

HOW ENVIRONMENTAL FACTORS AFFECT STOMATAL DENSITY AND CHLOROPHYLL IN  
TREES



by

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## ABSTRACT

This study is important because it shows how trees fit in the environment and how environmental factors affect trees. The environmental factors studied were light, temperature, water availability, and elevation. Some species comparisons were also made. There were six different species tested altogether. For each species the dependent variable was stomatal density and/or chlorophyll. The impressions of the stomata were made using nail polish to imprint the stomata on the back of the leaf. The imprint was used to calculate the stomatal density. Chlorophyll was measured using SPAD and a spectrophotometer. To compare the results a t-test was used to find a significant difference. Significant differences in stomatal density were found in *Q. rubra* when comparing light variations. There was also a significant difference in stomatal density between *F. grandifolia* and *L. tulipifera*. Overall, the environmental factors seemed to have an effect on stomatal density and/or chlorophyll. However, the different species reacted differently to each factor. Carbon dioxide levels are rising in the atmosphere due to increasing fossil fuel usage. Trees take in carbon dioxide and give off oxygen. Understanding more about how environmental factors affect trees may aid the scientific world in understanding how trees will react to the increase of carbon dioxide levels.

## INTRODUCTION

The sun is responsible for providing energy for all organisms. This study is important because it shows how trees fit in the environment and how environmental factors affect trees. Weather that must be considered includes temperature (hot, cold), amount of sunlight (sun, shade) and humidity (moisture in the air).

When temperature is amplified above 30 degrees, the respiration is augmented, which increases the internal carbon dioxide, causing the stomata to close temporarily (Raven, 1999). Should the temperature diminish to zero degrees, the process of photosynthesis stops and causes the stomata to close and shut down for winter.

Photons (particles of light, waves of energy) from the sun trigger the opening and closing of stomata. There are different colors of light, the main ones being red and blue. Red light stimulates the guard cells and is taken in by the chloroplasts to supply ATP that pumps potassium ( $K^+$ ) in the cells. Guard cells surround the stomata and also control the opening and closing of the stomata. When the guard cells swell up with water, the stomata open; when the guard cells are emptied, the stomata close. Blue light is absorbed by pigment in tonoplasts, the membrane of the vacuole (Raven, 1999). Blue light promotes  $K^+$  uptake and causes the opening in the morning. When the sun is shining, the stomata are open, and when the sun sets, the stomata are closed. The stomata's function is to open and close to regulate gas exchange (Spence, 1987).

While this study focuses mainly on the stomata, there are many other parts of the leaf. The cuticle, upper epidermis and lower epidermis protect the plant similarly to the way skin protects humans (Raven et al. 1999). The palisade mesophyll cells are used to capture the rays from the sun (Raven et al. 1999). The bundle sheath cell contains the xylem, which transports water

throughout the plant, and the phloem, which transports nutrients throughout the plant (Raven et al. 1999). Chloroplasts make chlorophyll, which gives the plant its green pigment (Raven et al. 1999). The spongy mesophyll is on the side of the leaf with the stomata (usually the underside), which as previously stated, allows carbon dioxide to enter the plant (Raven et al. 1999). The stomata are the openings in the leaf where the gas exchange (carbon dioxide for oxygen) takes place (Raven et al. 1999). The guard cells surround the stomata, allowing it to open and close (Raven et al. 1999).

Photosynthesis means putting together with light (Sussman, 2000); it is a process that plants use to get energy. The chloroplast traps the light's energy, and the water enters, being carried by the xylem from the roots (Raven, 1999). Carbon dioxide (CO<sub>2</sub>) then diffuses from the atmosphere to internal leaf space through the stomata (Raven, 1999). When CO<sub>2</sub> and water react with light's energy, it produces glucose, oxygen and water. Some of the sugar exits the leaf, and some is left in the leaf. The sugar that is left in the leaf is used for growth and metabolic activity (Sussman 2000). The more carbon dioxide present in the atmosphere, the less stomata present on the leaf. Since there is a higher concentration of CO<sub>2</sub> available, the leaves don't need a lot of stomata to bring in CO<sub>2</sub> for photosynthesis (Tognetti et al, 2000). Whereas, when there is a limited amount of carbon dioxide (CO<sub>2</sub>) present in the atmosphere, there needs to be more stomata to allow the plant to take in more CO<sub>2</sub> to complete photosynthesis. Therefore, in order for the intake of CO<sub>2</sub> there must be light present.

In this experiment we tested different plants including: *Betula lenta*, *Prunus serotina*, *Acer saccharum*, *Quercus rubra*, *Liriodendron tulipifera*, and *Fagus grandifolia*. All of the trees are deciduous, meaning they shed their leaves in the fall.

The *Betula lenta* (black birch) grows faster in moist soil but overall it is a slow growing, shade tolerant tree. It is a tree that loves to grow wild and takes advantage of the canopy gaps. The average temperature the *Betula lenta* grows in is about 7°C (45°F) in the north and about 13°C (56°F) in the southern Appalachians (*Lamson*). This tree lives beyond 120 years in the first stage of succession (*Hicks 1998*).

The *Prunus serotina* is also known as the black cherry. Black cherries are generally found in the Allegheny plateau of Pennsylvania, New York and West Virginia; the southern Appalachians of the eastern U.S; and the central hardwood region. They grow on acidic mesic sites, and, when exposed to adequate light, black cherries are capable of very rapid height and diameter growth. They can live up to 200 years.

