ABSTRACT

The Changes in Stomatal Conductance in Response to Rising Atmospheric Carbon Dioxide Levels

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Ever since the emergence of the first plant species, plants have been altering their physiology in response to changes in external stimuli. One important factor for plants is the continual change in atmospheric carbon dioxide levels throughout Earth's history. Stomatal conductance is one plant characteristic that has been affected by the atmospheric carbon dioxide levels at short and long-time scales. Stomatal conductance is the diffusivity of water vapor and CO2 across the distance of the stomatal pore. To assess the stomatal conductance of plants over approximately a century, herbarium specimen from the Baylor Herbarium (BAYLU) were selected and scanned under a 3-D laser microscope. *Osmunda regalis* was selected to represent the monilophyes, *Pinus taeda* for the gymnosperms, and *Rubus trivialis* and *Prunus serotina* for the angiosperms. This study hypothesized that the angiosperms would exhibit greater plasticity in their stomatal conductance in response to increasing atmospheric carbon dioxide, which meant significant changes would be made in their stomatal physiology and/or density. Upon analysis, it was found that only *Rubus trivialis* exhibited significant changes in stomatal conductance in response to increasing atmospheric carbon dioxide. Further analysis on vein density, water use efficiency, and plant response to carbon dioxide levels were made based on the findings.

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Changes in Stomatal Conductance in Response to Rising Atmospheric Carbon Dioxide Levels

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CHAPTER ONE

Introduction

Plants are the foundation of life. The vital role they play in ecosystems, nutrition, medicine, etc. is the reason for their widespread study and attest to the need for further research, especially as the current world is changing due to the prevalence of human activity and its consequences. Climate change is just one of these consequences, as the result of the increasing carbon dioxide emission into our atmosphere. As land plants have been altering their physiology in response to external stimuli ever since the emergence of the first plant species, it is predicted that they could continue to change and evolve (Franks and Beerling, 2009).

Atmospheric Carbon Dioxide Levels

The rise in carbon dioxide levels is not a novel occurrence in the long history of earth. There were many fluctuations in atmospheric carbon dioxide levels during the Paleozoic period. Within the Paleozoic period, the Devonian period (~400-350 Mya) was a time of high atmospheric CO_2 levels (Berner, 1990). It was also in this period where the atmosphere experienced a rise in the oxygen levels. Studies reveal that this oxygenation is correlated with the diversification of vascular plants (Dahl et al., 2010). At the end of the Devonian period the atmospheric carbon dioxide levels dropped, resulting in a period of low atmospheric CO_2 levels during the Carboniferous period (~350-300 Mya). Plants in the Carboniferous period are argued to have been very productive as indicated by their

high maximal stomatal conductance (Wilson et al., 2020). The same study revealed that plants in this era compensated for their low vein length per area by increasing the diameter of their tracheids, cells of xylem that conduct water. It was found that tracheids of land plants of this era had four to 30 times the width of extant species. In the Triassic period of the Mesozoic era, there was a rise in the atmospheric carbon dioxide levels since the Cretaceous period. It was in the late Cretaceous period where flowering plants rose to dominance and in the early Cenozoic era where the second radiation of these land plants took place (Collinson, 2000). Throughout the Cenozoic, angiosperms continued to diversify due to global cooling and floristic changes that occurred in this period (Condamine et al., 2020). Evidence shows that due to the radiation of angiosperm species, gymnosperms experienced a decline in the rate of diversification during the Oligocene. In the present, atmospheric carbon dioxide levels are on the rise again due to exponentially growing anthropogenic carbon dioxide levels (Hofmann et al., 2009).

Phylogenies of Plants

The earliest monilophytes – a phylum of vascular, seedless plants – have been predicted to have evolved around 400 Mya, in the lower Devonian period (Pryer et al., 2004). It was during this time when a divergence event occurred, separating the group that includes modern day lycophytes from the euphyllophytes, a group which includes modern day monilophytes, gymnosperms, and angiosperms. From the euphyllophytes, another separation was made dividing the monilophytes, or seedless plants, from spermatophytes (seed-bearing plants). One important difference between these two

divisions is the mode of reproduction. As seedless plants, monilophytes reproduced via spores, which are produced in reproductive structures called sporangia. They have retained this form of reproduction, despite some variability, since their evolution around 400 Mya (Pšenička, 2020). The spermatophytes include the gymnosperms and the angiosperms. Gymnosperms have been calculated to originate within the upper Devonian period, approximately 350 Mya (Clarke et al., 2011). Angiosperms have been found to have diverged from the gymnosperms around the late Permian, approximately 275 Mya (Salomo et al., 2017). One difference between gymnosperms and angiosperms is that while angiosperms can produce flowers as reproductive organs, gymnosperms do not. However, a finding indicated that gymnosperms possessed genes that regulate flower development in angiosperms (Melzer et al., 2010). The presence of these genes proved there is a common ancestor between the two phylogenies and indicated that phylogeny alone may not be sufficient in studying the evolution of various traits. A different study also finds that measures of phylogenetic signals is not an ideal way of calculating the evolution of traits over time (Ackerly, 2009). It finds that phylogenetic signals do not give specific insights into the rate or total amount of trait diversification; instead, it only measures the relative similarities between distant relatives.

The Stomata and Stomatal Conductance

Stomata are microscopic openings on the surface of plants that enable gas exchange between the plant and its environment. This allows for carbon dioxide to be taken in and ultimately fixed into sugars in a process called photosynthesis. While the

stomate is open for the intake of carbon dioxide, loss of water via transpiration simultaneously takes place. Phylogenomic analyses reveal that stomata are ancient structures that were present in the common ancestor for all land plants (Clark et al., 2022). However, not all land plants are stomatous, as the divergence of bryophytes saw a downregulation of stomata, especially in liverworts, where there was a complete loss.

