Sunlight and shadiness do not influence American Holly (*Ilex opaca)* stomatal density

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INTRODUCTION

There are many factors that contribute to plant stomatal density, including carbon dioxide, water, auxin, and light (Dietrich et al., 2001). This experiment will focus on the effect of sunlight and shadiness. Stomata function in gas exchange of carbon dioxide by opening and closing. This experiment allows for an understanding of factors that affect stomatal density in *Ilex opaca*. By comparing the stomatal density in a region with greater sunlight and a region with greater shade, it will allow conclusions on the effect sunlight on stomatal density of *Ilex opaca*.

Previous research found that *Betula lenta* and *Quercus rubra* leaves both had a greater stomatal density in the shade compared to the sunlight (Young et al., 2004). This is because stomata in leaves less exposed to sunlight can do gas exchange more efficiently by bringing in carbon dioxide without excess transpiration due to the sun's heat.

With previous research, and because stomata function in gas exchange, we were able to develop our hypotheses. Our null hypothesis is that abaxial stomatal density is not affected by sunlight and shadiness. Our null prediction is that abaxial stomatal density will not differ significantly between leaves in shady and sunny environments.
Our alternative hypothesis is that stomatal density is affected by sunlight and shadiness. Thus, our alternative prediction states that *Ilex opaca* leaves exposed to sunlight are more likely to have a smaller abaxial stomatal density than those exposed to shade, in order to conserve water and minimize transpiration in the plant.

METHODS

Obtain 20 *Ilex opaca* leaves that are positioned distally from the trunk and exposed to extreme sunlight and another 20 leaves from the same tree that are proximal to the trunk and exposed to minimal sunlight. Take an abaxial leaf peel of each of the 40 leaves by using clear nail polish and pulling the polish up with tape once it is dry. Put each sample on a coverslip and count the number of stomata under a microscope at 400x magnification from three different fields of view. The mean number of stomata should be recorded from these 3 values for each of the 40 leaves. Each mean value will be converted to stomatal density (stomata/mm^2) using a value of 0.16 mm^2, since the microscope magnification is 400x. The results will be analyzed using a t-test for independent samples in which mean abaxial stomatal density will be compared between sunny and shady environments. A bar graph of mean stomatal density +/- SE will display the results.

Independent Samples Test

		F	Sig	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Density	Equal variance assumed	17.545	0	-0.206	40	0.838	-2.187	10.603
	Equal variance not assumed			-0.206	26.022	0.838	-2.187	10.603

Table 1- Results from the independent samples t-test. Results show a p value of 0.838, indicating that there is no significant difference between stomatal density in the two environments.

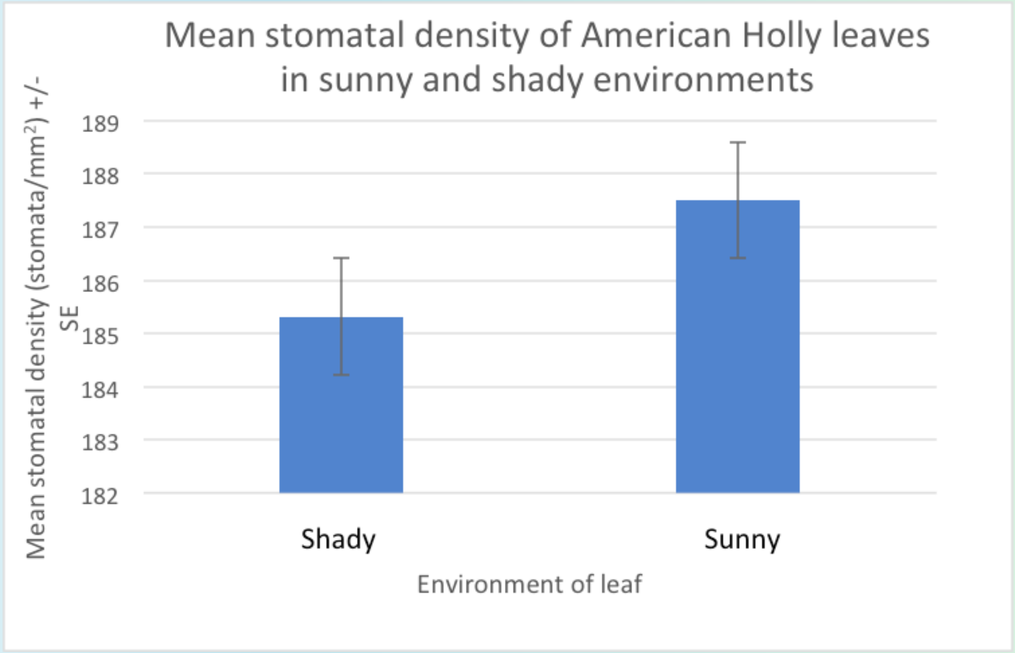

Figure 1- Mean stomatal density of leaves in shady and sunny environments. Overlapping error bars indicate that there is no significant difference between stomatal density in the two environments.

RESULTS

The independent samples t-test found that there was not a significant difference between the stomatal densities of leaves in shady and sunny environments, as shown by the p value of 0.838 in Table 1.

While the means did differ, as indicated in Figure 1, the difference was not large enough to be considered significant, and the error bars overlap between the two conditions, further indicating a lack of significance. Thus, we accept the null hypothesis that there is no difference between the stomatal density of leaves in shady and sunny environments.

DISCUSSION

The results disagree with our hypothesis, which stated that there would be a significant difference in abaxial stomatal density between the shady and sunny environments. They also disagree with the prediction, which was that leaves in the shady environment would have a larger abaxial stomata density than leaves in the sunny environment. This contradicts previous research such as that of Young et al, which leads us to believe that American Holly leaves have adaptations different from those of other plant species that cause their stomatal densities to not differ between shady and sunny environments.

Future research should examine stomatal aperture in sunny and shady environments. Previous studies have shown that the presence of light has an effect on stomatal aperture (Dietrich et al., 2001). This study may show that leaves in the shade have larger stomatal apertures than those in the sun, in order to minimize excess transpiration that would occur in leaves exposed to harsh sunlight, which would align with previous research.

CONCLUSION

- We found similar stomatal densities for leaves in shady and sunny environments
- American holly trees may have stomatal adaptations different than those of other species

LITERATURE CITED

Young T, Turner S, Torau S, et al. August 2004. How Environmental Factors Affect Stomatal Density and Chlorophyll in Trees [Internet]. [cited 1 Mar 2018].
Petra Dietrich, Dale Sanders, Rainer Hedrich; The role of ion channels in light-dependent stomatal opening, Journal of Experimental Botany, Volume 52, Issue 363, 1 October 2001, Pages 1959–1967