PLANT FUNCTIONAL TRAITS OF *CARAPA GUIANENSIS*, A WIDESPREAD TROPICAL TREE, ADAPTED TO LOCAL CLIMATE CONDITIONS AT TWO ELEVATIONS IN COSTA RICA

An Undergraduate Research Scholars Thesis

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ABSTRACT

Plant Functional Traits of *Carapa guianensis*, A Widespread Tropical Tree, Adapted to Local Climate Conditions at Two Elevations in Costa Rica

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Tropical rainforests are among the most biodiverse ecosystems in the world. However, plant species occurrences are highly dependent on localized topography, elevation, and climate. Convergent evolution has been studied extensively to identify key plant traits that vary along those gradients, which contributes to a greater understanding of how species adapt to climate variation. This project investigated leaf physiological and anatomical trait variation within a select tropical tree species, *Carapa guianensis* Aubl, which thrives in a wide range of elevations and climates within Central and South America. At two sites in Costa Rica, four leaves were sampled on each of three sample trees of *C. guianensis* growing at approximately 400-600 m (Texas A&M University Soltis Center) and approximately 830 m elevation (Pocosol Biological Preserve), in which we measured leaf photosynthesis and stomatal densities. We further assessed photosynthetic responses across a range of light intensities to determine the maximum potential photosynthesis of trees occurring at contrasting elevations. We found that stomatal density was higher at decreasing elevations, possibly as a means to compensate for less cloud cover. Furthermore, *C. guianensis* trees located in lower elevations had higher net photosynthesis rates (P_{Nmax}) than trees sampled at higher elevations, after accounting for differences in leaf

temperature. These findings suggest that lower elevations with higher light intensity can significantly drive critical plant processes. Hence, further experiments are needed, not only to explore plant functional traits in species adapted to occur across wide ranges of elevations, but climatic changes that drive them, especially in our current global scenario in which tropical forests are expected to have higher temperatures and longer dry periods.

DEDICATION

This thesis is dedicated to the mentors and students of the Texas A&M REU program and the professors of the Ecosystem Science and Management department who aided me in my academic pursuits and have inspired me to pursue a career in research.

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NOMENCLATURE

PAR	Photosynthetically Active Radiation
P_{gmax}	maximum gross photosynthetic rate
P_{Nmax}	maximum net photosynthetic rate
$P_{N(Imax)}$	maximum net photosynthetic rate obtained at $I = I_{max}$
I	photosynthetic photon flux density
I_{comp}	light compensation point
$I_{sat(n)}$	light saturation point at a specific percentile (n) of P_{Nmax}
I_{max}	light saturation point beyond which there is no significant change in P_N
$f_{(Icomp-I200)}$	quantum yield at the range between I_{comp} and I = 200 μmol photon m^{-2} s^{-1}
A_{net}	Net assimilation (photosynthetic rate)
R_D	Dark respiration

(Kaipiainen 2009, Lobo et al. 2013)

CHAPTER I

INTRODUCTION

Tropical forests are among the most biodiverse environments in the world (1971, Gentry 1992, du Toit et al. 2004) with many of their complex processes and internal interactions not understood (Malhi et al. 2010). Several defining features of this type of ecosystem are its varying environmental gradients and their effect on the life that has developed along them (Malhi et al. 2010). Gradients such as elevation, climate, and disturbance regimes vary significantly between microsites that make up tropical ecosystems and play critical roles in how plants develop, interact within a community, and adapt (Malhi et al. 2010). On a physiological level, environmental gradients have also been known to alter how plants function (Cornwell and Ackerly 2009). While these effects have been extensively explored in other ecosystems (Whittaker and Niering 1975, Franklin 1995, Callaway 1998, Malhi et al. 2010, Schöb et al. 2013), there is a considerable gap in literature analyzing the effects of environmental gradients on plant function and adaptation within the tropics (Malhi et al. 2010). Improving our understanding of these processes within tropical ecosystems is crucial for a number of reasons. Significant changes in microclimatic and topographic variation are common within these forest systems and can potentially give insight as to how biophysical processes of plants change not only with elevation, but also climate (Malhi et al. 2010).

These microclimatic variations often effect essential plant function by altering the major drivers of photosynthesis, light and CO_2 (Clark and Clark 1994, Griffin and Seemann 1996, Lloyd and Farquhar 2008). CO_2 assimilation by a plant's stomata allow for the Calvin Cycle to work in tandem with light reactions to carry out the photosynthetic process which yields the

sugars and O_2 we acknowledge as the products that fuel the majority of our ecosystems(Kirk 1994, Field et al. 1998, Clark et al. 2018). While this process is molecularly straight forward, external factors in a plant's surrounding environment often affect a plants ability to carry out photosynthesis (Teskey et al. 1995). These restrictions can come from nutrient availability, light availability, and temperature (Teskey et al. 1995, Loustau et al. 1999).