The stomatal apparatus consist of different cells, but the most prominent feature are the two guard cells, specialized parenchyma formed through a process that undergoes three precursor cells and involves asymmetric cell division (Nadeau and Sack, 2002). The first precursor cell is the meristemoid mother cell (MMC) and its asymmetric division produces the meristemoid and a larger sister cell. The meristemoid is the second precursor, which can divide a few more times before converting to a guard mother cell (GMC), the third and final precursor to guard cells. GMC, unlike MMC, divide symmetrically to produce two guard cells, which ultimately border the stomatal pore. Another study revealed the presence of stomata on the seed coat of some species, which suggest stomatal development can occur as early as seed development (Jernstedt and Clark, 2023). Another component of the stomatal apparatus are subsidiary cells, which are usually adjacent to guard cells and are morphologically distinct from other epidermal cells (Gray et al., 2020). Some of the predicted functions include elevating the guard cells from the epidermal surface, ion and water movement, and stomatal movement.

The opening and closing of the stomatal pore allow the plant to control the rate of gas exchange and thus the rate of carbon dioxide intake and water loss from the plant.

The diffusivity of CO_2 and water vapor across the distance of the stomatal pore is called the stomatal conductance. The opening and closing of the stomata are the result of changes in turgor pressure in the guard cells (Zeiger, 1983). The change in turgor pressure is caused by osmotic changes induced by moving ions in and out of the guard cells. Potassium ion was found to have the main role in influencing the osmotic gradient. The increase in potassium ions in guard cells during stomatal openings indicate that an influx of this ion leads to an influx of water, which increases the turgor pressure and opens the stomatal pore. Guard cell deflation caused by the efflux of water leads to stomatal closing. Some stimuli that affect stomatal opening and closing include blue light and abscisic acid (ABA) signaling. A study showed that under blue light, or a lower light intensity, the photosystems of guard cells exhibited a photoresponse that influenced stomatal opening and closing (Zeiger, 1984). It was also found that abscisic acid activates the release of calcium ions, which in turn activates calcineurin, a calcium ion-activated protein phosphatase (MacRobbie, 1998). Calcineurin was found to have deactivated potassium ion channels that are involved in stomatal opening. Thus, in the presence of ABA, stomatal closing was favored. Another ABA-induced mechanism of stomatal closure involves the activation of receptor-like kinase GHR1, which in turn activates SLAC1 anion channels to induce an anion current across the guard cell, which closes it (Engineer et al., 2016). The evolution of ABA-induced stomatal closure has been argued to have occurred around 360 Mya, after the divergence of ferns (Cai et al., 2017). Thus, it was proposed that the guard cells of ferns and lycophytes lack responsiveness to ABA signaling, which are exhibited by seed-bearing plants. Instead, it was found that ferns

close their stomata passively, induced by the dehydration of the guard cells (Brodribb and McAdam, 2017). This would mean the gymnosperms and the angiosperms would have better water-conservation capabilities during droughts.

Detection of Carbon Dioxide

As previously mentioned, the stomata can open and close in response to external stimuli to maximize carbon dioxide intake and minimize water loss. Carbon dioxide is another of these stimuli that can be detected by the plant as internal carbon dioxide level. CO_2 sensitivity refers to the response of stomatal opening and closing based on signaling mechanisms that involves factors such as carbonic anhydrase (Engineer et al., 2016). Carbonic anhydrase is an enzyme that catalyzes the conversion of one water molecule and one carbon dioxide molecule into bicarbonate and a proton. The bicarbonate and proton are used as secondary messengers, and a high enough concentration has been shown to activate anion channels. The activation of anion channels induces stomatal closing by creating a concentration gradient that favors the outflow of water from the guard cells.

Dimensions of the Stomata

Stomatal conductance is the diffusivity of gas molecules across the stomatal pore to the site of assimilation within the plant (Franks and Beerling, 2009). Stomatal conductance is determined by the stomatal density and the stomatal size, which are

determined by its dimensions. As this paper studies the sensitivity of stomatal conductance in response to rising atmospheric carbon dioxide levels, it is important to understand the stomatal dimensions that effect its conductance. The stomatal dimensions that will be covered are stomatal pore depth, stomatal pore length, stomatal pore width, guard cell length, and guard cell width. Stomatal pore depth is the distance between the atmosphere and the interior of the leaf, specifically the spongy layer, through the pore that the gas molecules must travel across. Stomatal pore length is the vertical distance between the two meeting points of the guard cells. Stomatal pore width is the horizontal distance between the two inner edges of the guard cells. Stomatal pore length and width determine the stomatal pore area, which is positively correlated with stomatal conductance. Guard cell length is the vertical distance from the furthest two points of the guard cells. If assuming an ellipsoidal shape, it would be the length of the major axis. Guard cell width is the horizontal length between the edge of the stomatal pore and the outer edge of the stomata. Although there are various stomatal shapes, the species that I studied specifically assumed an ellipsoidal shape, with one axis longer than the other.

Stomatal Conductance

As previously mentioned, stomatal conductance is the diffusivity of gas molecules across the stomatal pore. It is through this pore where the exchange of gas molecules, intake of carbon dioxide and loss of water vapor, occurs. When the stomata are open for the intake of carbon dioxide, they simultaneously lose water to the environment through transpiration. A few factors determining the stomatal conductance are the stomatal

dimensions and density. Stomatal pore depth is inversely related to stomatal conductance, as increasing the stomatal pore depth is increasing the distance and the time it takes for gas molecules to travel across (Franks and Beerling, 2009). Stomatal pore length, stomatal pore width, guard cell width, and guard cell length are all directly related to stomatal conductance, as increasing the area of the pore would allow more gas molecules to travel across. Stomatal density is also directly related to stomatal conductance, as increasing the number of available pores for the gas molecules to pass through would increase stomatal conductance.

Importance of Stomatal Conductance on Plant Gas Exchange

A higher value of stomatal conductance indicates a greater intake of CO_2 from the atmosphere into sites of assimilation. A lower value of stomatal conductance would indicate the opposite: lesser intake of CO_2 to sites of assimilation. Thus, stomatal conductance is a limiting factor on the plant's photosynthetic capabilities and rates of assimilation (McElwain et al., 2016). However, this would also mean that a higher stomatal conductance value would increase rate of water loss while a lower stomatal conductance value would decrease the rate of water loss. Therefore, plants must balance stomatal size and density within the theoretical maximum to improve water-use efficiency (Franks and Beerling, 2009). The study of this balance between size and density in determining stomatal conductance and its relation to carbon dioxide levels has been used to better understand the responses of stomatal physiology and the mechanism of its evolution.

