend was observed. This is surprising because previous studies have shown that leaf width affects the leaf boundary layer and thus influences heat transfer between leaves and their surroundings. Leaves with smaller width are expected to be cooler compared to wider leaves. This may be explained by the amount of transpiration, because although wider leaves will have greater stomatal density, their boundary layer thickness will also be greater, which will reduce their transpiration rate. Thus, these two factors cancel each other out.

5.Leaf Width vs. Temperature for Vaseline

No trend was observed. Here, since stomata are closed, the primary factor affecting temperature should be boundary layer thickness. We would expect temperature to increase with increasing leaf width; however, this did not occur

6. Leaf Width vs. Temperature Difference

This is the only conclusive relationship in our study, as evident from $R^2 > 0.8$. The increase in leaf width correlating with an increase in temperature difference is a direct consequence of the increase in boundary layer thickness. When stomata are covered, the mechanisms for heat transfer become restricted to convection with air. However, a thick layer of still air near the leaf surface reduces heat transfer, thereby increasing the temperature difference.

7. Leaf Length vs. Temperature for Non-Vaseline and Vaseline

No evident trend was observed. This implies that leaf length has no role in heat transfer mechanisms, particularly concerning boundary layer thickness. Previous studies also ignore the length factor in the calculation of boundary layer thickness.

8. Leaf Length vs. Temperature Difference

No trend was observed, with very large error bars.

2. Some extra comments:

1. About boundary layer-Boundary layer refers to the thin zone of calm air that surrounds each leaf. The thickness of the boundary layer influences how quickly gases and energy are exchanged between leaf and surrounding area. (Source-the boundary layer and its importance, Erik Runkle, Michigan State University)
2. Some other assumptions-

1.Temperature is constant over entire leaf

2. Atmospheric temperature considered constant at 30 degrees Celsius due to small duration of experiment

3.The saplings were kept in optimum conditions in the context of fertilizer content, water content, and abiotic conditions; hence, we assume that the stomatal regulation of temperature is nullified across the four groups.

4. Negligence of some other factors like vein vasculature,leaf appendages

3. Errors and limitations

All our conclusions and results are based on very few sample points from each species. Furthermore, several confounding variables have affected the reliability of data. Some of them are as follows:

1. The distance of leaves from the ground varied in each measurement. Radiation from the ground may have influenced the leaf temperature measurements. Since the intensity of radiation falling on leaves varies inversely with distance from the ground, leaves closer to the ground would have higher temperatures than expected due to solar radiation alone, and vice versa.
2. Ideally, to compare the effect of Vaseline application, the control and treatment leaves should have the same width, length, and area. However, since this was not practically feasible, we had to deduce the relationship. A

better approach would be to test the same leaf, with half of it covered for a more controlled comparison.

3. Similarly, to deduce a causal relationship between leaf temperature and transpiration, width, length, or area, it is essential that the angle each leaf makes with the Sun is the same so that within a specified time, each leaf receives the same solar radiation. This is a limitation of our experimental design.

4)While taking measurements, the device used was getting heated (probably due to the large number of measurements taken and high ambient temperatures). Although not certain, there is a possibility that this might have affected our results.

5)The temperature measurements were taken from at least 20-30 cm away from the leaves. It is possible that infrared radiation emitted from neighboring leaves or other objects might have led to higher temperature readings than the actual leaf temperature.

6)Ideally, the application of Vaseline should be uniform to ensure that transpiration is completely blocked. However, since different individuals performed the treatment, it is highly likely that the application was non-uniform. Although this error cannot be quantified, we hypothesize that it led to a smaller temperature difference between control and subject leaves than what we might expect if transpiration were completely blocked.

Conclusions

Our study only gave two evident relationships which are as follows-

1. Temperature of leaves with application of Vaseline was always greater than temperature of control leaves
2. Width of the leaves is directly proportional to the temperature difference between subject and control leaves.

References-

1.The influence of leaf size and shape on leaf thermal dynamics:does theory hold up under natural conditions?- A.Leigh et al
2.The Boundary Layer and It’s Importance- Erik Runkle
3.NASA’s Goddard Institute for Space Studies (GISS)
Note that all the text was written by us. Only the font,paragraph spacing,formatting and correction of alphabetical errors was performed by AI.