Positive Relationship between Sun Exposure and Transpiration Rate of the *Psidium gujava* Tree

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Abstract

At the start of this experiment, I was curious on whether a human could survive off the amount of transpiration water collected from a tree for a few hours. My focus was determining the strength of the relationship between sun exposure and transpiration. Transpiration is the way an autotroph, such as the guava tree *Psidium gujava,* simultaneously absorbs necessary carbon dioxide from its external environment and releases water vapor and oxygen through its leaves (Jones, 1998). An increased rate of transpiration means an increased rate of water loss due to stomata being unable to control how much water is lost once open (Jones, 1998). There are many environmental factors that influence the rates of transpiration, such as temperature and level of exposure to sun radiation (Léchaudel, Lopez-Lauri, Vidal, Sallanon, & Joas, 2013). Many tropical trees, such as the common guava tree, have adapted to varying amounts of direct sun exposure due to living in lush forests with many overlapping neighboring plants (Mooney, Field, & Vázquez-Yánes, 1984). I collected the water vapor released by the leaves of my *Psidium gujava* tree for five days, 7 hours each day. I compared the volumes of water collected at each collection site day by day, top layer to bottom layer, outer layer to inner layer, number of leaves, and average daily temperature. My findings demonstrated transpired water volume had the strongest relationship with sun exposure, as represented by the different site layers. Investigating other potential factors that influence transpiration would aid in the attempt to understand, and hopefully restore, the habitats of autotrophs that provide us with food, medicine, shelter, and countless other items we depend on for survival.

Introduction

Leaves have microscopic pores called stomata which serve as the entry and exit point for gas exchanges between the plant and its surroundings, this is known as transpiration (Willmer and Fricker, 1996). When more transpiration occurs, more carbon dioxide comes into the plant and increases the amount of energy that can be created by the plant through photosynthesis (Knapp and Smith, 1990). Unfortunately, an increased rate of transpiration means an increased rate of water loss due to stomata not being able to control how much water is lost once they are open (Jones, 1998). There are many environmental factors that influence the rates of transpiration, such as temperature and level of exposure to the sun radiation (Léchaudel, Lopez-Lauri, Vidal, Sallanon, and Joas, 2013).

The *Psidium gujava* is the common guava tree native to the tropical and subtropical environments in the Southern parts of North America and South America (Pommer and Murakami). Many of these trees have adapted to different levels of direct sun exposure due to living in ecosystems that have many overlapping plants, like the rainforest (Mooney, Field, Vázquez-Yánes, 1984). The less direct sun exposure these tropical plants have, the less transpiration occurs (Léchaudel et al, 2013). Less sun exposure in shaded areas of the same mango tree, another tropical tree, led to smaller and thinner-skinned fruits adapt (Léchaudel et al, 2013), indicating that decreased sun exposure effects on a tropical plant does not end at lower transpiration levels but lower quality health and produce for the less-exposed sections of the tree, posing greater threats to these trees if these levels were to change drastically faster than these tropical species' ability to adapt.

To test the effects of sun exposure and temperature on the *Psidium gujava* tree's rate of transpiration, I collected the water released by the leaves of 12 different branches from 4 different sections of the tree. These sections were divided into the bottom-outer layer, bottom-inner layer, top-inner layer, and the top-outer layer, each containing 3 different transpiration collection sites (branches). I hypothesized the top layer sites would have significantly higher amounts of water released than the bottom layers sites, and that the outer layer sites

would have significantly higher amounts of water released than inner layer sites. This was predicted because the top layer sites were on the highest parts of the tree, limiting the amount of foliage that would block sun exposure from these sites, and similarly the inner layer sites had more obstacles decreasing their sun exposure than the outer layers.

Methods

In order to measure how much water was released by the transpiration of the leaves of the guava tree *Psidium guajava*, there had to be consistent equipment, consistent timing, consistent transpiration collection areas, and meticulous measurements taken.