Hypothesis

For this study, I hypothesized that all plants would adjust their stomatal dimensions and density to decrease stomatal conductance in response to increasing atmospheric carbon dioxide levels. However, as changes in maximal stomatal conductance were shown to have significant discrepancies between ferns, gymnosperms, and angiosperms I anticipate *the angiosperm species* will exhibit the greatest change in stomatal conductance compared to ferns and gymnosperms (Klein and Ramon, 2019). Also, as stomatal conductance is a balance between minimizing water and maximizing carbon dioxide intake, plants with greater vein density will exhibit greater response to rising atmospheric carbon dioxide levels than those with smaller vein densities(Franks and Beerling, 2009).

CHAPTER TWO

Methods and Materials

Estimation of Atmospheric Carbon Dioxide Levels

Records of atmospheric carbon dioxide levels were retrieved from Mauna Loa Observatory, which measured CO_2 concentration directly from air measurements since 1958 (Sundquist and Keeling, 2009). Atmospheric carbon dioxide levels prior to this date were estimated from CO_2concentration from gas captured in ice cores. The data from these sources were used to determine the atmospheric carbon dioxide levels throughout the time frame of this research which ranged from 1900's to 2010's. During this time frame, an exponential rise in atmospheric carbon dioxide level was observed due to an increase in anthropogenic CO_2 emission. This significant rise was observed since the industrial revolution which began around the 1800's (Hofmann et al., 2009). However, the time frame of the research was limited by the number of herbarium specimens available in the Baylor herbarium.

Selecting Herbarium Specimens

All of the specimens considered for data collection were available from the Baylor herbarium (BAYLU). The herbarium specimens were selected based on the range of their collection dates and the distribution of available specimens within the research time period. Specifically, species with specimens from each decade since the 1900's to the 2010's were selected to identify any progression in stomatal expression. Although the exponential rise in carbon dioxide levels began since the industrial revolution in the

1800's, the herbarium specimen only date as far back as 1840's to the present. However, this range was further truncated to the 1900's because the species that were collected in the 1840's did not have specimens collected throughout the rest of the research time period, preventing examination of any progression in changes in stomatal dimensions. The year each specimen was collected and the number of specimens from the respective year were recorded. Then, each specimen was graphed against the year it was collected in order to characterize the sampling frequency across the research time period.

Ideally, the species with at least one specimen for each decade of the research time period would be selected for further observation. The selection process was carried out for most of the available plant family in each phylogeny: monilophytes (ferns), gymnosperms and angiosperms. The selection of monilophytes and gymnosperms was limited by the number of species with at least two specimens available across the research period. For the monilophytes, there were two species that had nearly one sample for each decade of the research time frame of 1900's to the present. For the gymnosperms, there was only one species that nearly had one specimen per decade. However, for angiosperms, I initially identified six species with sufficient number of specimens distributed across the time period, meaning these species had at least one specimen collected from each decade from 1900's to the present or very close to this distribution. Thus, there was a greater number of angiosperm specimens available to provide a broader observation of the similarities and differences in stomatal conductance within this phylogenetic group.

Microscopy

A 3D Laser Scanning Microscope (Olympus LEXT 3000, Shinjuku Monolith, 3-1 Nishi-Shinjuku 2-chome, Shinjuku-ku, Tokyo, Japan) was selected to scan the herbarium specimens. For each herbarium specimen, images were taken on 10 different leaves that were selected at random. When the specimen did not contain this many leaves, samples were retrieved from repeating leaves to gather at least ten samples from each specimen. Also, only the leaves with their abaxial surface exposed were sampled which is where the majority of stomata were located.

The images were taken in a cuboidal frame, each side having a length of 259 micrometers (μm) which gave an area of 67,081 μm^2. The area of the image between the two sides of the frame was later used to calculate stomatal density. The height of the image frame was dependent on the depth of the sample leaf and, thus, was variable for each sample.

Stomatal Measurements

After counting the number of stomata in each image frame, one stoma was selected at random for a closer measurement of its dimensions. These measurements for each image were made using the Olympus OLS5100 Analysis Application. For each stoma, a single digital line was drawn through the middle of the major axis using the 'line' tool assuming an ellipsoidal shape of the stoma (Figure *1*). Another line referred to as the minor axis was then drawn digitally on the image, perpendicular to the first. By drawing these lines, the analysis application scans the surface the specimen for a graph of

is topography along the line. These two lines were used to measure the depth of stomatal pore, length of stomatal pore, width of stomatal pore, length of guard cells, and width of guard cell in the respective order. Individual dimensions were measured not only to calculate the maximal stomatal conductance but to also identify which component of stomatal dimension expressed the most plasticity.

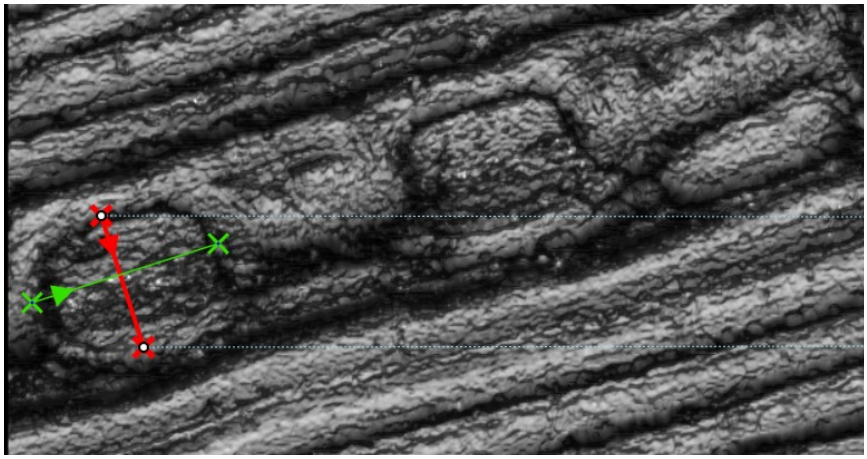


Figure 1 – The green line is the major axis of the assumed ellipsoidal shape of the stomata. The red line is the minor axis.