The ability of a plant to adapt to these external drivers as an individual within a single generation is denoted as its phenotypic plasticity (Bradshaw 1965). Phenotypic plasticity can be observed as the variation of one genotype and has been recognized to express itself as either morphological or physiological depending on what external factors are driving the change (Sultan 2000). For external factors effecting photosynthetic rates of plants, developmental plasticity has been explored in order to observe how plants react to differing environmental conditions (Sultan 2000).

A study conducted by (Oberbauer et al. 1985) explored the effects of CO_2 on two differing species, *Ochroma lagopus* and *Pentaclethra macroloba* which are native to Costa Rica and are a pioneer and climax species respectively. This experiment observed development of these plants as seedlings within controlled environments under constant intensities of light and increased CO_2 levels. For both plants, an increase in CO_2 yielded a measurable increase in plant biomass with less photosynthetically capable tissues and reduced photosynthetic outputs. A similar study conducted by (Ziska et al. 1991) explored the effects of increased CO_2 levels on the photosynthetic output of nine tropical plant species within Panama . By exposing nine different species to double their normal amount of CO_2 for three months this study reported an increase in both photosynthetic output and biomass for all C_3 plants tested. Responses of tropical plants to differing light intensities have also been explored. A study conducted by (Adamson et al. 1991)

analyzed how a shade-tolerant plant *Tradescantia albiflora* is able to acclimate to increased sunlight conditions and what morphological differences take place when this plant is grown in full sunlight. By growing separate *T. albiflora* under low and high light intensities, this study reported that *T. albiflora* grown under direct sunlight contained higher amounts of biomass than shade grown plants but had a lower capacity for photosynthesis.

While these studies focus on phenotypic responses of tropical plants under controlled environments, they do not study how changes of CO_2 and light alter plant development and function across an elevation gradient in nature. Recognizing this, we decided to utilized a widespread tropical plant *Carapa guianensis* for this study.

A commercially significant plant referred to as Royal Mahogany or Crabwood within Costa Rica (HARTSHORN and MAcHARGUE 1983, Hall et al. 1994, Dayanandan et al. 1999, Zuchowski and Forsyth 2007), *C.guianensis* is a late successional shade tolerant plant (Aparecido et al. 2017) whose range is documented as 700m (although we found it at higher elevations) within Costa Rica. A phenotypically plastic plant (Chazdon et al. 1996, Aparecido et al. 2017), *C.guianensis* is commonly found in intermittently flooded swamps and is utilized commercially as a pseudo mahogany for furniture creation. The oil produced from its seeds are also utilized for medicinal purposes (Zuchowski and Forsyth 2007). Some have considered *C. guianensis* and *C. nicaraguenensis* as separate species (Zuchowski and Forsyth 2007), but for the purpose of this study, we consider them synonymous.

Utilizing photosynthetic and stomatal density analysis across two distinct elevations within differing microclimatic sites along the altitudinal gradient of a novel tropical forest in Costa Rica, this study seeks to explore how *C. guianensis*, has adapted to microclimatic and elevational differences within a highly biodiverse and novel tropical environment in Costa Rica.

CHAPTER II

METHODS

Study Sites

This experiment was conducted at two sites that are both located within the Children's Eternal Rainforest, a tropical evergreen broadleaf pre-montane forest ecosystem consisting of 22,000 acres of protected forest in north-central Costa Rica (Langholz et al. 2000). The first study site is located at the Soltis Center for Research and Education located in the Alajuela Province (See Figure 1). At 10°22'59.84''N, 84°37'03.21" W, the Soltis Center sits within an elevation between 400m and 600m with a mean annual precipitation of approximately 4200 mm (Teale et al. 2014, 2017). Measurements for this site were conducted at the edge of the Children's Eternal Rainforest within secondary forest. The second site also located in the Alajuela Province, was located in at the Pocosol Biological Preserve at 10°21'05.64''N, 84°40'03.04" W, at approximately 830m above sea level with an annual rainfall of 4674 mm (Montenegro 2010) (See Figure 1). Measurements for the Pocosol site were conducted within primary forest.

For each study site, a total of three *C. guianensis* saplings were identified and tagged along hiking trails for data collection. Each sample tree had two leaflets from two separate leaves chosen for photosynthetic measurements. Careful selection for fully expanded green leaves receiving sunlight were made for each sample tree.

Figure 1. This map above displays both sample sites where data was collected.

Photosynthetic Measurements

During July of 2018, photosynthetic measurements from both sites (hereafter referred to as Soltis and Pocosol) were taken utilizing a Li-6400XT photosynthesis machine with a 6400-02B LED light source (Biosciences 2012) in natural environments with variable tree sizes. Light curve analysis was conducted on four different leaflets from each sample tree. Each light curve consisted of exposing all leaflets to PAR intensities of 100, 300, 400, 600,700, 900, 1100, 1200, 1300, 1500, 1700, 2000, 2300, 2500, 2600, and 3000 μmol photons m^{-2} s^{-1}. The duration of each PAR level was two minutes and each measurement would begin and stop at 1500 μmol m^{-2} s^{-1}. At each PAR level, photosynthetic activity was empirically measured by the Li-6400XT through leaf gas exchange measurements of CO_2. assimilation. A total of four light curves were conducted from three sample trees at each site from July 5th to July 13th, 2018.