The equipment used to collect the transpiration data included: a full-grown common guava (*Psidium guajava)* tree, 60 clear fish bags, 72 strings of strong bright yarn, labels, a ladder, a tape measure, a jewelry scale, scissors, a 25 mL granulated cylinder, a 100 mL beaker, gloves, water containers, a smart phone (clock and weather source), a black permanent marker, a journal, and a pen. Large-sized transparent bags, strings, and a nontoxic tree in direct sunlight were the recommended tools to collect transpiration water (Ninja) The clear fish bags were chosen as the transpiration collection bags as they are known to hold water well and prevent the loss of liquid data. The jewelry scale was chosen because it detects weight to the nearest 0.01g, making it the ideal weight measuring device to detect the minute differences in weight water droplets would add to the bags.

The experiment took five days total, each collection site was utilized for a total of 7 hours. In the morning, the first site was bagged at 11:30 am each day and the last site was bagged at 12:00 pm, each site's bag took approximately 2.5 minutes to set up. In the evening, the first site's bag was removed at 6:30 pm each day and the last bag was taken down at 7:06 pm. The timing of this step varied from the morning setup timing because there was the extra step of measuring and recording each bag's collected water volume, making the average data collection time for each site increase to 3 minutes. This timing was chosen after several previous failed attempts to collect enough water to measure from each site. From 10:00 am to 4:00 pm, the sun exposure is at its peak height and highest level of radiation (Ultraviolet (UV) Radiation, 2020), which I thought would make it the ideal time interval to collect transpiration water excretions since the guava tree would have the most sun exposure.

This idea worked out for the collection sites on the outer sections of the tree, but not for the sites in the inner layers of the tree because the collection bags from these sites typically came back bone-dry. The highest level of water collected from the top and bottom-layers of the guava tree were from 4:00pm to sunset, but this time-period tended to be colder and testing the sun's radiation exposure on the tree would be difficult. When I combined my data from my previous attempts, I decided to choose a time frame that would be ideal for both my experiment and all sections of the tree. That is the reason why my transpiration collection time-period was planned as follows: 11:30 am-12:00 pm to set up all the bags and collect the first set of data and 6:30 pm-7:06pm to take down my completed transpiration collection and record the last set of data for the day. The evening time frame was chosen since sunset was predicted to be around 6:00 pm my five chosen days.

The first three courses of action were completed the afternoon before the first day of experimentation. The first one was selecting all the branches that would be the designated water collection sites for the entirety of the experiment. I chose twelve branches, each spread out at least two feet across from one another. There were three designated collection sites on the bottom-outer layer of the guava tree (Sites 1,2,5), three sites on the bottom-inner layer (Sites 3,4,6), three sites on the top-inner layer (Sites 7,8,9), and three sites on the top-outer layer (Sites 10, 11, 12) as seen in Figure 1. There was the limitation of the combination of my height, arm's reach, and ladder were not tall enough to get to the highest points of the tree, but I went as high as was possible. The heights of each of these sites and the total height of the guava tree were measured with a tape measure and recorded in the journal (Table 1). I tied a label with a number from 1-12 to each selected collection site's branch with a piece of brightly colored yarn that would help me find the sites easily amongst

the dense leaves. These tied labels would not only prove to be useful to have consistent collection sites, but they also served as dividers between the leaves that would be bagged and the ones that would not, giving me confidence that the number of leaves in each collection site would remain consistent as well throughout the experiment.

Figure 1: Photos of Designated Transpiration Collection Sites. The yellow circles on the left photo represent the top and bottom-outer layer collection sites. The green circles on the right photo represent the top and bottom-inner layer collection sites.

Site Number	Height (in)
1	66
2	65
3	63
4	78
5	82
6	81.5
7	101.5
8	119
9	106.5
10	124
11	130
12	119

Table 1: Recorded heights for each of the selected transpiration collection sites. Sites 1-6 were on the bottom half of the tree and Sites 7-12 were on the top half of the tree. The total height of the tree was 188.25 inches.

The second preparation was and organizing all the clear collection bags. The 60 bags were divided into 5 groups, and each of the bags in a group would have a number from 1-12 written on it with a permanent marker and well as a number from 1-5 to label the day of the group they were in. Although reusing the plastic bags would have been more environmentally friendly, I could not risk an undetected tear costing me the accuracy of the water data collected each day.