Stomatal pore depth was determined using the height measuring tool in the analysis application. For each image, depth was measured from the highest point on the guard cell to the deepest part of the stomatal pore. The deepest part of the stomatal pore was identified from the scanned topography of the specimen as an invagination between the guard cells. However, in gymnosperms, the stomatal pores were surrounded by guard cells with ‘lip-like’ structures that were identified as vertical protrusions extending from

within the stomatal chamber. For these species, the deepest surface was the area, between the lip-like protrusions. Guard cells of monilophytes and angiosperms did not possess this structure.

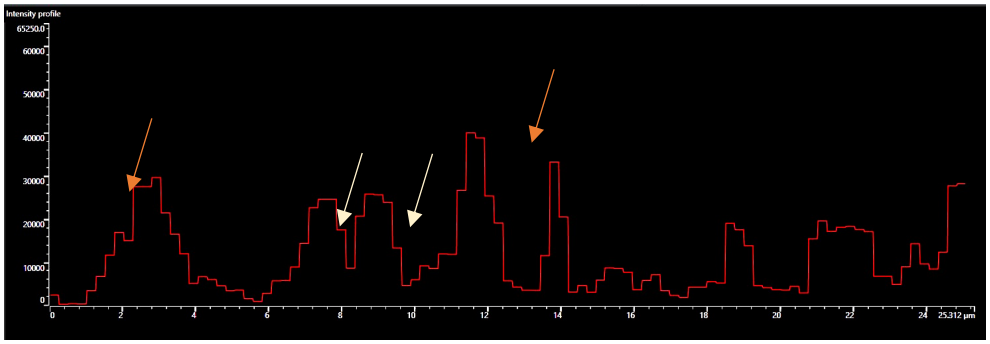


Figure 2 – This represents a scanned topography of *Pinus taeda* (pita_a_048573). This figure shows the lip-like projections (indicated by the yellow arrows) that lie between the border of the two guard cells (indicated by the orange arrows).

Stomatal pore length was evaluated as the vertical distance from the ends of the stomatal pore. It was measured along the major axis from the points where the guard cells merged at the top and bottom of the pore. Similarly, stomatal pore width was determined by the horizontal distance of the pore measured along the minor axis from the inner edge of one guard cell to that of the other. In gymnosperms, the stomatal pore was measured from the edge of one lip-like projection to the other.

Guard cell length was quantified as the vertical distance from the long ends of imaged guard cells measured along the major axis from the ends of the guard cells. Guard

cell width was measured as the horizontal distance along the minor axis from the outer border of one guard cell to the inner border of the same guard cell that borders the stomatal pore. The guard cell that was more clearly defined on the topography was selected for the measurement.

All measurements of stomatal dimension were made in micrometers (μM) and in the following order – stomatal pore height, stomatal pore length (along major axis), stomatal pore width (along minor axis), guard cell length (along major axis), and guard cell width (along minor axis) – for all specimens from the three phylogenies.

Calculation of Stomatal Properties

Using the number of stomata counted per image and the area of the image frame, the density of stomata was calculated. Next, stomatal pore length and pore width was used to estimate the maximum area of the open stomatal pore (a_{max}) in μm^2 which was calculated as $\pi(\frac{P}{2})^2$ where p is the length of stomatal pore. These specific measurements were made to calculate the maximal stomatal conductance (g_{wmax}). The maximal stomatal conductance was calculated from the equation (Franks and Beerling, 2009):

$$g_{wmax} = \frac{d}{v} \cdot D \cdot \frac{a_{max}}{(l + \frac{\pi}{2}\sqrt{a_{max/\pi}})}$$

where d is the diffusivity of water vapor in air ($m^2 \cdot s^{-1}$), v is molar volume of air ($m^3 \cdot mol^{-1}$), D is the stomatal density, and l is the stomatal pore depth. With each specimen, approximately ten samples were collected. Some specimens had less than ten stomatal samples if most of the stomata expressed were obstructed by a stomatal plug or fungi.

The measurements of these stomatal samples were averaged to calculate a single maximal stomatal conductance value per specimen. Finally, stomatal size, S was obtained by the product of guard cell length and guard cell width collected for each image. These values were also used to assess whether the expression of S changed throughout the research time frame.

Because the calculated stomatal conductance value was found to be sensitive to stomatal pore depth values, stomatal conductance was calculated using three different stomatal pore depth values. The first calculations used the measurements made directly from the images. The second calculation was made using the mean value of the stomatal pore depth value of each of the species, which was done to account for any measurement errors. The third calculation made used a predicted stomatal pore depth value. Assuming the "guard cells [inflated] to a circular cross-section" (Franks and Beerling, 2009), the pore depth value was estimated to be equal to the guard cell width. The predicted value of pore depth was used too because the herbarium specimen was pressed and dried before mounting, which meant the stomatal pore could have been compressed.

CHAPTER THREE

Results

Selection of Herbarium Specimens

From the herbarium, *Osmunda regalis* and *Polypodium polypodioides* were selected to represent the monilophytes. However, *P. polypodioides* could not be considered due to unclear images of the stomatal dimensions. *Pinus taeda* was selected for the gymnosperms. For monilophtes and gymnosperms, the number of specimens was limited by their availability in the herbarium. The species not chosen for measurement lacked sufficient numbers of specimens within the time frame of interest. *Quercus stellata, Q. falcata, Q. virginiana, Crataegus viridis, Prunus serotina,* and *Rubus trivialis* were selected for the angiosperms. However, *Q. stellata*, *Q. virginiana*, and *C. viridis* could not be used for imaging because the view of the stomata were obstructed by trichomes, hair-like projections on the surface of leaves. *Osmunda regalis* had 31 samples, *Polypodium polypodioides,* had 36, *Pinus taeda* had 56, *Quercus stellata* had 69*, Quercus falcata* had 33*, Quercus virginiana* had 33*, Crataegus viridis* had 29*, Prunus serotina* had 52*,* and *Rubus trivialis* had 73.