Fixed parameters for each measurement consisted of a fixed flow rate = 500 mol s^{-1} CO_2 concentrations ≅ 400 μmol CO_2 m^{-2} s^{-1}**,** and leaf area = 6cm^2. All measurements were taken during the day time between the hours of 1000 and 1700. All measurements were conducted on a dry portion of each leaflet that, if containing any moisture, was dried before data collection was conducted. Due to the high percentage of humidity present at each study site, chamber levels were maintained between each measurement utilizing desiccant. By doing this, most measurements conducted were kept between 40 and 90 percent RH with several outliers occurring.

Upon completion of measurements, all data was manually reviewed and unusable data was identified and omitted based on negative photosynthesis readings (i.e. higher plant respiration than photosynthetic activity), false readings of conductance, and anomalous drops in light curve data that occurred due to unstable field conditions and issues with the Li-6400XT.

Once initial data omission was conducted, all data was run through nine non-linear regression models from (Kaipiainen 2009, 2013). The model with the lowest average standard error was chosen for further analysis to estimate key ecophysiological traits as described below. See Equation 1 and Figure 2 for a visual representation of the variables utilized within the model:

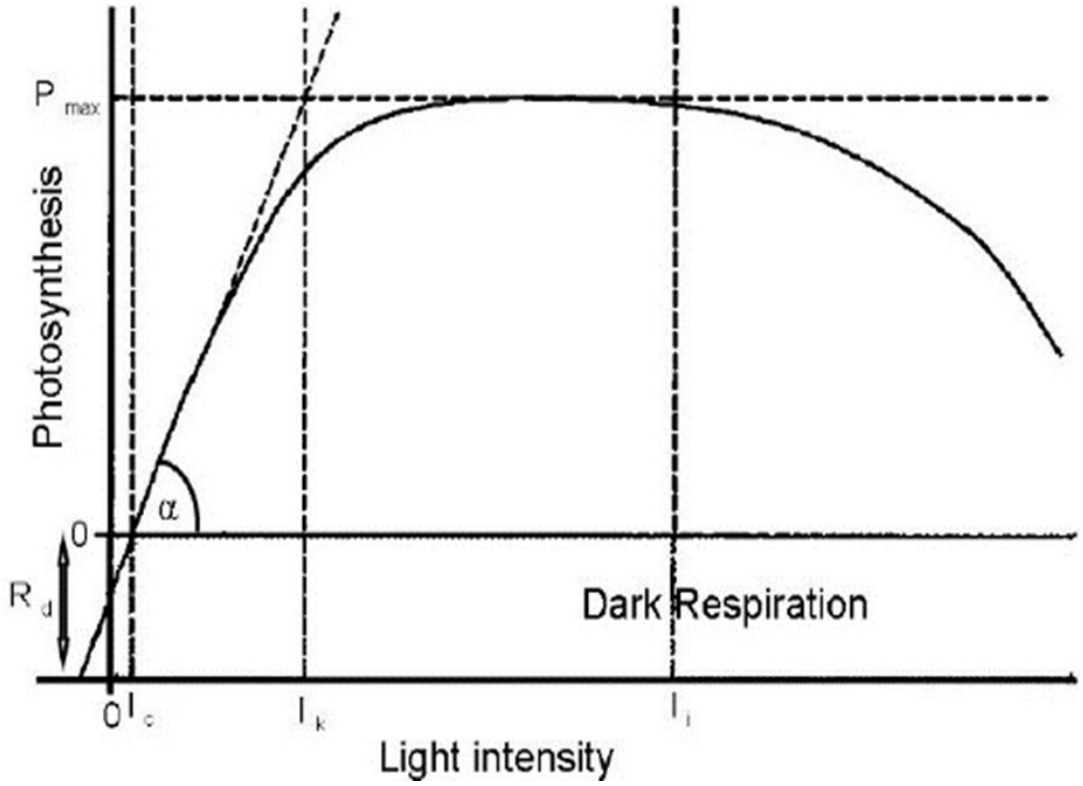

Figure 2. Graphical representation of a light curve concept with labeled parameters (Iluz et al. 2012)

$$\text{Equation 1: } P_N = \frac{I \times P_{gmax}}{I + I_{50}} - R_D$$

The model chosen for this experiment is based on the Michaelis-Menten rectangular hyperbola equation where P_N = net photosynthesis rate, P_{gmax} = maximum gross photosynthesis rate, I = photosynthetic photon flux density, $I_{(50)}$ = Light saturation point for $P_N + R_D$ equal to 50% of P_{Nmax}, and R_D = dark respiration (Kaipiainen 2009, Lobo et al. 2013). This model allowed for

data collected by the Li-6400 to be converted into parameters that allowed for the comparison of physiological function within plants. Upon completing this, data was then again manually reviewed and additional omission was conducted to ensure all measurements being utilized were an accurate representation of each site. Once all data was assessed, it was then compiled into an output table.

Other data taken during light curve analysis such as ambient temperatures for the sample leaflet and air were consolidated in a raw data output table.