The final preparation was to set up two tables per day in my journal, one table for the morning data and one table for the evening data. The morning data table included the initial temperature of the set-up period, the bag number, the initial weight of the bag, the initial time the bag was tied in place at its designated site, the number of leaves each bag contained, and the final temperature of the set-up period. The evening data table included the initial temperature of the bag collection period, the bag number, the time the bag was removed from its

designated site, the final weight of the bag, the volume of the water collected, and the final temperature of the bag collection period. Once all these components were prepared, I began my 5 days of data collection.

All 5 days had the preparation procedure done the mornings and the same water collection procedures in the evenings. The mornings consisted of setting up the collection sites and taking note of any differences there might have been from the previous days. I would check the temperature on my phone and record this information before setting anything up. All the tools I would need were contained in a small cardboard box which included: the 12 fish bags of the day, 12 strings of brightly colored yarn, the jewelry scale, the journal, a pen, and my phone. Each bag would be gently rolled up and then placed on the scale. After this information was recorded, I would carefully engulf the leaves of the designated collection site with the bag of the same number and tie the bag in place with a piece of yarn, counting the number of leaves that would go inside. The guava tree's branches have leaves that are staggered in pairs which allowed me to tie the bags and labels securely in place without causing damage to the leaves outside or within the bag. As soon as the bag was set-up, I recorded the time I saw on my phone in my journal and write down any small details that could affect the final volume of water collected. This small specs of dirt having fallen from the leaves into the bag, a few ants stuck on the leaves inside of the bag, and other oddities that could have affected my results. For the taller sections of the tree, I had an assistant help me go up on a ladder and place the bags on those collection sites. This assistant would also be my main scribe in these moments so that I would not risk falling off the ladder. I once again glanced at my phone to check and record the temperature at the end of this set-up period. The bags were then left alone for 7 hours in the sun.

To gauge the average temperature of my transpiration collection period each day, I would check the temperature 3.5 hours after the initial setup time and 3.5 hours after the final set-up time. After the 7 hours were completed, the bags were removed carefully, and their contents were measured. My toolbox would contain its evening procedure equipment which included: the jewelry scale, scissors, a 25 mL granulated cylinder, a 100 mL beaker, gloves, water containers, the journal, a pen, and my phone. This time had the tendency of being colder than the mornings, enough to where gloves were necessary for me to have nimble enough fingers to remove the bags without spilling a drop. Once again, I would check the temperature on my phone and record this information before beginning the evening routine. I would then record the time the moment before I removed the now-filled water collection bag from each branch. Some water would remain on the leaves, but not enough the show the difference between the collection sites.

Once a bag was removed from the collection site, I gently pressed out all the air in the bag, pushed all the water droplets down to the bottom of the bag by pinching the sides of the bag and dragging downwards, rolled up the bag (there was not enough water to spill out when this was done, otherwise I would have tared a cup on the scale to weigh the bag on), and recorded the final weight of the collection bag. All the water droplets would then be pushed to one corner of the bag and a small incision was made with scissors over the 25mL granulated cylinder. Once the water was drained from the bag, I recorded the amount of water collected by the cylinder and then dumped that water into a larger 100 mL beaker to repeat the process for the remainder of the bags. For the taller sections of the tree, I would ask my assistant to again help me use the ladder carefully and write down the times of those 6 bag removals (he also had gloves on). After all the collection bags were weighed and drained, I would check and record the final temperature of the day and clean-up the experiment site.

At the end of each day, the used bags would be thrown into recycling, the yarn would be saved to reuse the next day, the water was dumped into a water container labeled with the number of the day and placed in a dark, dry cupboard, and the data was inputted into my laptop. On my laptop, I recorded all the data I recorded in my journal and calculated the estimated average temperature of the day (Fahrenheit) and the estimated volume of water collected per site (mL). The estimated volume of water was calculated by taking the difference between the total weight of each bag minus the initial weight of each bag, to get the grams of water collected,

and then converting those grams into milliliters with the conversion factor 1 g = 1 mL. This was the estimated volume of water collected because the weight of the water included the weight of any dirt, ants, or leaf debris that had fallen into the collection bag. The actual amount of water collected per site was measured in the granulated cylinder after having pushed any large debris away from the water drainage site, which was later compared with the estimated water volume collected at the end of the 5 days. The two-sample t-test was attempted for each individual pair of comparisons (Top-Outer – Top-Inner, Bottom-Outer – Bottom-Inner, Top-Outer – Bottom-Outer, Top-Inner – Bottom-Inner).