Data Summary

The calculated mean and standard deviation values are shown in

Table *1*. These data indicate that of the four species, *O. regalis* had the greatest mean pore depth (3.24 μm), mean pore length (26.63 μm), mean guard cell length (45.49 μm), and mean guard cell width (10.64 μm). On the other hand*, R. trivialis* had the lowest

mean pore length (10.09 μm), mean guard cell length (16.45 μm), and mean guard cell width (4.49 μm). However, the small stomatal dimensions of *R. trivialis* are contrasted by this species having the highest stomatal density (17.05×10^{-7} stomata/m^2) out of the four species. With the smallest mean pore depth and greatest stomatal density, *R. trivilalis* was calculated to have the greatest mean stomatal conductance (3.28 mol m^{-2} s^{-1}). Also, the four species did not have equal sample sizes or equal distribution across the hundred-year time period (

Table *2*; Table *3*). This may have led to some discrepancy in the results, as it could have led to unequal variances between species.

Table 1 – Mean and one standard deviation values are shown for all stomatal characteristics measured for each species. Mean of stomatal conductance is measured.

	Osmunda regalis	*Pinus taeda*	*Rubus trivialis*	*Prunus serotina*
Number of plants	21	21	18	15
Number of stomata	211	77	142	150
Mean of Pore Depth (μm)	3.24	2.24	1.80	3.14
Mean of Pore Length (μm)	26.63	13.40	10.09	14.31
Mean of Pore Width (μm)	9.33	2.46	4.81	4.59
Mean of GC Length (μm)	45.49	21.71	16.45	21.70
Mean of GC Width (μm)	10.64	3.91	3.65	4.49
Mean of Stomatal Density (stomata/ m^2)	72382036.0	87495178.5	586882617.1	170540093.3
Mean of Stomatal Conductance (mol m^{-2} s^{-1})	0.97	0.35	3.28	0.97
Mean of Stomatal Conductance with mean of all pore depth values within the speices (mol m^{-2} s^{-1})	0.95	0.34	3.08	0.93

Mean of Stomatal Conductance with pore depth value estimated from pore width (mol m^{-2} s^{-1})	0.64	0.27	3.09	0.83
SD of Pore Depth (m)	1.31	1.56	0.58	1.16
SD of Pore Length (μm)	2.89	3.26	2.03	1.91
SD of Pore Width (μm)	1.84	0.90	1.06	1.11
SD of GC Length (μm)	5.14	4.62	3.55	2.67
SD of GC Width (μm)	1.65	1.04	0.84	1.19
SD of Stomatal Density (stomata/ m^2)	21056099.7	23126034.9	122580851.3	44490031.7
SD of Stomatal Conductance (mol m^{-2} s^{-1})	0.42	0.15	1.26	0.42
SD of Stomatal Conductance with avg pore depth (mol m^{-2} s^{-1})	0.38	0.12	1.16	0.41
SD of Stomatal Conductance with predicted pore depth (mol m^{-2} s^{-1})	0.26	0.08	0.99	0.32

Table 2 – The number of samples scanned under the 3D laser microscope per decade for each of the four species.

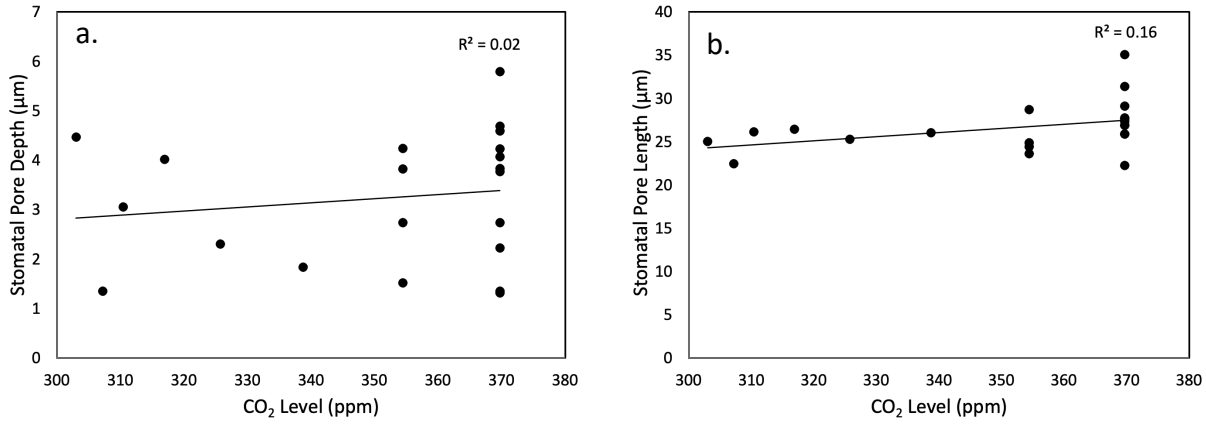
Number of Samples	*Osmunda regalis*	*Pinus taeda*	*Rubus trivialis*	*Prunus serotina*
1910	0	1	1	0
1920	1	0	3	0
1930	1	0	2	2
1940	1	0	2	1
1950	0	0	1	2
1960	1	0	3	2
1970	1	1	0	1
1980	1	2	2	2
1990	4	9	2	2
2000	11	5	2	2
2010	0	0	0	1

Table 3 – The number of stomata scanned under the 3D laser microscope per decade for each of the four species.

Number of Stomata	*Osmunda regalis*	*Pinus taeda*	*Rubus trivialis*	*Prunus serotina*
1910	0	4	10	0
1920	11	0	18	0
1930	10	0	20	20
1940	10	0	11	10
1950	0	0	5	20
1960	10	0	18	20
1970	10	2	0	10
1980	10	11	20	20
1990	40	31	20	20
2000	110	28	20	20
2010	0	0	0	10

Osmunda regalis

Low correlation was found between all the stomatal dimensions and atmospheric carbon dioxide levels for *O. regalis (*Figure 3). No stomatal characteristics for *O. regalis* had significant correlation with atmospheric carbon dioxide levels.