The *Acer saccharum* (sugar maple) is more prevalent in the northern hemisphere because it is shade tolerant (*Hicks 1998*). It is a slow growing organism which grows on an extensive variety of sites, especially on sandy soils (*Hicks 1998*). The tree lives a long time, reaching the age of 300-400 years (*Hicks 1998*). Acidic decomposition, nitrate saturation, ozone saturation and elevation can cause the *A. saccharum* population to decline due to stress (*Smith 1987*).

The *Quercus rubra*, commonly referred to as red oak, can grow under various conditions and is one of the fastest growing oaks. It grows from southern Canada to southern Alabama and from the Atlantic coast to the edge of the Great Plains (*Hicks 1998*). *Q. rubra* can grow in many soil types: inceptisols, alfisols, mollisols, ultisols and entisols (*Hicks 1998*). It grows best in deep well drained soils of concave slopes. Red oaks are somewhat shade tolerant, which enhances the trees chance of survival and germination.

*Liriodendron tulipifera* is commonly known as the yellow poplar. The yellow poplar is a fast growing plant that is found in the U.S., east of the Mississippi River in hardwood regions

(Hicks 1998). It can grow up to 1.5ft and adds to its diameter at about .25 units per year up to a hundred years of age (Hicks 1998). The yellow poplar grows best on the deep and well drained sites of the region, “mixed mesophytic association” (Hicks 1998). It invades disturbed areas where light is available. Yellow poplar often displays rapid height growth and develops in pure, even aged, stands (Hicks 1998). The plant monopolizes; its sprouts usually exceed seedlings in growth rate (Hicks 1998). The sprouts near the root are less prone to decay than those arising higher on the stump (Hicks 1998). Yellow poplars are shade intolerant; they need nearly full sunlight to grow well (Hicks 1998). It can live up to 300 years, but it usually stops growing between 150 and 200 years of age (Hicks 1998).

*Fagus grandifolia*, the American beech is a widely distributed species that grows to a large size in eastern states and central hardwood regions; it is highly prone to damage (Hicks 1998). It is found at low elevations in the north and high elevations in the south of the Appalachians (Hicks 1998). American beech sprouts well from cut stumps, but sprouts better when tree stumps are less than 4 inches in height (Hicks 1998). The plant is shade tolerant, its seedlings, sprouts and suckers can grow under a completely closed canopy (Hicks 1998). American beech is capable of living more than 300 years (Hicks 1998).

There were six hypotheses made based on the multiple environmental factors. The independent variables were species, light, water availability, elevation and temperature. What was tested was stomatal density and chlorophyll. When a tree does not get much light there is more stomata, so that the intake of CO<sub>2</sub> is enough to complete photosynthesis. On the other hand, a plant that gets a lot of light has a low stomatal density because it is open more during the course of the day and is able to complete photosynthesis more often. Therefore, it was

hypothesized that leaves of shade intolerant trees will have lower stomatal density than leaves of shade tolerant trees.

Plants in the shade have a greater stomatal density because all parts of the leaf are not hit with sunlight. To conduct photosynthesis and bring in carbon dioxide more stomata are needed to regulate gas exchange throughout the day. A leaf in the sun is open more and is able to bring in more carbon dioxide with less stomata. Hypothesis two is: Leaves of trees growing in shade should have a greater stomatal density than leaves in direct sunlight.

The more light entering the leaf the more chlorophyll is present. Since there is more light entering the leaf the tree has more energy which enables it to make more chlorophyll. Hypothesis three is: The *Quercus rubra* in areas with a high amount of light should have a higher amount of chlorophyll than those in areas with a low amount of light.

The process of photosynthesis speeds up when more water is available; therefore, the leaf needs more carbon dioxide, causing it to have a higher stomatal density. Hypothesis four is: Leaves of trees growing in areas with higher water availability will have higher stomatal density than those growing in lower water availability.

In high elevation the temperature is cooler so there will be a lower stomatal density because the stomata won't have to close as often. In low elevation the temperature is hotter so there are more stomata, since the stomata close when it is too hot. Hypothesis five is: The *Acer saccharum* in high elevation will have a lower stomatal density and higher chlorophyll than that in low elevation.

Stomata in lower temperatures stay cooler and the process of photosynthesis slows down. Therefore it has a lower stomatal density, but wide, thin leaves to compensate. Leaves in higher temperature can evaporate water, speeding up the photosynthesis process, and will have a higher

stomatal density. Hypothesis six is: Leaves of trees growing in area with low temperatures will have a lower stomatal density than those growing in an area with high temperatures.

#### METHODS

The four sites tested were Frostburg University Campus, St. Johns Rock and high and low elevations at Fernow Experimental Forest. The elevation for Frostburg State University is 2000 ft., Fernow Experimental Forest low elevation is 1700ft. and high elevation is 2700ft., and St. Johns Rock is about 2800ft. Research was conducted at these sites during the time period of 10:00 am. through 5:00 pm.

There were five sun and five shade leaves, and five high and five low elevation leaves for each species. Five *Fagus grandifolia* leaves were used to measure water proximity. The different distances to water were determined by whether the tree was less than 10m from water or greater than 10m from water. Five *Prunus serotina* leaves were used to measure temperature in 64° fahrenheit and in 72° fahrenheit. The temperature was taken from the months of May and June because that is when the leaves begin to develop.

