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The Effect of the Stomatal Index on the Net Rate of Photosynthesis in the Leaves of *Spinacia oleracea*, *Vinca minor*, *Rhododendron spp*, *Epipremnum aureum*, and *Hedera spp*

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Summary

Climate change, caused by global warming and an increase in atmospheric CO_2 concentration, poses significant challenges to plant life in a rapidly changing environment. To be able to survive in a changing environment, plants must learn to adapt (1). The stomatal indexes, or density of stomata relative to the epidermal cell count, of plants are inversely correlated to paleo-atmospheric CO_2 concentration and present day CO_2 concentration (2) as a species-specific adaptation resulting from not only genetics but also environmental pressures (3). In order to understand the effects that rising CO_2 levels will have on the photosynthetic rate of plants in the coming decades we need to research how plants adapt through stomatal indexes to maximize photosynthesis. This study investigated the effect of the stomatal indexes of five different plant species on their derived net rate of photosynthesis. Leaf disks from the plant species were placed in a vacuum, so that bicarbonate solution would enter the disks and provide the carbon for photosynthesis. As photosynthesis proceeded, the leaf disks floated due to oxygen accumulation. The effective time for the disks to rise was used to derive the net rate of photosynthesis. We found a correlation (R^2=0.5625) between the stomatal index and photosynthesis, and that a higher stomatal index correlates to a higher rate of photosynthesis in a non-water stressed environment. The results could be used to further study how environmental changes due to climate change influence the stomatal index and thereby the net rate of photosynthesis of plants.

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Introduction

Photosynthesis in plants is the process by which plants convert water and carbon dioxide, in the presence of light, to sugars and oxygen (7). Stomata, pores in the leaf surface of plants, are essential in photosynthesis. They are key to gas exchange, opening and closing to allow the diffusion of gases in response to ion accumulation in guard cells surrounding the stomata (5). Carbon dioxide levels are known to play a key role in the rate of photosynthesis (6). One of the purposes of the stomata is to obtain carbon dioxide for the Calvin cycle, the part of photosynthesis (7) during which the plant performs carbon fixation and eventually produces the sugar G3P. As carbon dioxide input increases, the rate of photosynthesis also increases until it reaches the maximum amount of intake, which causes the rate to plateau (6). However, plants face a dilemma, as stomata are not only a source of carbon dioxide intake, but also water loss (1). According to Darwinian principles, plants have evolved traits that enable them to grow faster while maintaining their probability of survival (1). Therefore, the stomatal density in plants has evolved based on their environmental conditions to provide for optimal growth (photosynthesis) while keeping water loss under control.

This study tested these predictions using the net rate of photosynthesis as an indicator of optimal growth and compared these rates to the stomatal indexes of a variety of plants. Since the stomatal density in plants, or the stomatal index, is species specific (3), we experimented on five different species of plants. It was hypothesized that a higher stomatal index would correlate with a higher net rate of photosynthesis. This is due to the large availability of carbon dioxide via the larger number of

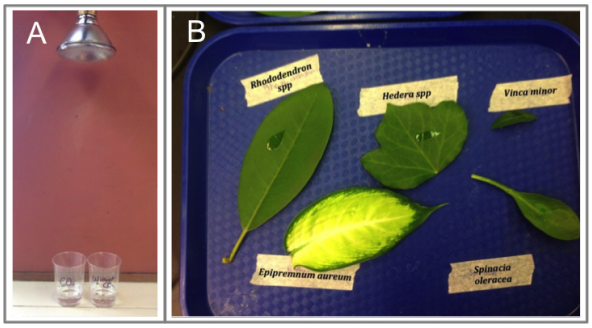

Figure 1: Measuring Photosynthesis Rate in Five Plant Species. A) The leaf disks and light setup. B) The leaves used with nail polish applied.