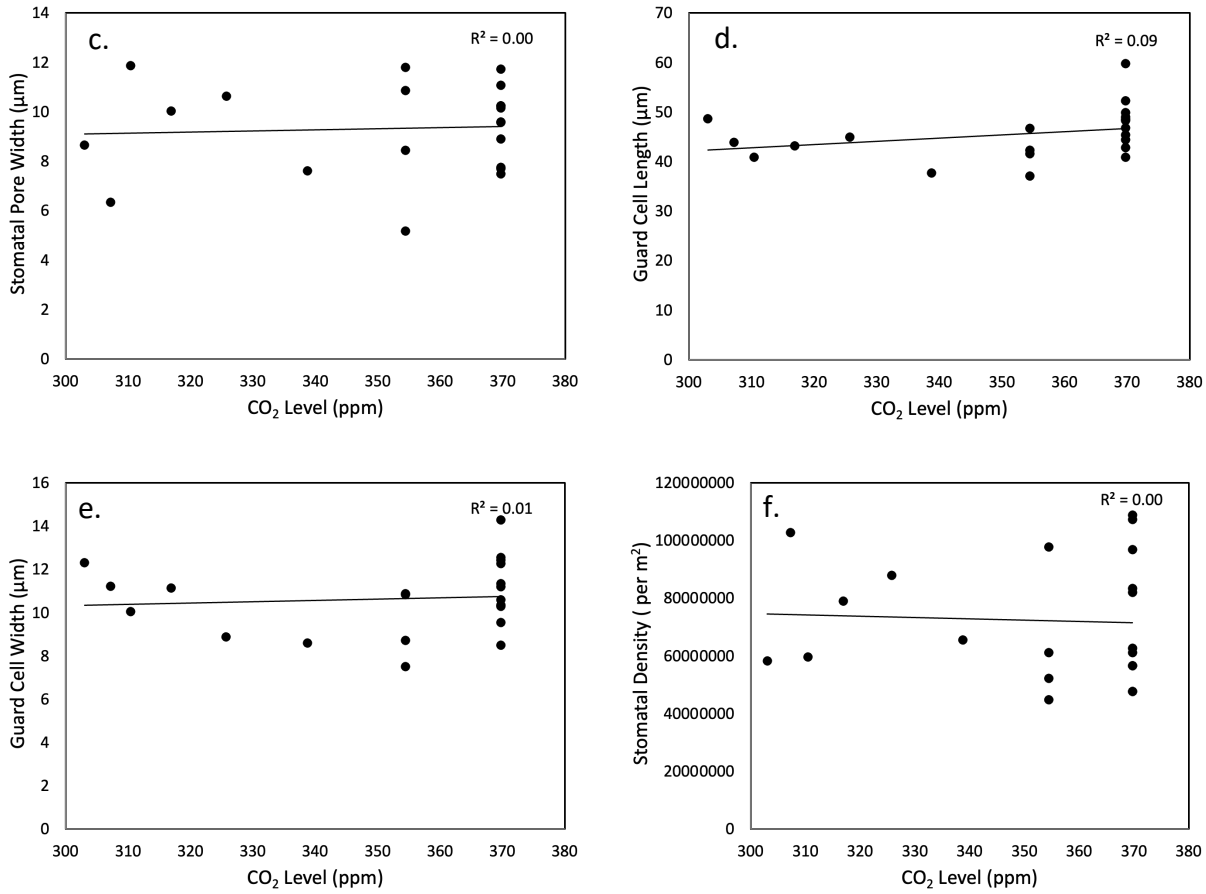
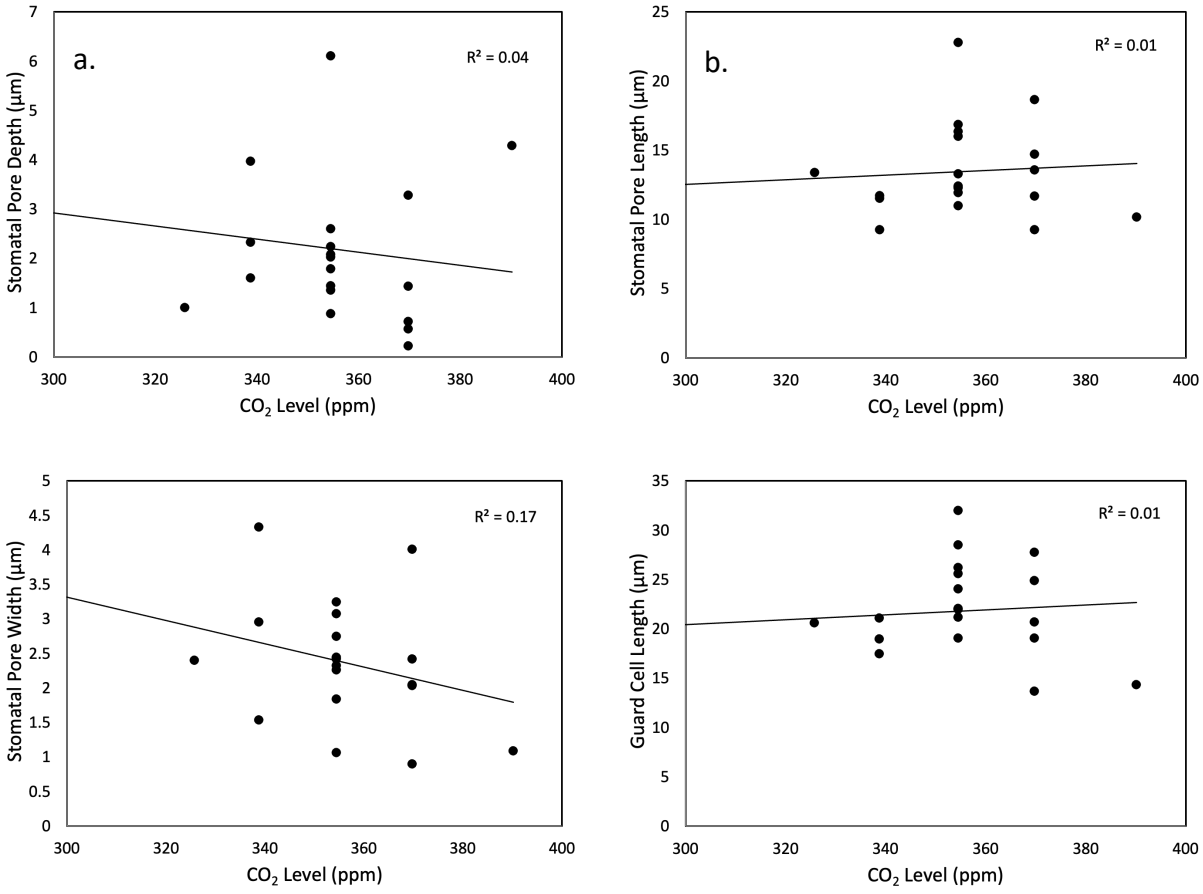