To locate the species the trees were flagged with yellow flagging tape. The researchers used a compass to find east and west. This was done to trace the path of the sun throughout the course of the day and to see if there were objects blocking the sun from the leaves. To take light measurements, two light meters were used. One was used to measure a sun leaf, while the other measured a shade leaf. The sensor was placed on top of the leaf and simultaneous measurements were taken. Simultaneous measurements were needed because to compare the leaves accurately, the leaves needed to be under the same environmental conditions. This was done three times, and the light measurements were recorded.

To take the impression of the stomata, nail polish was used. It was important that the leaf was polished while on the tree because once the leaf was pulled from the tree the stomata may have closed, thus preventing an accurate count of the stomata.

Once dried, the polish was peeled off the back of the leaf and taken back to the lab to be placed on a slide marked in millimeters. The slide was placed on the microscope, to find the diameter. The diameter was found at 100x magnification. It was then divided by two; this was to get the radius. The equation used to find the area was  $\pi R^2$ . The stomata were counted under the 400x magnification, therefore the diameter was divided by 4.

To test the soil moisture, a hydrometer was used. A tape measure was used also to measure the distance from the stream to the tree. In order to get an accurate distance from the plant to the stream, the tape measure was placed directly at the trunk of the tree and directly at the stream.

Once field work was completed the specimens were taken back to the lab to be tested. To test chlorophyll, SPAD and a spectrophotometer were used. Chlorophyll is measured by using crushing leaf tissue in liquid nitrogen and extracting the chlorophyll with acetone. The spectrophotometer is a machine that calculates the amount of transmittance or absorbance in chlorophyll. The transmittance was inversely proportional to the chlorophyll. The light wavelength was set at 680nm because it is the peak absorbance wavelength of chlorophyll A. Chlorophyll A is where the reaction starts. The SPAD index was measured three times from one leaf and averaged. Then it was averaged together with all four leaves that were taken from high or low elevation. Calculating the SPAD three times from various spots in the leaf provided more replication to make the data as accurate as possible. The statistical analysis used to test the hypothesis is the t-test. To be 95% confident we used the critical value of .05.

## RESULTS

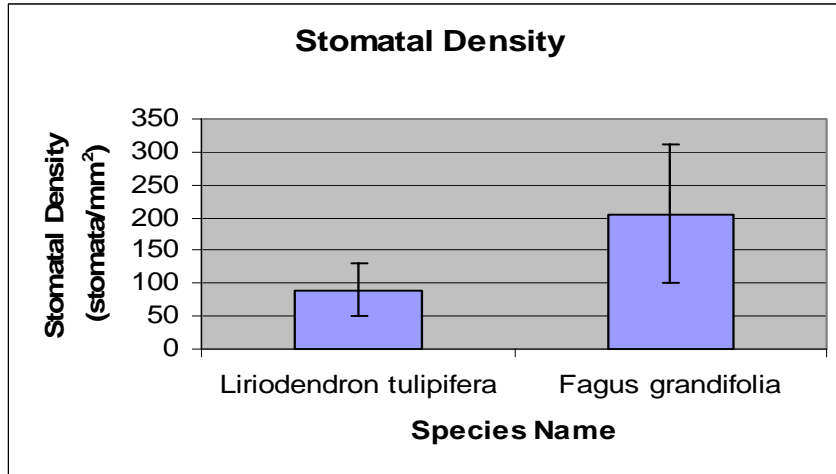


Figure 1. The difference between stomatal densities

Figure 1 shows the difference between the stomatal density of the *Liriodendron tulipifera* and *Fagus grandifolia*. The average stomatal density for the *Liriodendron tulipifera* is 89.5 and the *Fagus grandifolia* is 205.4. A t-test was calculated to see if there is a significant in stomatal density of *L. tulipifera* and *F. grandifolia*. The t stat value was -3.196 while the t critical value was 1.782 ( $p = 0.0038$ ).

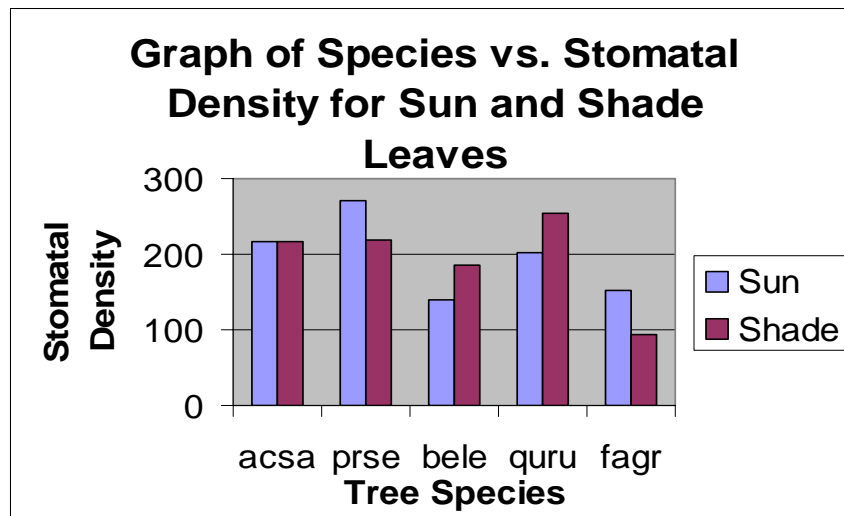


Figure 2. This graph shows the average stomatal density for the sun and shade leaves of each tree.