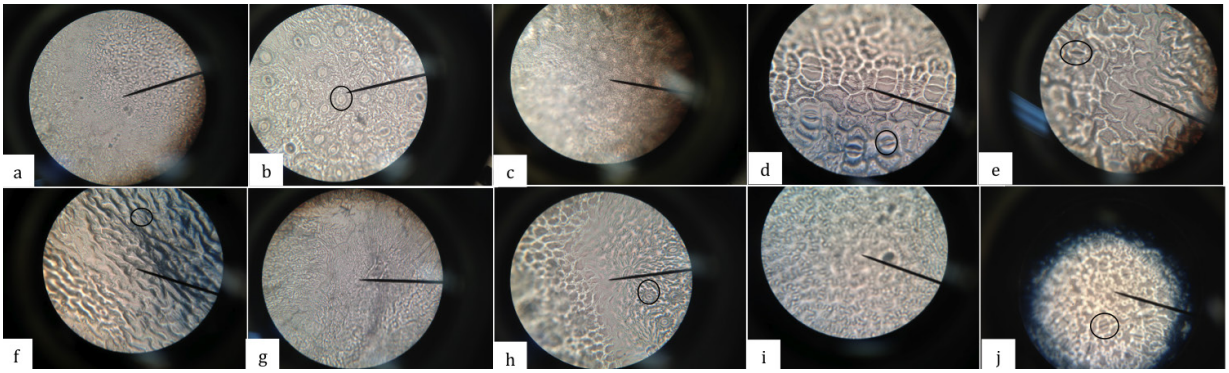

Figure 2: Microscopic Views of the Upper and Lower Epidermis of the Measured Plants. The images are of the imprints of the leaf surface onto the nail polish. Outlined in black is a stoma in each picture where there are stomata present. A) Upper and B) Lower *Hedera spp.* C) Upper and D) Lower *Epipremnum aureum*. E) Upper and F) Lower *Spinacia oleracea*. G) Upper and H) Lower *Rhododendron spp.* I) Upper and J) Lower *Vinca minor.*

stomata in the leaf, resulting in higher levels of available carbon dioxide for photosynthesis and, consequently, a higher rate of photosynthesis.

In this investigation, the floating leaf disk technique was used, and a vacuum was created with the leaf disks to draw out the air bubbles from the spongy mesophyll and allow the space to be filled by the bicarbonate solution (4). This resulted in the leaf disks sinking and starting photosynthesis due to the carbon present in the bicarbonate solution. The leaf disks floated due to the accumulation of oxygen as a byproduct of photosynthesis (**Figure 1A**). The rate of photosynthesis was indirectly measured based on the time for the leaf disks to rise. The leaves were then painted with nail polish (**Figure 1B**) and the epidermis imprints on the nail polish was observed under the microscope (**Figure 2**) to determine the stomatal index on the upper and lower epidermis of each plant. The species used were *Spinacia oleracea*, commonly known as spinach, *Vinca minor*, *Epipremnum aureum*, *Hedera spp*, and *Rhododendron spp*. The symbol spp is used to represent that the plant was one species within a specific genus. These species were used as they were grown under similar conditions in the classroom and thus had the same exposure to noise and light.

We found that a higher stomatal index is correlated with a higher net rate of photosynthesis in the plants tested.

Results

The net rate of photosynthesis was calculated from the effective time, or the time for half the leaf disks to rise. The net rate of photosynthesis (**Table 2**) was then compared to the stomatal index (**Table 1**) of the different plants to determine a correlation (**Figure 4**). The lower epidermal stomatal index was used, since the *Spinacia oleracea* leaf was the only one to have stomata on the upper epidermis. A correlation was found between the lower stomatal index and the net rate of photosynthesis for the plants tested (**Figure 4**). For example, the *Rhododendron spp* leaf that we observed had a lower stomatal index of 6.04 and consequently a lower net rate of photosynthesis of 0.07, compared to the *Vinca minor* with a stomatal index of 9.09 and a net rate photosynthesis of 0.08 (**Tables 1 & 2**, **Figure 4**). We found that a higher number of stomata increases the net rate of photosynthesis, since the leaf disks with more stomata rise at a quicker rate. The Coefficient of Determination (R^2) was 0.5625: 56.25% of the total variation in the net rate of photosynthesis is accounted for by the stomatal index.

	Number of Stomata				
Types of Leaf	Upper Leaf	Stomatal Index (SI)	Lower Leaf	Stomatal Index (SI)	Upper: Lower SI Ratio
Spinacia oleracea	7	4.76	13	11.50	0.41
Rhododendron spp	0	0	9	6.04	0
Vinca minor	0	0	13	9.09	0
Epipremnum aureum	0	0	8	11.76	0
Hedera spp	0	0	26	7.98	0

Table 1: The number of stomata and stomatal index for each type of plant. The microscopic pictures taken of the different plants under the microscope to determine the stomatal index can be seen in Figure 2.

The controls used in this experiment played a major role in our ability to reach this conclusion. To demonstrate that plants need to uptake CO_2 from the stomata to conduct photosynthesis, we created a negative control where the leaf disks of the *Epipremnum aureum* plant were placed in water and not a bicarbonate solution. In this experimental treatment, the leaf disks did not rise (**Figure 3K**), demonstrating that CO_2, or more specifically carbon, is needed for photosynthesis to occur and for the leaf disks to rise due to the accumulation of oxygen

as a waste product. Similarly, a control without light was used to demonstrate that the leaf disks needed light to conduct photosynthesis. The results of the experimental treatment (**Figure 3J**) demonstrated that light was necessary for the leaf disks to conduct photosynthesis and rise.