Figure 3 – The relationship between stomatal dimensions of *O. regalis* and atmospheric carbon dioxide level. (a) Stomatal pore depth (µm). (b) Stomatal pore length (µm). (c) Guard cell width. (d) Guard cell length (µm). (e) Guard cell width (µm). (f) Stomatal density (stomata/m^2).

Pinus taeda

A low correlation was found between all the stomatal dimensions and atmospheric carbon dioxide levels (Figure 4). Of these, stomatal pore width had the highest level of correlation ($r^2 = 0.17$). After a Jarque-Bera test for skewness and kurtosis, the stomatal densities for *P. taeda* were found not to be normally distributed and were

positively skewed. Therefore, the square root of the stomatal densities was calculated which transformed the data into a normal distribution.



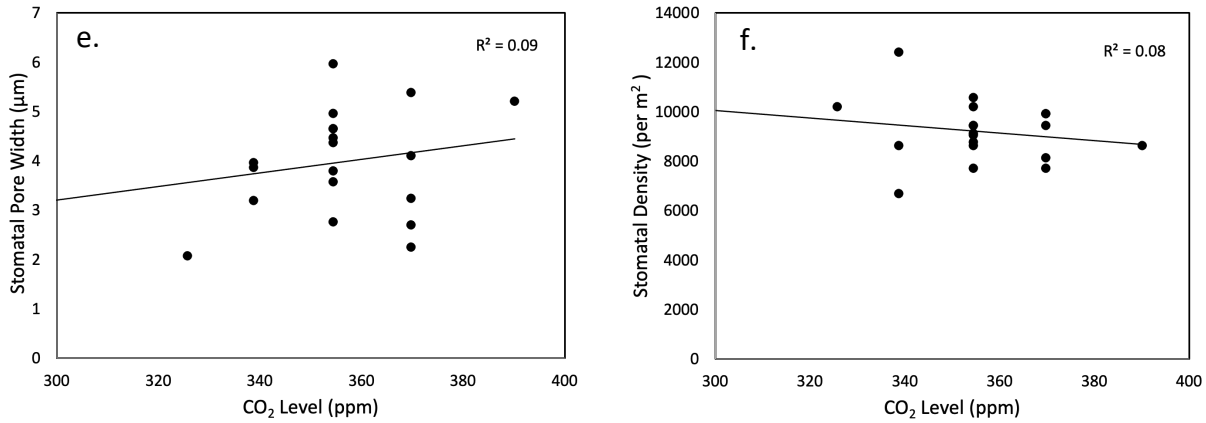


Figure 4 – Relationship between stomatal dimensions of *P. taeda* and atmospheric carbon dioxide levels. (a) Stomatal pore depth (μm). (b) Stomatal pore length (μm). (c) Guard cell width. (d) Guard cell length (μm). (e) Guard cell width (μm). (f) Stomatal density (stomata/m^2).

Rubus trivialis

In comparison to the other plant species studied, *R. trivialis* had the highest correlation among measured stomatal dimensions and atmospheric CO_2. Stomatal pore length had the highest correlation calculated ($r^2 = 0.65$) with an inverse relationship with CO_2. Stomatal pore width, guard cell length, guard cell width, and stomatal density also had moderate correlation with atmospheric CO_2 with r^2 values of 0.42, 0.60, 0.38, and 0.53, respectively. Stomata pore width, guard cell length, and guard cell width were inversely related while stomatal density was positively correlated.