Figure 2 shows the average stomatal density of the sun and shade leaves for *Acer saccharum*, *Prunus serotina*, *Betula lenta*, *Quercus rubra* and *Fagus grandifolia*.

A t-test was calculated for *Q. rubra* and it was found that leaves developing in a shade environment have a statistically greater stomatal density than the leaves in the sun. In the t-test the t stat value was 2.594 while the t critical value was 2.132 ( $p = 0.03$ ). Another t-test was done using *P. serotina* and it was found that there is no significant difference between leaves developing in sun or shade areas. In the t-test the t stat value was 0.677 and the t critical value was 2.132 ( $p = 0.268$ ).

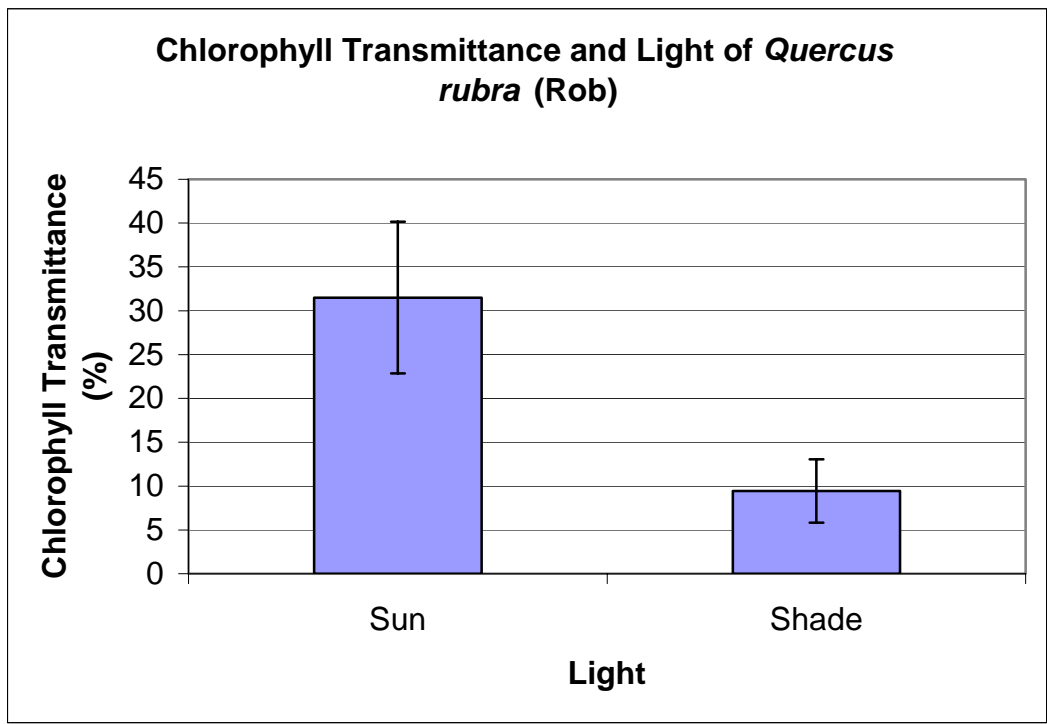


Figure 3 shows the percentage of chlorophyll transmittance and standard deviation of the sun and shade leaves of the *Quercus rubra*.

This graph relates to the hypothesis saying that the *Quercus rubra* (red oak) in areas with a high amount of light will have a higher amount of chlorophyll than those in areas with a low amount of light. The standard deviation for the sun leaves was 8.6417591, while the standard deviation for the shade leaves was 3.606245.

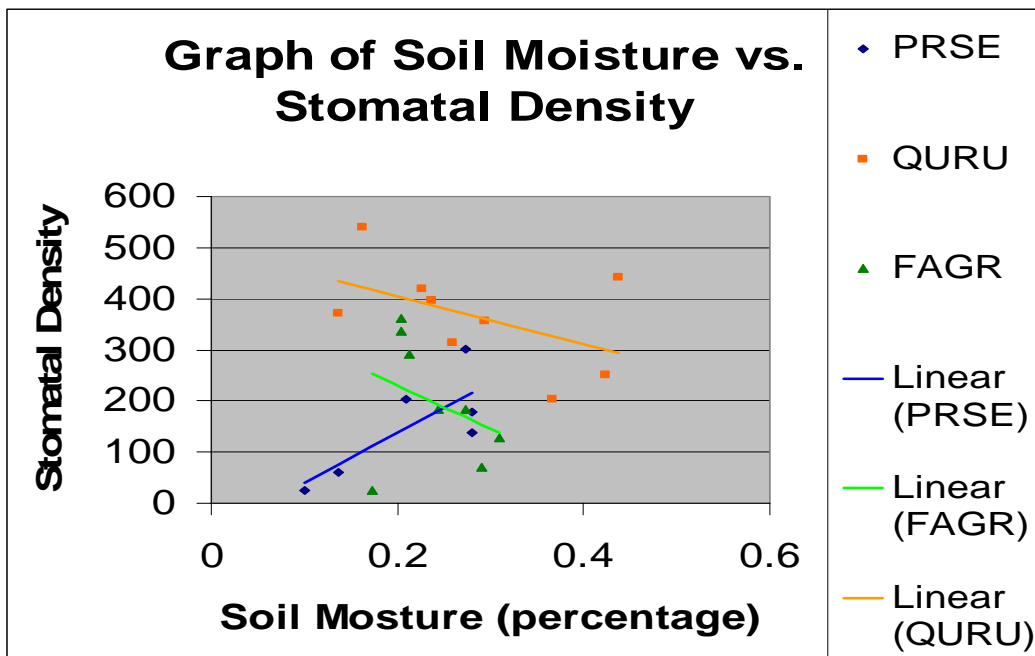


Figure 4. This scatter plot represents the comparison of soil moisture to stomatal density for the *Quercus rubra* (red oak), *Prunus serotina* (black cherry), and *Fagus grandifolia* (American beech)