Stomata Density Measurements

Utilizing methods from (Hilu and Randall 1984, Aparecido et al. 2017), stomatal density was measured through stomatal impressions using acrylic paint. Being that *C. guianensis* is hypostomatous (Aparecido et al. 2017), the underside of each leaflet used for photosynthetic measurements was collected and had films taken from the abaxial side of each leaflet. Due to film damage, a representative amount was selected from each study site where sub-samples from three trees were utilized from each site. From these stomata films, four 0.06 x 0.06 mm^2 high resolution images were taken utilizing a Zeiss Axiophot (Zeiss Axiophot, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) light microscope at 20x magnification paired with MetaView image capturing software at the Texas A&M Microscopy and Imaging Center (Aparecido et al. 2017). These images were then processed in ImageJ using the cell counter tool to count the number of stomata present. Once all stomata were counted, stomata density was found by scaling up the area captured by the microscope to $1mm^{-2}$.

Statistical Analysis

Statistical analysis of all measurements (see table 1) was conducted in excel and consisted of a one tailed two-sample t-test assuming unequal variances with a P-value of 0.05.

All measurements taken were compared between sites as averages and grouped accordingly per measurement before statistical analysis was conducted.

CHAPTER III

RESULTS & DISCUSSION

Photosynthesis Analysis

The non-linear regression model (Kaipiainen 2009, Lobo et al. 2013) utilized to process light curve data yielded 7 comparable parameters as seen in Table 1 and Figures 3 and 4. Maximum net photosynthetic rate obtained at $I = I_{max}$ ($P_{N(Imax)}$) was 1.45 times higher at the Soltis Center (7.2 ± 0.70 μmol CO_2 m^{-2} s^{-1}) than Pocosol (4.9 ± 0.60 μmol CO_2 m^{-2} s^{-1}, $P = 0.017$, Table 1). Maximum gross photosynthesis rate (P_{gmax}) was 1.60 times higher at the Soltis Center (8.5 ± 0.97 μmol CO_2 m^{-2} s^{-1}) than at Pocosol (5.3 ± 0.80 μmol CO_2 m^{-2} s^{-1}, $P = 0.015$, Table 1). Quantum yield at the range between I_{comp} and I = 200 μmol photons m^{-2} s^{-1} ($f_{(Icomp\_I200)}$) was 1.31 times higher at the Soltis Center (0.021 ± 0.002 μmol CO2 μmol^{-1} photons) than at Pocosol (0.016 ± 0.002 μmol CO2 μmol^{-1} photons, $P = 0.055$). Light saturation point for $P_N + R_D$ equal to 50% of P_{Nmax} ($I_{sat(50)}$) was 3.12 times higher at the Soltis Center (189.099 ± 78.763 μmol CO_2 m^{-2} s^{-1}) than at Pocosol (60.575 ± 5.661 μmol CO_2 m^{-2} s^{-1}, $P = 0.067$). Light saturation point for $P_N + R_D$ equal to 85% of P_{Nmax} ($I_{sat(85)}$) was 3.04 times higher at the Soltis Center (1016 ± 412 μmol CO_2 m^{-2} s^{-1}) than at Pocosol (354 ± 32 μmol CO_2 m^{-2} s^{-1}, $P = 0.065$). Light saturation point for $P_N + R_D$ equal to 90% of P_{Nmax} ($I_{sat(90)}$) was 3.03 times higher at the Soltis Center (1607 ± 650 μmol CO_2 m^{-2} s^{-1}) than at Pocosol (529 ± 51 μmol CO_2 m^{-2} s^{-1}, $P = 0.065$). Light saturation point for $P_N + R_D$ equal to 95% of P_{Nmax} ($I_{sat(95)}$) was 3.03 times higher at the Soltis Center (3378 ± 1364 μmol CO_2 m^{-2} s^{-1}) than at Pocosol (1114 ± 108 μmol CO_2 m^{-2} s^{-1}, $P = 0.064$) (Kaipiainen 2009, Lobo et al. 2013).