We also used a negative control experiment to determine whether the plants have to be alive for photosynthesis to occur. The leaves used for the experiment were fresh but there were four controls for the aging of the leaves. When leaves age, they experience water loss and eventually shrivel up and die. To model the effect that age would have on the leaf disks, they were heated for 2, 4, 6, and 8 minutes. As seen in **Figure 3F–I**, as the leaves were heated for longer, they were able to conduct photosynthesis less effectively compared to the unheated *Epipremnum aureum* (**Figure 3E**) leaves, and under the 6 and 8 minute treatments, the leaf disks were unable to conduct photosynthesis and thus did not rise.

	Time for the Disk to Rise, min (±0.5min)				
Disk Number	Spinacia oleracea	Rhododendron spp	Vinca minor	Hedera spp	Epipremnum aureum
1	2	8	9	5	5
2	2	11	11	6	6
3	3	12	13	9	6
4	3	16	13	10	6
5	3	17	14	10	7
6	4	20	17	--	9
7	5	--	18	--	--
Average	3.1	14.0	13.6	8.0	6.5
Effective Time (ET_{50})	3	14	13	9.5	6
Net Rate of Photosynthesis ($1/ET_{50}$)	0.33	0.07	0.08	0.11	0.17

Table 2: The time for the disk to rise, effective time, and net rate of photosynthesis for each type of plant. For some disks, there is no value as the disk would not rise and therefore was considered an outlier and not included in the average calculations. The effective time (ET_{50}) is the median of the data, or the time it takes for 50% of the disks to rise. It is a measure of the central tendency of the disks to rise and was discovered by Steucek and Hill (4).

Discussion

The results support the hypothesis that a higher stomatal index would lead to a higher net rate of photosynthesis. In **Figure 4**, 56.25% of the total variation in the net rate of photosynthesis is accounted for by the stomatal index.

A potential limitation of this study is the method of measurement of the stomatal index. While the leaves used in the study were from the same plant and stomatal index is a species-specific trait, there was a possibility that they had slightly different stomatal indexes based on the age of the specific leaf on the plant and the location of the leaf on the plant. In future experiments, this potential limitation could be avoided by conducting multiple trials to increase the likelihood that the stomatal index observed would be reflective of the stomatal index in the leaf disks used. Other limitations to this investigation are that we only used five species, so there could be other explanations for the differences in photosynthesis of these species. Furthermore, this study did not take into account the effects of stomatal conductance on the stomatal index and derived net rate of photosynthesis.

There are many practical applications for the results of this investigation and further research in the area. These results show that a higher stomatal index correlates with a higher net rate of photosynthesis. Therefore, from this investigation higher stomatal densities could

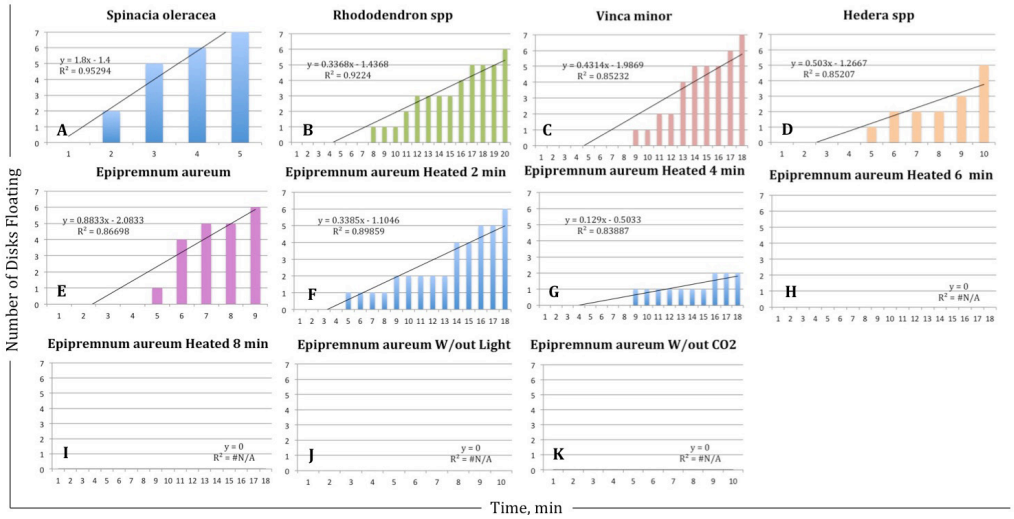

Figure 3: Number of Leaf Disks Floating vs. Time. The graphs are a visual representation of Table 2 and the negative controls done with the *Epipremnum aureum* leaf. Graphs A-E summarize leaf disk behavior for different types of plants. Graphs F-K are negative controls.