Stomatal Density of *Fagus Grandifolia* Compared to Distance from Water (Kendra)

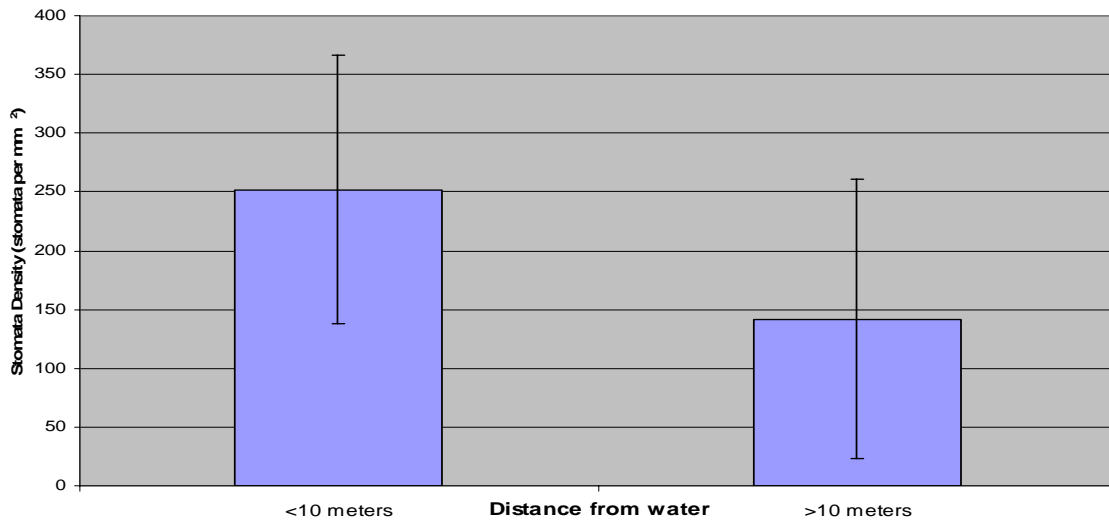


Figure 5 represents the data collected on the stomatal density to its distance from the spring for the *Fagus grandifolia*.

Figures 4 and 5 relate to the hypothesis that leaves of trees that grow in areas with higher water availability will have higher stomatal density than those growing in areas with lower water availability. The standard deviation for the leaves that were closer to water was 114.4884. The standard deviation for the leaves that were further from water was 118.6786.

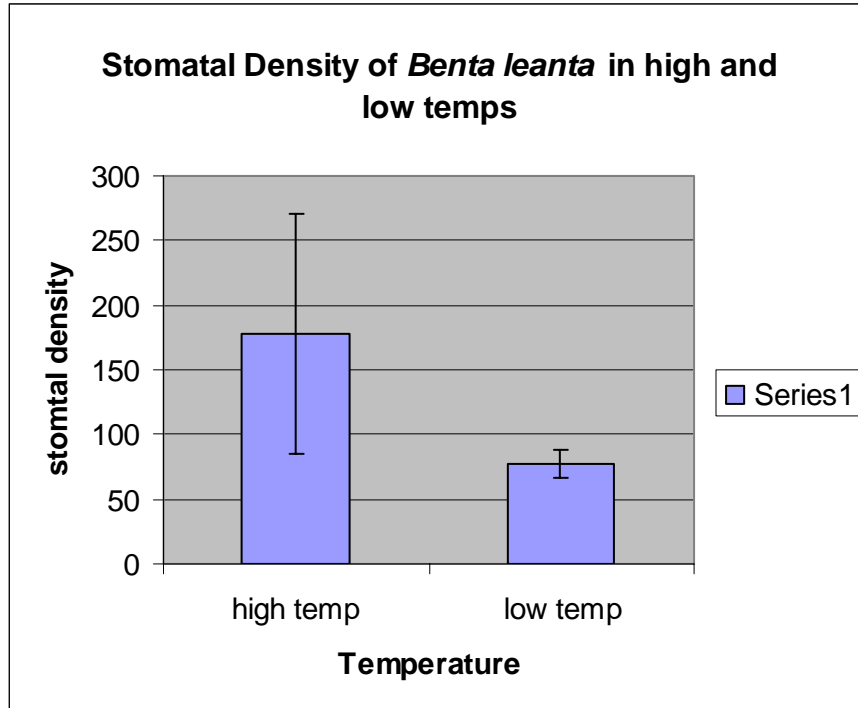


Figure 6 shows the average stomata density in high and low temperatures. The standard deviation for low temperature is 11.021 and high is 84.407.