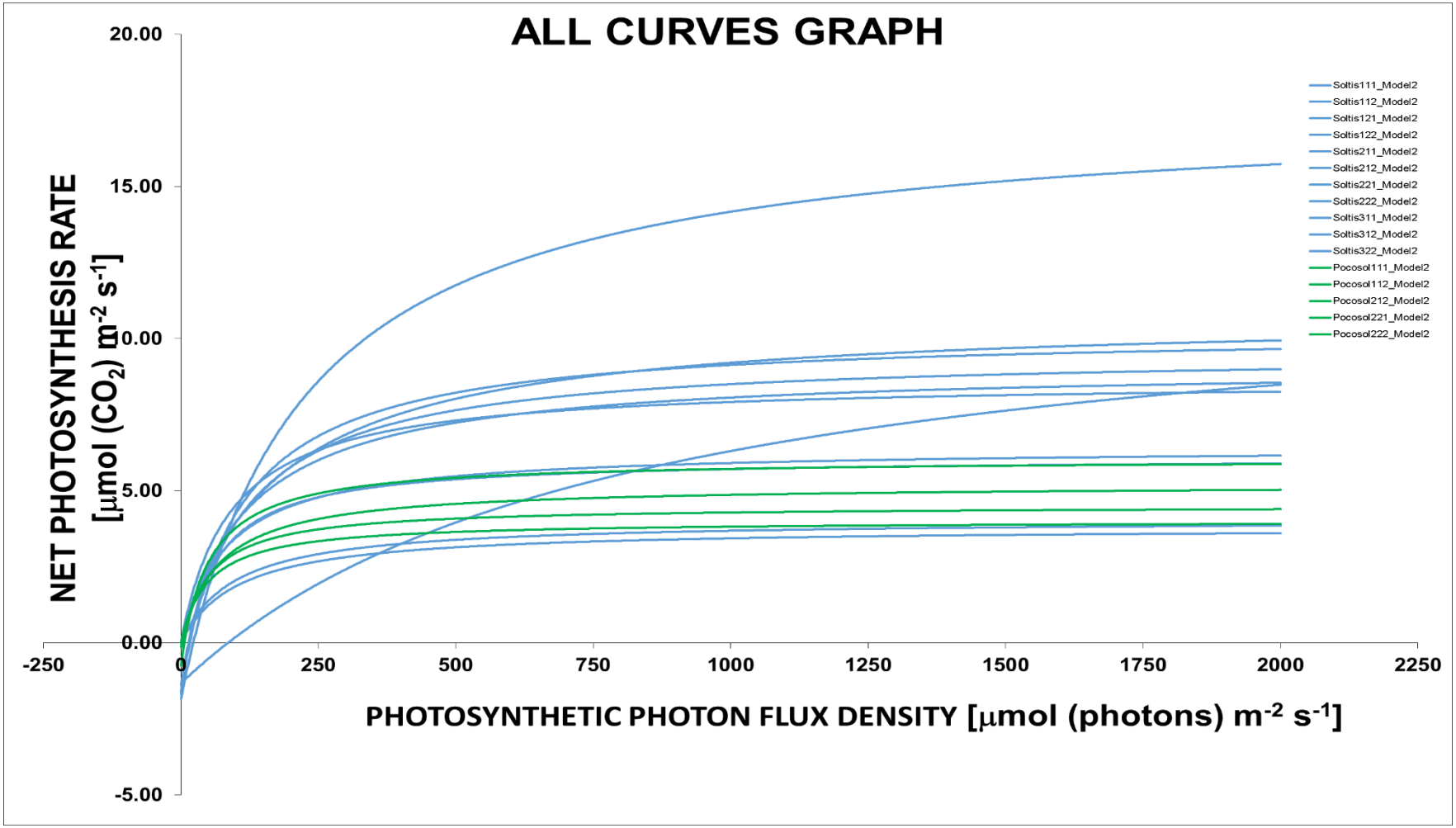

Figure 3. All data from both sites overlaid on the same graph depicting 15 points of light and respective photosynthetic output for that point after first round of data omission.

Table 1. Results of statistical analysis of compared parameters between high elevation (Pocosol) and low elevation site (Soltis Center), $P = 0.05$

Compared Variable	One-Tailed p-value	Soltis	± Standard Err.	Pocosol	± Standard Err.
P_{gmax} µmol m^{-2} s^{-1}	0.017	8.5	0.96	5.3	0.8
$P_{N(Imax)}$ µmol m^{-2} s^{-1}	0.015	7.2	0.7	4.9	0.6
$f_{(Icomp\_I200)}$ µmol CO2 µmol^{-1} photons	0.055	0.021	0.002	0.016	0.002
$I_{sat(50)}$ µmol m^{-2} s^{-1}	0.067	189.099	78.763	60.575	5.661
$I_{sat(85)}$ µmol m^{-2} s^{-1}	0.065	1016	412	334	32
$I_{sat(90)}$ µmol m^{-2} s^{-1}	0.065	1607	650	529	51
$I_{sat(95)}$ µmol m^{-2} s^{-1}	0.064	3378	1364	1114	108
Stomata Densities mm^{-2}	0.027	447.3	99.78	171.57	19.19