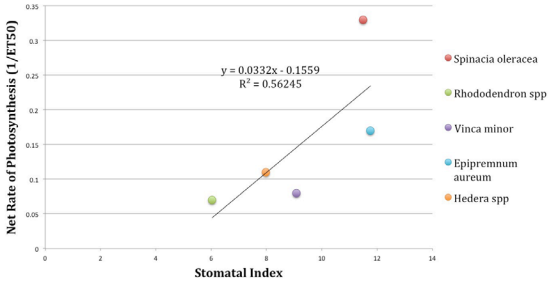

Figure 4: Net Rate of Photosynthesis vs. Stomatal Index for the Measured Plants. Note: the measurement of the rate of photosynthesis is referring to the net rate of photosynthesis as cellular respiration and photosynthesis are being conducted in the presence of light in plants.

be evolutionary advantageous to the five plant species investigated assuming there is no water stress. Further research into factors that affect the stomatal index and the net rate of photosynthesis could be applied to the growing issue of climate change and how pollution affects the rate of photosynthesis.

Although the stomatal index is species specific, it is subject to the influence of environmental factors such as temperature, CO_2 concentration, and humidity (9). Stomatal index in plants can be an indicator of paleo-atmospheric CO_2 concentration, as well as present day CO_2 concentration, as it correlates inversely with CO_2 concentration (2). The study of the effects of environmental changes on the stomatal index, as well as other aspects that affect photosynthesis across plant species, can give us insight into how different plant species will be able to adapt to environmental changes caused by global warming. Studies have shown that in higher CO_2 concentrations, plants are able to maintain a high photosynthetic rate with low stomatal conductance (8). The simulation of environments that mimic predicted changes due to global warming, such as higher CO_2 concentrations, provides an opportunity to study the effects of these environmental changes on the stomatal index.

In conclusion, we found a correlation between the stomatal index and the net rate of photosynthesis in plants. Further investigations could test the limiting effects of stomatal index on photosynthesis, given that stomata are not only a means of carbon dioxide intake but also water loss (1). The effect of different environmental conditions on the stomatal index and rate of photosynthesis can be studied to determine how the stomatal index and photosynthetic rate affect adaptation to increasing environmental changes caused by global warming.

Methods

The leaf disk technique from the College Board's "AP Biology Investigative Labs: An Inquiry - Based Approach" was used. The plants were grown in the classroom and thus were exposed to similar levels of noise and light. The first stage of the experiment involved creating a vacuum using a syringe to draw out the air in the spongy mesophyll, allowing the leaf disks to sink and the solution to be absorbed into the leaf. Seven leaf disks with a diameter of 6 mm were made using a cork borer. The disks were cut from the center of the leaf while avoiding major leaf veins. These leaves were then placed in a syringe and a small volume (5 mL) of 0.2% sodium bicarbonate was added. The plunger was then inverted and moved so that no air remained. A vacuum was then created by placing a finger on the syringe opening and drawing the plunger back, holding for 10 seconds, and then releasing. The leaf disks then fell to the bottom of the inverted plunger because the air had been drawn out of the spongy mesophyll and replaced with the solution. The disks were then poured from the syringe into a clear plastic cup with bicarbonate solution. This procedure was done for each of the plant types and the negative controls. For the negative control 'without CO_2', water and not bicarbonate was used to create the vacuum, and there was only water in the plastic cup.

A 660W floodlight was set up approximately 50 cm above the cups with the leaf disks to provide the light necessary for photosynthesis (**Figure 1A**). The time that the leaf disks were under the light was recorded and after each minute the number of leaf disks floating was counted until all seven disks had risen. The leaf disks rose due to the accumulation of oxygen in the leaf as a waste product of photosynthesis. The net rate of photosynthesis based on the time it took for the leaf disks to rise was calculated based on the Effective Time (ET_{50}), which is the time it took for half the leaf disks to rise. The net rate of photosynthesis of each plant was measured using the same technique by creating vacuums for each of the different plant's leaf disks, measuring the time to rise, and using the Effective Time to calculate the net rate of photosynthesis.

The second stage of the experiment was determining the stomatal index for each of the plants. Nail polish was applied to the center of the upper and lower epidermis of the *Spinacia oleracea*, *Vinca minor*, *Rhododendron spp*, *Epipremnum aureum*, and *Hedera spp* leaves (**Figure 1B**). After the polish had dried it was peeled off and placed under a microscope at power 400X to observe the number of stomata and determine the stomatal index in the upper and lower epidermis of each plant (**Figure 2** and **Table 1**). The stomatal index was calculated using Salisbury's equation for stomatal index:

$$SI = \frac{\text{Stomata \#}}{\text{Epidermal Cell \# + Stomata \#}} \times 100$$

The stomatal index of the lower epidermis was then compared to the net rate of photosynthesis, as many plant types did not have stomata on the upper epidermis.

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