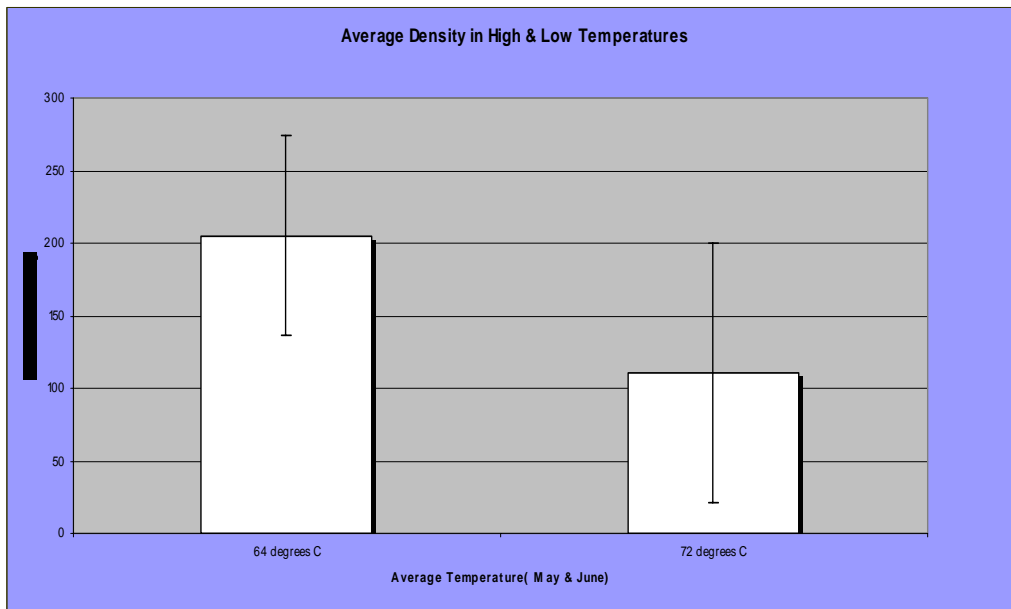


Figure 7 shows the average temperature and standard deviations for Fork Mt and Nursery bottom.

Daily temperature was collected from both sites. That data was then averaged out to give an average temperature between the two sites. The graph shows an 8° difference between the two sites. The standard deviation value for low temperature is 69.365 and high temperature was 89.343.

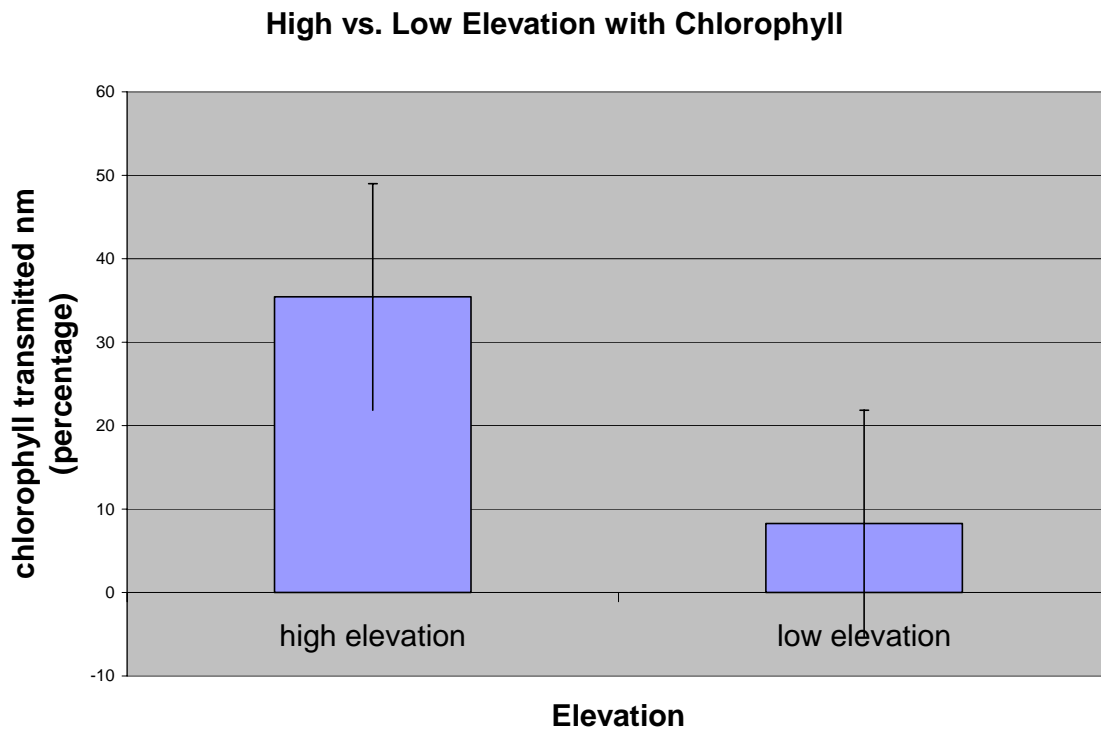


Figure 8 shows chlorophyll transmittance which is the percent of light that the plant didn't capture compared to elevation.

Using the spectrophotometer to measure the amount of chlorophyll that was extracted from the leaves in high and low elevation the graph presents that there was more absorbance in the leaves found in low elevation. The graph shows the average of 6 samples that were measured once in the spectrophotometer, then averaged together from high and low elevations. To measure the chlorophyll not only was the spectrophotometer used but the SPAD as well. The next graph shows the amount of chlorophyll the leaf had in three different spots. There is a difference of 27

stomata per mm<sup>2</sup> difference. The standard deviation shows that there is a possibility of a significant difference.