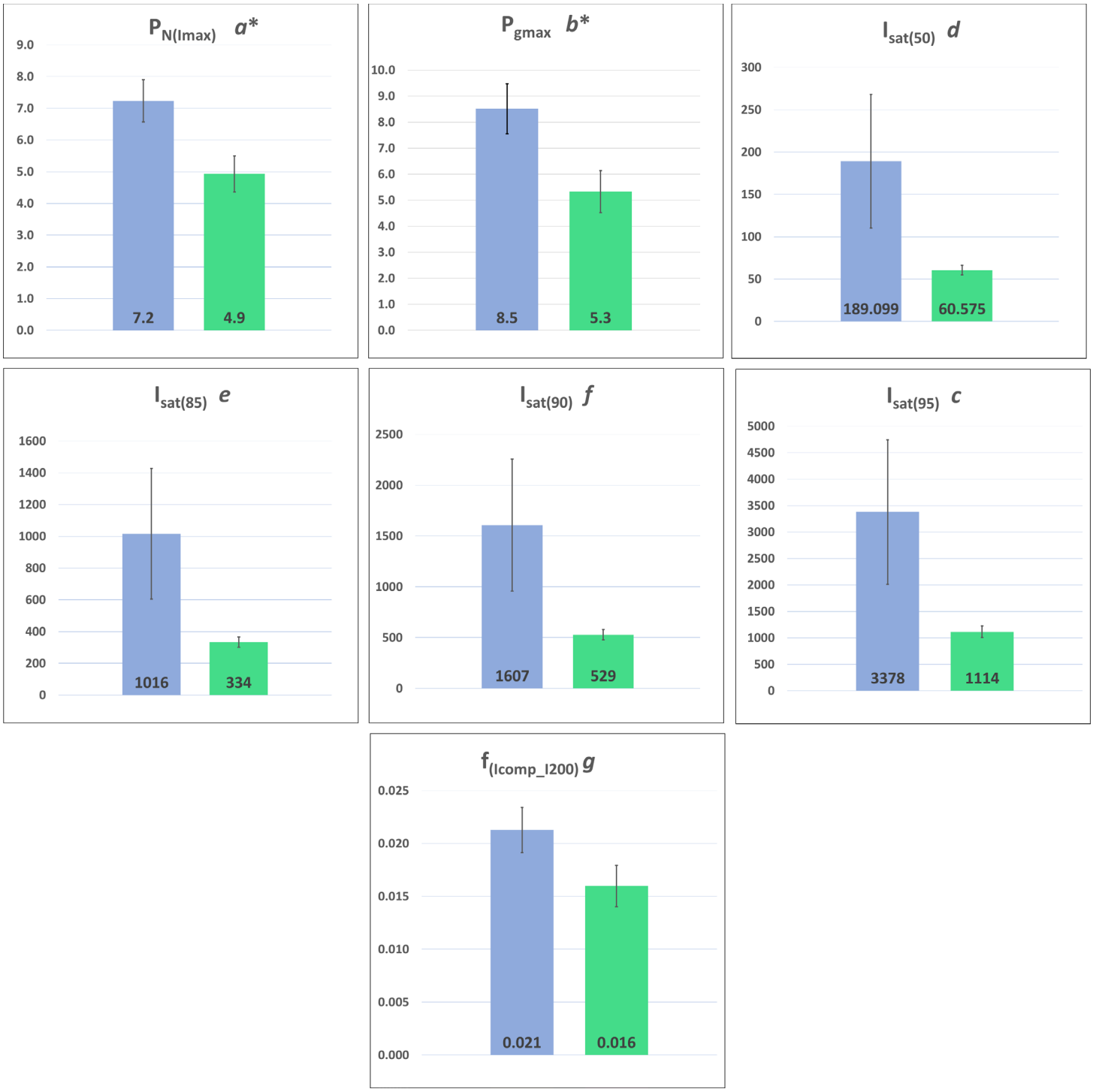

Figure 4. Graphs depicting light curve parameters (*a-g),* *= Significant

Stomata Density Analysis

Results for stomatal densities showed that our sample trees at Soltis had 2.61 times more stomata (447.30 ± 99.78 mm^{-2}) than Pocosol (171.57 ± 19.19 mm^{-2}, $P = 0.027$, Table 1 and Figure 5). Example of the images used for stomata analysis can be seen in Figure 6.