Results

Several independent factors were compared to the dependent actual water volume collected (mL) at each site. The independent factors were temperature, number of leaves and layer of the site. The most significant ones were number of leaves bagged and layer of the site (Top-Outer [T-O], Top-Inner[T-I], Bottom-Outer[B-O], Bottom-Inner[B-I]). The least influential factor was temperature of the day. As the average temperature increased, the actual water volume slightly decreased, but there was no significant change (Figure 2, Table 2).

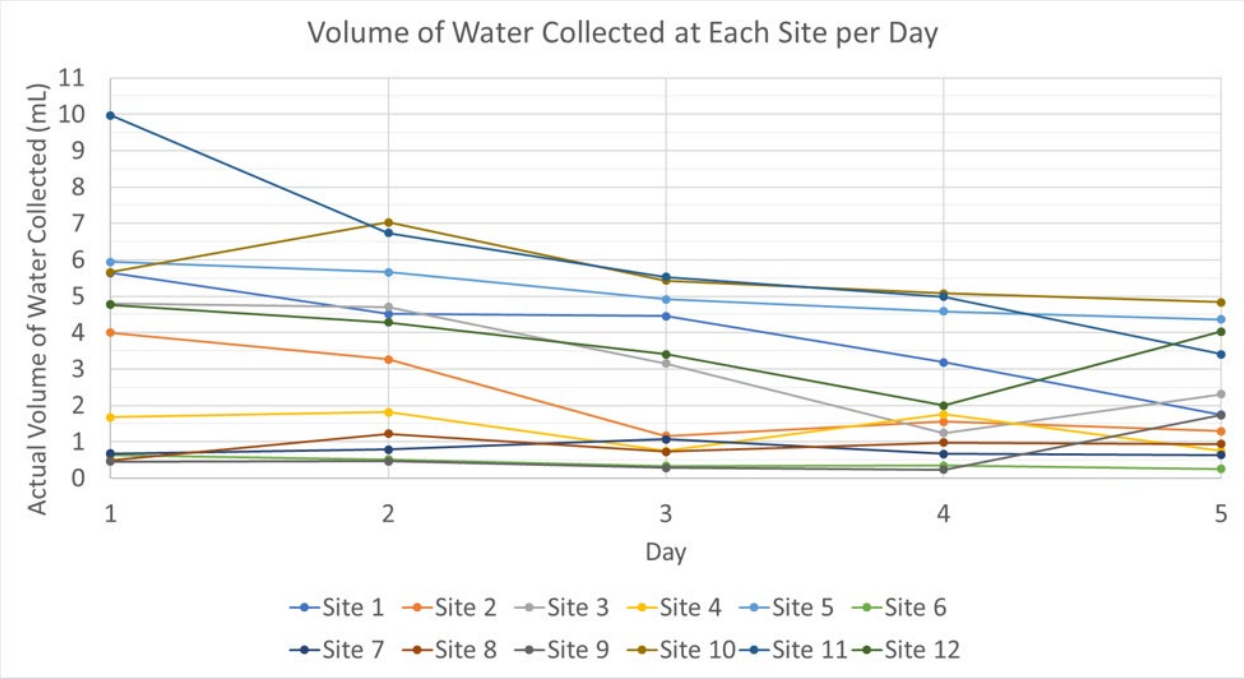

Figure 2: Actual Volume of Water Collected at Each Site Per Day. Each Day represents that Average Day's temperature (Table 2). Each colored line represents the changes in actual water volume collected for each site per day. Site 11 is an outlier on the first day

Day	Avg. Temperature (F)
1	64
2	66
3	66
4	67
5	75

Table 2: Average Daily Temperature per Day

The above sum was calculated by taking the sum of all 6 of the temperatures recorded per day (two in the morning, two at the half-way point, and two in the evening) and dividing them by 6 to get the average for the day. As temperature increased slowly as each day passed, there was little change in the actual volume of water collected (Figure 2).