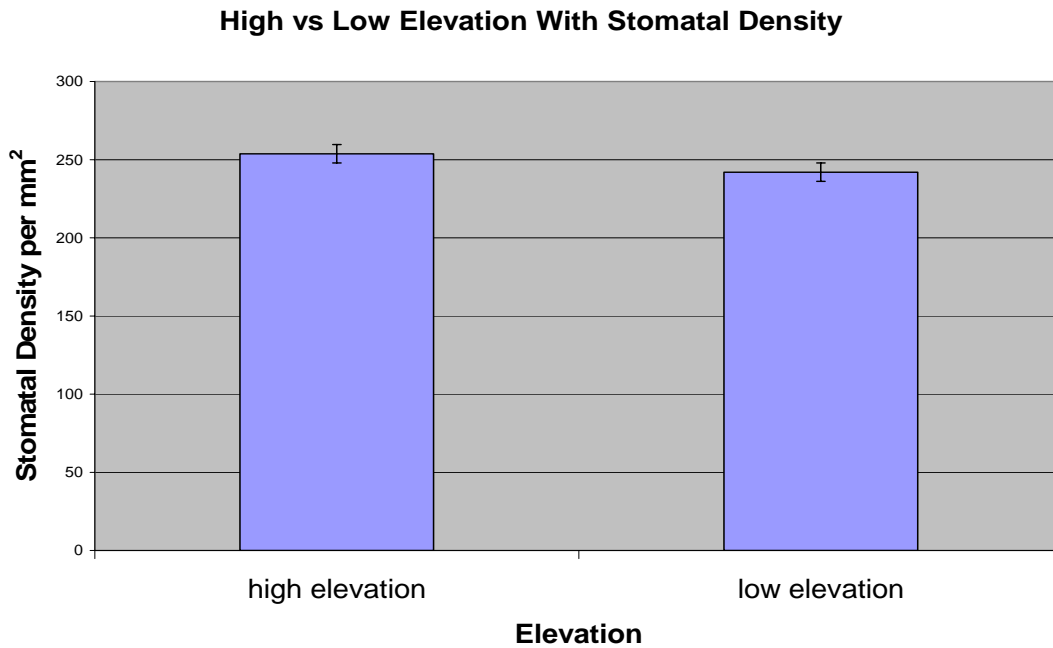


Figure 9. Difference in high and low elevation measuring stomatal density.

The graph shows that in a high elevation that there is a higher stomatal density than in a lower elevation. Even though there is a slight difference when compared to low elevation the high elevation has 11.75 stomata per mm<sup>2</sup>. The graph presents a standard deviation that overlaps, such that there is a low possibility of a significant difference.

#### DISCUSSIONS AND CONCLUSIONS

The first hypothesis was that leaves of shade intolerant trees will have lower stomatal density than leaves of shade tolerant trees. This hypothesis was supported due to the large difference between stomatal densities (as shown in figure 1) and the results of the performed t-test. The *Liriodendron tulipifera* is a shade intolerant tree. Figure 1 shows that it had a lower

average stomatal density than *Fagus grandifolia*. *L. tulipifera* had an average stomatal density that was below 100 stomata/mm<sup>2</sup>. *F. grandifolia* is a shade tolerant tree and has a higher stomatal density than *L. tulipifera*. Figure 1 shows that *F. grandifolia* had an average stomatal density of about 200 stomata/mm<sup>2</sup>. The t-test performed showed a p value less than the critical value of .05. This leads to the conclusion that there is a statistical difference between the *F. grandifolia* and *L. tulipifera* stomatal densities. The t-stat value, 3.195, was higher than the t-critical value, 1.7822. This supports the hypothesis. The higher stomatal density of *F. grandifolia* could be due to the fact that it is more shade tolerant. The tree would need more stomata to account for the lack of light. When light hits the leaf the stomata open; when there is a higher stomatal density and a lower amount of light there will be more stomata to open when light hits the leaf. The lower stomatal density of the *L. tulipifera* may be due to the tree being found mostly in the sun. This species needs less stomata to achieve the same amount of carbon dioxide as the *F. grandifolia*. There is more sunlight hitting the leaves, so the stomata are open more often. This means that there doesn't need to be as high a stomatal density as a tree found in the shade. Tognetti et al found that there is a decrease in stomatal density with the increase of an environmental factor (2000).

The hypothesis that leaves growing in the shade will have a greater stomatal density than leaves in direct sunlight was not supported for all species. *Acer saccharum* showed equal stomatal densities for both sun and shade. *Prunus serotina* and *Fagus grandifolia* showed higher stomatal densities in the sun. This does not support the hypothesis. However, *Betula lenta* and *Quercus rubra* did support the hypothesis by showing higher stomatal densities in the shade (Figure 2). T-tests were performed on the *Quercus rubra* and *Prunus serotina* data. *Quercus rubra* showed a statistical difference between the stomatal densities of sun and shade leaves,

whereas the *Prunus serotina* did not show a statistical difference. Different species are adapted to different things in an ecosystems so that all the species can live together.