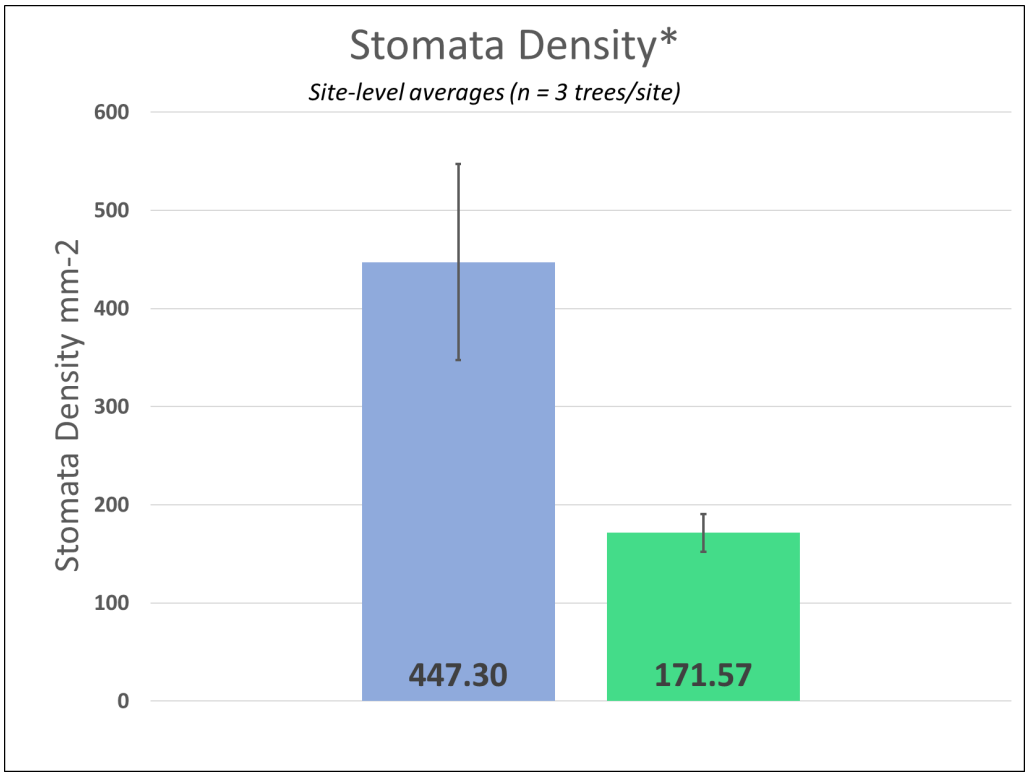

Figure 5. Stomata Densities of both Soltis and Pocosol scaled to mm2. * = Significant

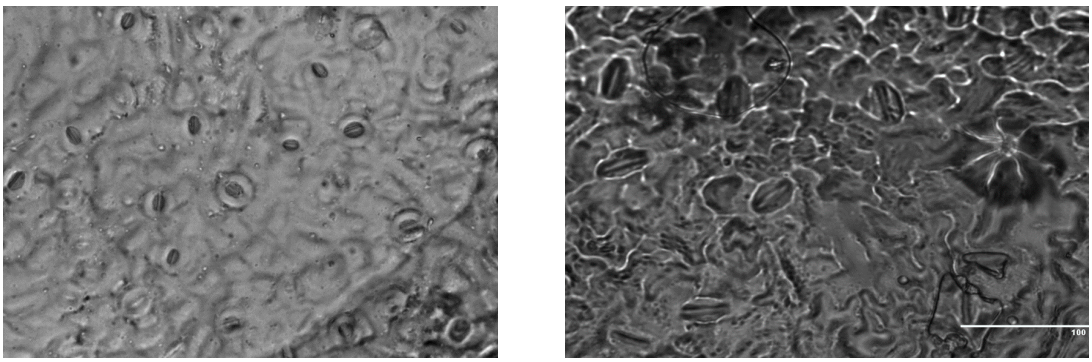

Figure 6. Example of imaging captured for stomata density analysis from Soltis (left) and Pocosol (right)

Leaf Temperature Analysis

The LI-6400XT also provided leaf temperature data from both sites. This allowed us to compare leaf temperature and evaluate if this parameter could potentially have had any impacts on the photosynthesis data. (See Figure 7). Leaf temperatures recorded from the Li-6400XT depict minimal variance in leaf temperature between either site. This suggests that leaf temperature itself does not have significant effects on the photosynthetic output of the sample trees we studied between our study sites.

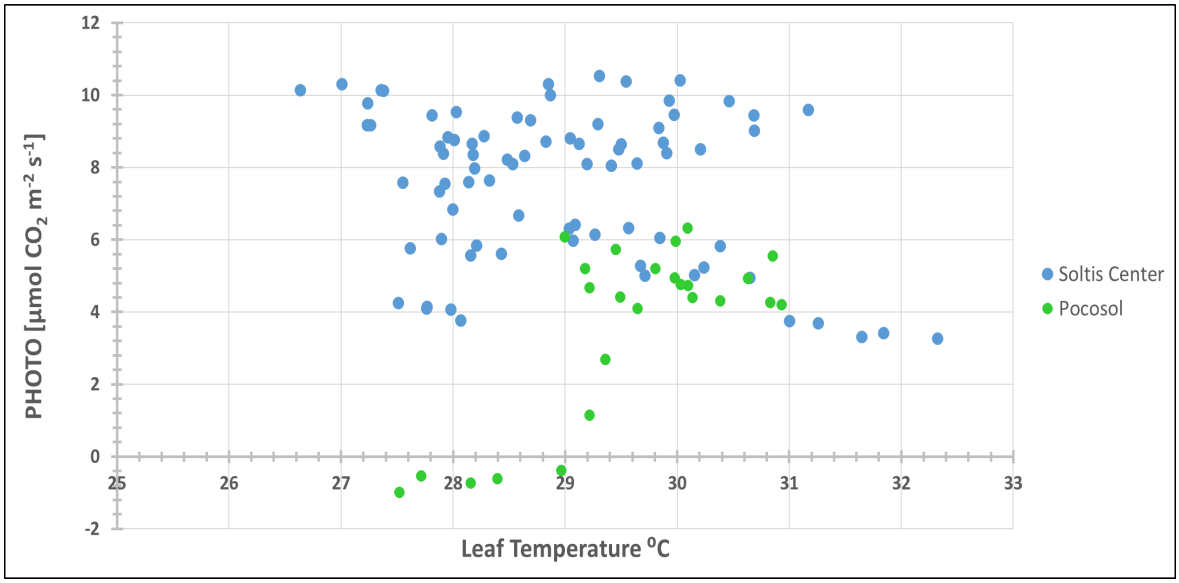

Figure 7. Leaf Temperature plotted against photosynthetic output (µmol CO_2 m^{-2} s^{-1})

Discussion

These results show us that there is are differences in the way that *C.guianensis* is able conduct photorespiration at different elevations. While (P_N) and (P_{gmax}) clearly show that sample trees within the Soltis Center site have a higher rate of photosynthesis rate, the data for light saturation at x% shows that for each measurement taken of I_{sat}, sample trees from Pocosol consistently yielded lower values for all parameters tested. This means that *C.guianensis* at

higher elevations not only had lower rates of photosynthesis but reached their peak amount of photosynthesis at lower light intensity.