The number of leaves bagged had a positive relationship with the actual water volume collected. As the number of leaves increased, the actual volume did as well. Figure 3 demonstrates this steady increase of water volume with the slight differences in number of leaves. The regression line had a slope of 1 showing the positive relationship between these variables. In order to further investigate the affect the number of leaves had on the actual water volume, I calculated the average actual volume of water per leaf per layer. This showed the layer of the site had a stronger positive relationship with the actual water volume than the leaf count (Figure 4).

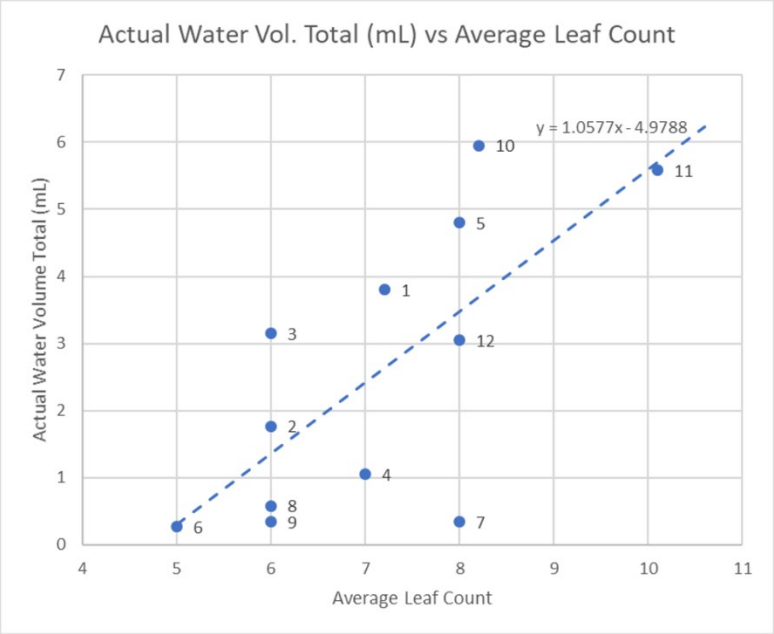

Figure 3: Actual Water Volume Total vs Average Leaf Count. Demonstrates the positive direct relationship the leaf count has with the water volume collected. When one increases the other does too.

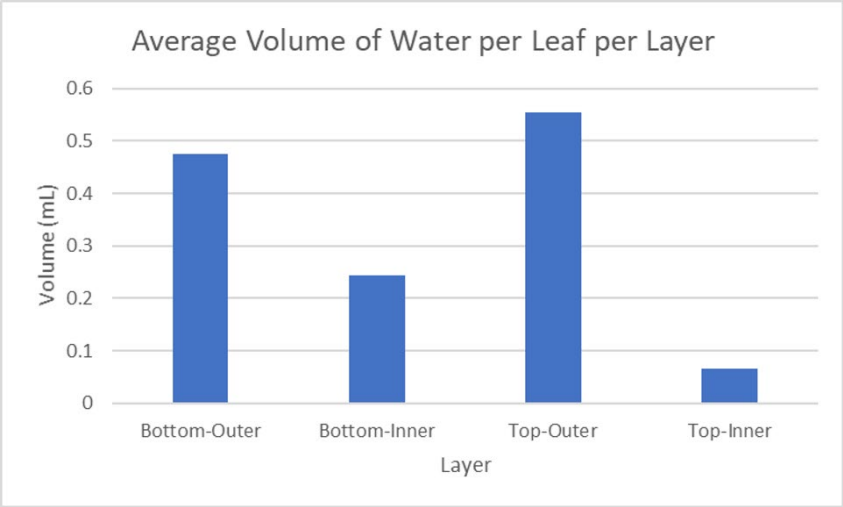

Figure 4: Average Volume of Water per Leaf per Layer. This shows the relationship between the average amount of water collected per leaf given the layer.

The layer of the sites had the largest influence on the actual water volume collected. The relationship between these two variables was also positive, but there is a clear visual statistical difference between T-O and T-I, B-O and B-I, T-O and B-O, and T-I and B-I (Figure 5). The estimated water volume from the calculated water weight is also demonstrated in Figure 5. This estimate and the actual water volume collected after debris was

removed show to be slightly different, but not in a significant manner, the estimate always being higher in value than the actual volume (Figure 5).