The hypothesis that *Quercus rubra* growing in full sun will have more chlorophyll than *Quercus rubra* growing in shade was not supported. Figure 3 showed that the chlorophyll of the leaves in the shade absorbed more light than the leaves that were exposed in the sun. Shaded leaves held more chlorophyll within the leaves. Leaves in the shade need more chlorophyll in order to capture as much light as leaves in the sun. Sun leaves received more light, so they invest less in light capture. The chlorophyll and light transmittance was higher in the sun leaves, which was 32%. The Chlorophyll and light transmittance was lower in the shade leaves, which was 9%. The standard deviation for the sun leaves was 40; the shade was 13.

As the soil moisture increased, the *Prunus serotina* stomatal density increased. The *Quercus rubra* soil moisture decreased the stomatal density. The *Fagus grandifolia* stomatal density decreased with soil moisture as well. *Fagus grandifolia* stomatal density was lower in leaves of trees that are farther from water. Thus, hypothesis 4 was not supported because stomatal density of trees in areas of high water availability was not higher for all species tested.

Another hypothesis was that the *Acer saccharum* in a high elevation will have a lower stomatal density and a higher chlorophyll concentration than that in a lower elevation. This hypothesis had two different conclusions. The chlorophyll concentration was higher in the higher elevation, however the stomatal densities were not statistically different. Figure 6 shows that in the high elevation the chlorophyll transmitted was at about 35%, whereas at the lower elevation the chlorophyll transmitted was about 8%. There were two t-tests performed on the chlorophyll data. One t-test was conducted on the data from the spectrophotometer. The p value was lower than the critical value (.05), showing a statistical difference and supporting the

hypothesis. The second t-test was on the data from the SPAD. The p value was higher than the .05 critical value, showing no statistical difference. Figure 7 shows that the stomatal density at the higher elevation was about 250 stomata per square millimeter and the stomatal density at the lower elevation was just below 250 stomata per square millimeter. There was a third t-test performed on the stomatal density data. The p value was higher than the critical value of .05. This shows no statistical difference and does not support the hypothesis. Some reasons for these conclusions include that the amount of carbon dioxide in the higher elevation were similar to the carbon dioxide levels in the lower elevation, the amount of sunlight and temperature may not have been very different in the two elevations, and the SPAD meter is not as accurate as the spectrophotometer.

The hypothesis that the stomata density of the *Prunus serotina* would be lower in lower temperature than in a higher temperature was supported. From collecting and researching leaves from different sites, diverse data was collected and tested to suggest that the hypothesis was correct. Two sites that were tested were Fork Mt, which is high elevation and Nursery Bottom, which is a low elevation. The average temperature in the month of May and June were calculated based on the data collected from Fork Mountain and Nursery Bottom. In the higher elevation, the temperature was lower. The stomata density in the higher temperature (72° F) was higher for both *Betula lenta* and *Prunus serotina* than the stomatal density in the lower temperature (64° F). In a higher temperature the water evaporates off the leaf surface to cool the leaf, so having the higher stomatal density also helps cool the leaf. None of the sites tested went above the critical temperature of 30-35°C.

A two-way ANOVA was performed to see if there were interactions between two environmental factors. The two factors tested were light and elevation for the *A. saccharum*.

Since the significance value for LIGHT \* ELEVATION is above 0.05 then there is no statistical interaction. Also a two-way ANOVA was used with light and elevation for *Q. rubra*. The significance value for LIGHT \* ELEVATION is below 0.05 so therefore there is an interaction between the two factors. *Q. rubra* and *A. saccharum* were analyzed because they were the only two species found at all elevations.

The environmental factors are linked together for many reasons. The light is like the water, it helps the tree grow.

The study was limited by many factors, including the length of study, one week. Another limiting factor is repetition: only four sites were tested with only a few leaves collected of each species from each site.

The study could be lengthened, which would give adequate time to collect more specimens for repetition. Another improvement is to take light measurements throughout the entire day and study. Visiting more sites and collecting more leaves so the results of the experiment could be more precise. Then, having a computer count the number of stomata on each leaf, which would decrease the error in counting.

Some of the questions that were raised are: how do multiple environmental factors interact to affect stomatal density? Of the different environmental factors that have been tested, which will be different if tested on different species? What affect would high and low temperatures have on the rate of photosynthesis? The results of this study and investigation into the new posed questions will help explain how ecosystems may respond to changing environmental factors in the context of global climate change.

#### **LITERATURE CITED**

Hicks, Ray. 1998. Ecology and Management of Central Hardwood Forests. John Wiley and Sons. New York.

Raven, Peter, Evert, Ray, and Eichorn, Susan. 1999. The Photosynthesis, light, and Life. Pages 126-153. *Biology of Plants*. W.H. Freeman and Worth. New York.

\_\_\_\_\_. 1999. The Movement of Water and Solutes in Plants. Pages 750 -758. *Biology of Plants*. W.H. Freeman and Worth. New York.

Spence Richard D. 1987. The Problem of Variability in Stomatal Responses, Particularly Aperture Variance, to Environmental and Experimental Conditions. *New Phytologist* 107: 303-315.

Sussman, Art. Dr. Arts Guide to Planet Earth. San Francisco: West Ed, 2000, chp. Life Webs.

Roberto Tognetti, Antonio Minnocci, Josep Penuelas, Antonio Raschi, Mike B. Jones. 1999. Comparative field water relations of three Mediterranean shrub species co-occurring at a natural CO<sub>2</sub> vent. *Journal of experimental botany* vol. 51: 1135- 1146