While few studies have attempted to analyze the effect of an elevation gradient on single species adaptations, there are studies that provide support for our results as well as possible explanations for the differences in physiology observed between our sample sites.

Our study is not the first to study the rates of photosynthesis of plants across an altitudinal gradient. One study conducted by (Cabrera et al. 1998) observed the photosynthetic trends of two tropical species in the Andes, *Acacena cylindrstachya* and *Senecio formosus* along an increasing elevation gradient. By also conducting leaf gas exchange analysis, this study observed a negative trend between photosynthetic rates and their tested elevation gradients between both sample plants. Some main difference between this study and ours is that reductions in photosynthetic output were accredited to differences in either atmospheric or soil temperature between sites and differences in either of these measurements were correlated with leaf temperatures within their study plants.

The effects of CO_2 exposure on plants have also been studied and while not identified to be directly linked to altitudinal variation (at least within the scope/literature review of this study), a positive relationship between CO_2 and the photosynthetic rates of plants has been established (Ainsworth and Long 2005) disproving the initial hypothesis that prolonged exposure to increased CO_2 levels reduced photosynthetic rates of plants (Kramer 1981, Hogan et al. 1991, Ainsworth and Long 2005) Along with this, literature on stomatal development and the environmental factors that drive stomatal density have also been explored. A controlled laboratory study conducted by (Woodward 1987) observed the effects of increased CO_2 on 100 herbarium species and found that over half yielded lower stomatal densities when exposed to

elevated levels of CO_2. However, light intensity levels have also been shown to significantly affect stomatal numbers and size as well. A study exploring plant physiological differences in *Withania somnifera* (L.) under differing light intensities found that stomata numbers increased with higher light intensities as well as decreased in both numbers and physical size when light levels were increased beyond a certain light level (Lee et al. 2007). Upon reviewing these studies, hypotheses may be developed from our study results.

Being that atmospheric CO_2 between our two sites is unlikely to be significantly different, and its increased presence has been recorded to yield higher rates of photosynthesis within plants (Ainsworth and Long 2005), it seems unlikely that elevated CO_2 levels are the reasons for the distinct differences in photosynthetic activity of sample plants between our study sites. Instead, variation in light intensity and climate are more likely to be the main drivers behind our results and should be explored further within tropical environments (Cabrera et al. 1998, Lee et al. 2007)

While the afore mentioned studies do support our results and allow us to speculate over possible reasons for the relationship between elevation and tropical plant physiology, the limitations of this study should be acknowledged. Due to all measurements having been taken under natural conditions within the Children's Eternal Rainforest during the wet season, the standardization of measurements across an elevation gradient was perhaps the biggest obstacle of this study. Daily rainfall greatly reduced the rate at which measurements could be taken and the effects that it had on the activity of our sample plants was heavily represented in our data (i.e. negative rates of photosynthesis and false readings of conductance). Additionally, controls such as tree age, canopy level, and canopy gap could not be replicated across study sites and the small

sample size that could obtained is potentially responsible for the lack of significance across many of our tested parameters as well as any possible type II errors.

CHAPTER IV

CONCLUSION

The findings from this study show a correlation between an elevation gradient and plant physiology of *C.guianensis*. Our lower elevation site yielded the higher rates of gross maximum photosynthetic rates (P_{gmax}) and maximum net photosynthetic rate (P_{Nmax}) as well as stomata density. All other light parameters tested by the Li-6400XT yielded results that were consistently higher than our lower elevation site yet were not significantly different (most likely due sample size). Leaf temperature analysis from both sites suggest that leaf temperature had no effects on our sample data. While there are many variables that could have affected these measurements, i.e. canopy cover, weather, herbivory, etc., the drastic difference in stomata density and photosynthetic activity between study sites seems to allude to light intensity and climate having a drastic effect on the way our sample plants behaved. Whether this difference comes from a concrete relationship between altitudinal gradients and climate, varied light intensity in the forest canopy, or as a result of a lurking variable remains unknown.

Despite this, the results of this study succeed in showing how drastically plant function and adaptation can be altered along elevation gradients as well as how more emphasis should be placed in exploring more rigorous analysis of this phenomenon within tropical ecosystems. If such studies were completed across multiple functional groups within tropical ecosystems, a greater understanding of how plant function differs across an altitudinal gradient could be obtained and potentially provide us with more insight on the complex relationships between plants and other environmental gradients as well as how these gradients drive ecosystem processes.

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