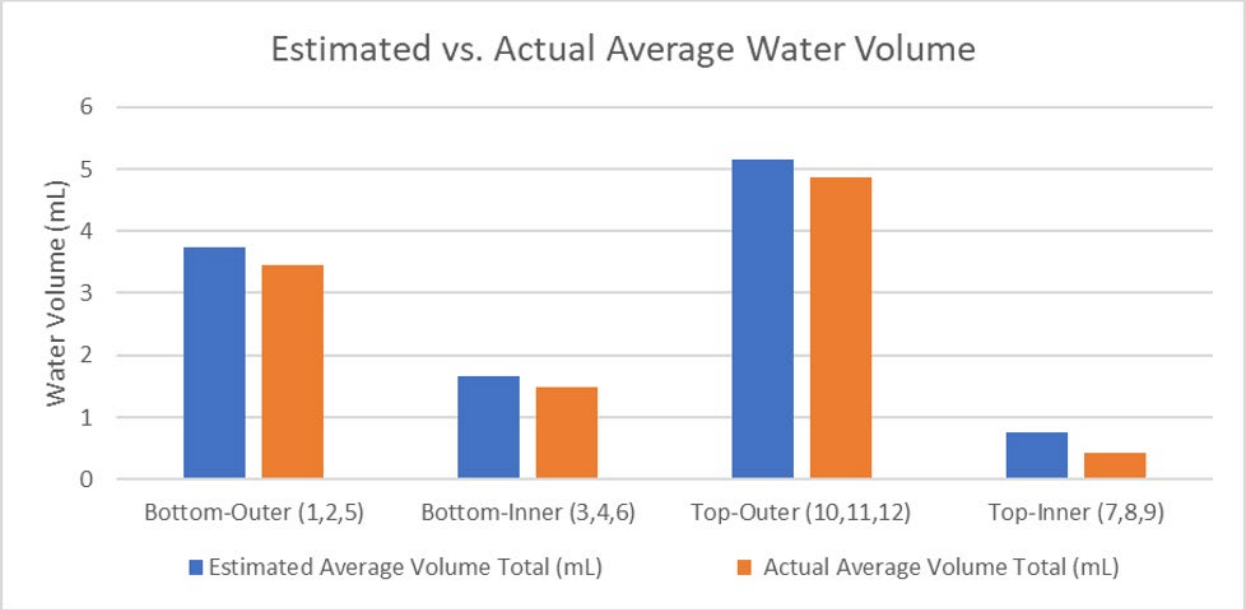

Figure 5: Estimated vs. Actual Average Water Volume. This graph compares the small difference between the estimated and actual water volume values, as well as the large positive influence the position of the tree the site was on with the aforementioned volumes.

Taking into account each of the four layers of the tree (T-O, T-I, B-O, B-I), there were a total of 15 samples available for each layer at the end of the 5 days. From this, I continued with a 2-sample t-test to test for statistical significance, using the appropriate hypotheses for each instance. I compared the differences between top and bottom, and outer and inner, leading to the following four possible sets of hypotheses (Table 3).

$H_{0,1}$: B-O ≤ B-I	$H_{A,1}$: B-O > B-I
$H_{0,2}$: T-O ≤ T-I	$H_{A,2}$: T-O > T-I
$H_{0,3}$: T-O ≤ B-O	$H_{A,3}$: T-O > B-O
$H_{0,4}$: T-I ≤ B-I	$H_{A,4}$: T-I > B-I

Table 3: Four possible sets of hypotheses that I would test. H0,1 refers to the null hypothesis in the first instance, HA,1 refers to the alternate hypothesis to be tested in the first instance, etc.

The goal for the collected data was to disprove each null hypothesis and support the alternative hypotheses. Given these hypotheses, one could easily perform the 2-sample t-tests, however, there was the restriction of having a limited sample size which made these results not statistically viable. Having more individual samples at each test site (something more easily tested with a larger scale experiment given more materials were available, a taller ladder at one's disposal, and a larger guava tree) would make a statistical significance test more reliable and relevant to the data at hand. This would decrease the standard deviation given more individual points of data in each sample, be more representative of the population (each site's layer) and prove more reliably the results shown above with more significance than possible here given the constraints of this experiment.

Discussion

The common guava tree is a well-built tropical tree with many medicinal properties in its leaves, health benefits in its fruit, and refreshing shade quality on a hot summer day (Gutierrez, Mitchell, and Solis, 2008). The *Psidium gujava* tree can endure high disturbances such as intense hurricanes, high dry or humid temperatures,

and extreme rainy seasons with high survival rates (Pommer and Murakami). Once I organized and graphed my results, it came to no surprise that a few degree differences in the average daily temperatures (F) had no profound effect on the transpiration rates of the resilient guava tree (Figure 2). There was a slight decline in the transpiration rate of the guava tree when the temperature steadily increased through the 5 days, but not a significant amount. Temperature has shown to have a significant difference in the transpiration rates of other tropical fruit trees, such as decreasing the rate of the mango tree as it increases, but this measurement of temperature was determined by how much direct sun exposure vs. shade the mango tree had (Léchaudel et al, 2013). This provokes the question whether temperature was the main determining factor of the declining mango transpiration rate or if direct sun exposure was.

The two significantly impactful factors in this experiment were the amount of leaves bagged in each site and the layer where the site was situated. The number of leaves contained in each site varied a little due to the structures of each chosen branch varying in density of leaves. Although the leaves seem to grow in pairs along the guava tree branch, they had different sizes and patterns of proximity to one another. On average I tried to have 6-8 leaves in total, but I had a few outliers in this pattern; and at the end of the experiment, a few leaves had fallen from the sites, but not enough to have a large difference in the data collected (1-2 leaves). Once this data was graphed, it was noticeable that more leaves led more transpiration, as was expected, but since the visual evidence on Figure 3 would have failed to support the original hypothesis, more analyses had to be made of the leaf count.

The next data analysis of the leaf data count again showed the positive relationship between the number of leaves and the average amount of water collected, but it mainly highlighted the layer of the site the leaf was on was the most important factor tested in determining how much water was released (Figure 4). This data supports the hypothesis by emphasizing how strong the influence of the layer of the site (T-O, T-I, B-O, B-I) was that it even stood out when compared to a stable positive relationship with a consistent upward trend. In previous experiments on tropical trees in their natural environments, greater leaf surface area has a positive relationship with transpiration as well, indicating that this pattern is not just present in guava trees (Mooney, Field, Vázquez-Yánes,1984). This adaptation has been made for several reasons in these tropical plants, one of them being the density of foliage the sun's light must get through in order to reach the plants in the lower canopies (Mooney, Field, Vázquez-Yánes,1984). This rainforest experiment agrees that sun exposure is a crucial element in levels of photosynthesis and transpiration (Mooney, Field, Vázquez-Yánes,1984), supporting my original hypothesis.

The strongest positive relationship in all the data I collected was between the transpired water volumes and the layer of the tree the site was on (Figure 5). The bottom-outer and the top-outer layers had the two highest amounts of water collected out of the four layers. This indicated that the high direct sun exposure these two layers had increased their rate of transpiration. The top-outer layer sites still had higher rates than the bottom-outer layer sites due to the shade caused by the top-outer layer's foliage blocking the direct sun exposure to the B-O layer. Both inner layers had significantly less transpired water than their outer-layer counterparts, which was predicted because these layers would have more obstacles in their way of direct sun exposure.

Most of the patterns in Figure 5 support my hypothesis except for one. Bottom-inner layer sites had more transpiration occur than top-inner layer sites. This was not an expected result at all. After many sleepless nights, I finally understood that this outlier to my results was only supporting my hypothesis more. This pattern emerged because the top-inner layer sites had a larger density of the foliage and other obstacles blocking direct sun exposure from passing through than the sparse B-I leaves did. This resulted T-I in having significantly lower numbers of water volume than all the other layers. Figure 5 provided the largest support for my hypothesis not only by demonstrating 3 out of 4 patterns I predicted in my hypothesis, but also by remaining consistent with the last data set comparison (T-I<B-I). The last data set comparison did not match

my last prediction in my hypothesis, but it emphasized the positive relationship between direct sun exposure and transpiration rates the most.

I could have improved by my experiment by having collected more data and having a taller ladder. I could have chosen more collection sites or have done the experiment one whole week instead of only 5 days, or both, to have had more data to analyze. Having more data would have allowed me to complete a successful 2-sample t-test and statistically show the significance of sun exposure levels and transpiration rates. Having a taller ladder would have allowed me to reach higher points in my guava tree, possibly collecting even more distinct data to compare layers of the tree than I collected this time around. Outside of those two main differences, my methods were simple and easy to carry out each day, so it was not difficult to remain consistent and it was easy to collect accurate data. There might be better tools and equipment for a researcher to use for this type of experiment, but none that I found in other transpiration research studies.

At the start of this experiment, I was curious on whether a human could survive off the amount of transpiration water collected for a few hours. I was sorely mistaken. The estimated amount of water necessary for an adult individual to survive is a little more than 1000mL per day, on my best day I collected only 40.9 mL of water (Day 2), which is only 4.09% of a survivable amount (Piantadosi, 2003); so in reality, setting up transpiration bags could possibly be lethal if you sweat out too much water during the process. This curiosity was brought on because of all the chaos that has happened this entire past year of 2020. The poor reaction large groups of people have had towards several severe situations, such as refusing to wear masks during the current pandemic, has reminded me of the irresponsible reaction we have had towards other serious issues such as the ozone layers and the resulting climate change. The ozone layer is a layer of unstable O_3 molecules that protect the Earth's surface from the sun's strong ultraviolet radiation that can lead to detrimental effects on our natural surroundings and humans as well (Rutledge et al, 2011). The current damage in the ozone layer has already shown to have drastic effects on the tropical climate zones by increasing the rainfall amounts drastically as well as decreasing the temperatures (Wu and Polvani, 2017). The guava tree being as resilient as it is, cannot be bred in many parts of the united states due to its sensitivity to lower temperatures (Pommer and Murakami). If a tree as disturbance tolerant as the *Psidium gujava* can face a high risk of damage by lowering temperatures, other less-resilient tropical trees will also be largely damaged by the declining tropical temperatures. An increase in sun exposure caused by an increased amount of damage to the ozone layer could also cause immense amounts of problems for tropical plants. If the significant affect the sun exposure had on the guava tree is like other tropical trees, increased sun exposure could lead to greater water loss in these species, which in turn could lead to drastically diminishing populations due to dehydration and slow adaptation of these species in relation to the rate of sun exposure increase.

Future experiments could help determine how we can improve living conditions for tropical autotrophs, as well as non-tropical ones, by having more testing done on how profoundly the effects of temperature and the level of sun exposure are for transpiration rates. As I was preparing for my research paper, I noticed there were few articles on transpiration and sun exposure levels, many were fixated on temperature. I believe more papers should be done on sun exposure levels and they should not only be compared with temperature-transpiration studies, but also there should be transpiration studies differentiating the two of them. One future experiment could test to see if sun exposure has a higher effect than temperature on a plants transpiration by comparing three species of plants, all with high levels of sun exposure in their native environments, but each of their native environments being drastically different temperatures. An example of this would look like: a plant from a tundra, another one from a desert and the last one from an open-spaced tropical environment (like the guava tree). Investigating other potential factors that influence transpiration would also aid in the attempt to understand and hopefully restore the living environments for the multitudes of primary producers that provide us with food, medicine, shelter, and countless other items we depend on for survival.

Conclusion

For 5 Southern Californian autumn days, I collected the water vapor released by the leaves of my *Psidium gujava* tree for 7 hours each day. After recording the volumes of water that were released into my clear plastic fish bags at each selected collection site, I compared the volumes day by day, top layer to bottom layer sites, outer layer to inner layer sites, number of leaves, and to average daily temperature. My findings demonstrated transpired water volume had the strongest relationship with sun exposure, as represented by the different site layers. The relationship between these two variables was positive, so when one increased the other did too (Figure 5). My original hypothesis was supported with the data I collected, with one exception. The top-inner layer sites released less water than the bottom-inner layer because I thought the top layer sites would have less obstacles blocking sun exposure. The further I investigated this matter, I realized that the top-inner layer had much more foliage and branches occluding these sites from the sun. The bottom-inner layer had slightly more sun exposure since the bottom was sparser than the top (Figure 1). This exception only strengthened the support for my hypothesis further. It is evident that higher levels of sun radiation exposure lead to a higher transpiration rates for a tropical tree, such as the guava tree *Psidium gujava.*

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