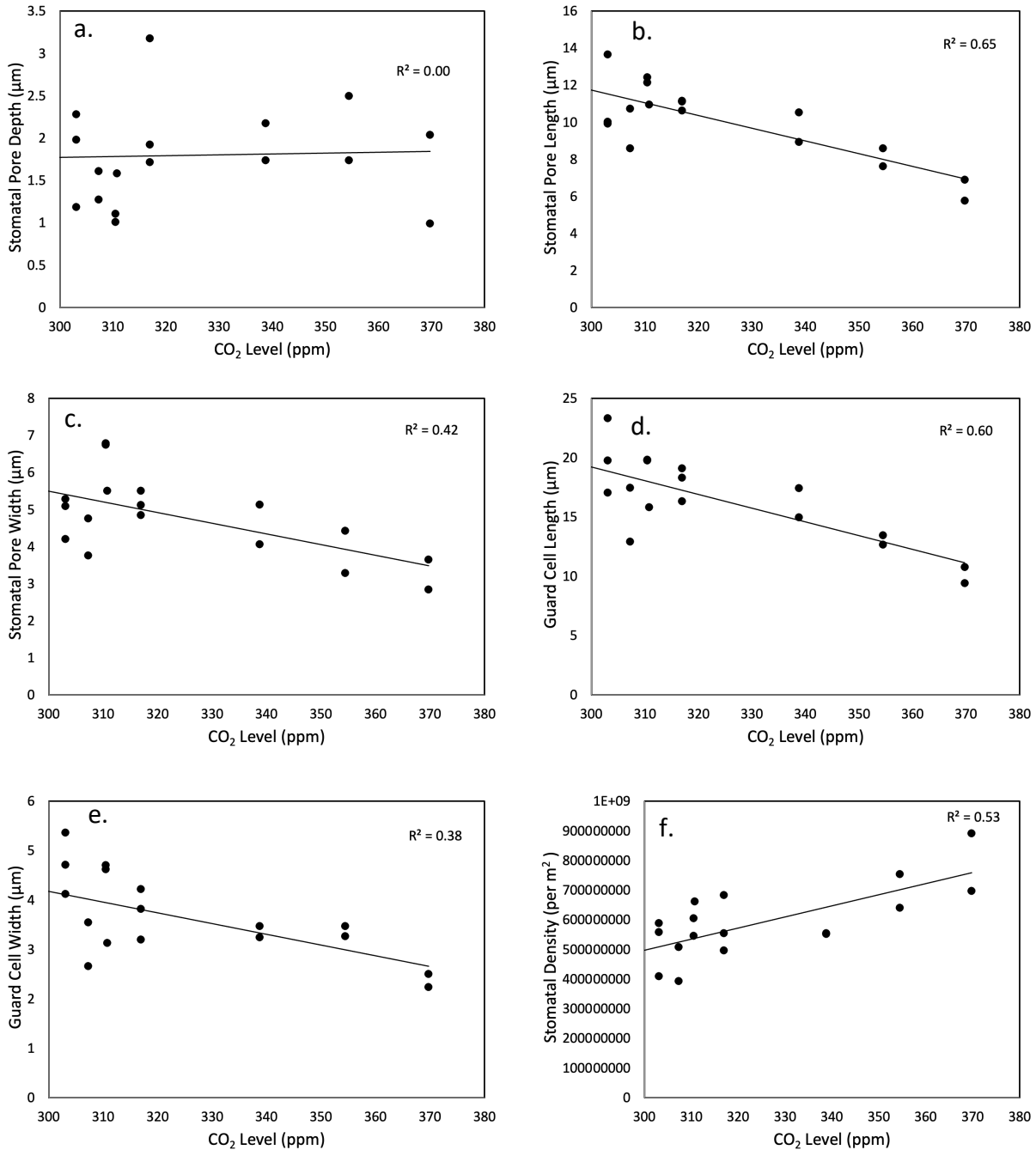
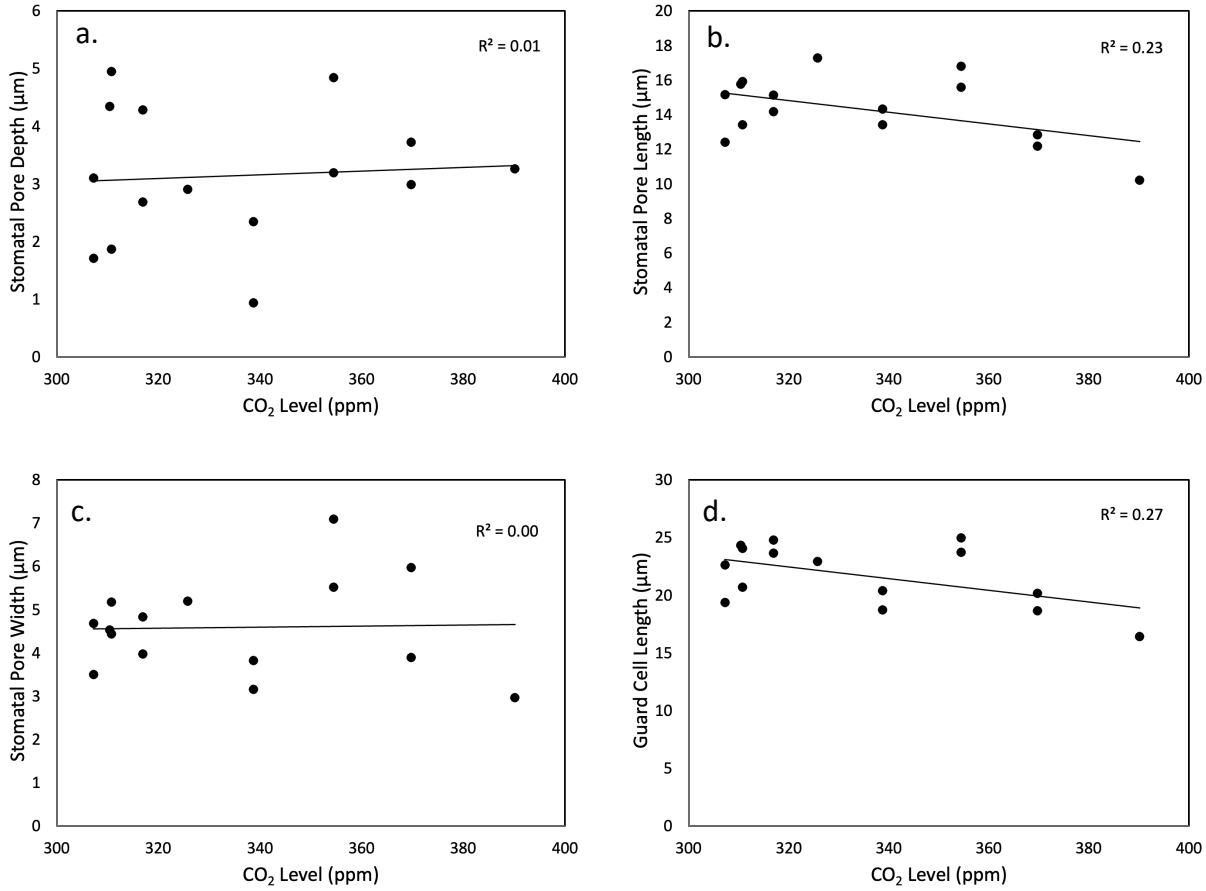


Figure 5 - Relationship between stomatal dimensions of *R. trivialis* and atmospheric carbon dioxide levels. (a) Stomatal pore depth (μm). (b) Stomatal pore length (μm). (c) Guard cell width. (d) Guard cell length (μm). (e) Guard cell width (μm). (f) Stomatal density (stomata/m^2).

Prunus serotina

Amongst *P. serotina* specimens measured, guard cell length exhibited the highest level of correlation with atmospheric CO_2 level with an r^2 value of 0.27. Stomatal pore length was correlated with atmospheric CO_2 ($r^2 = 0.23$).



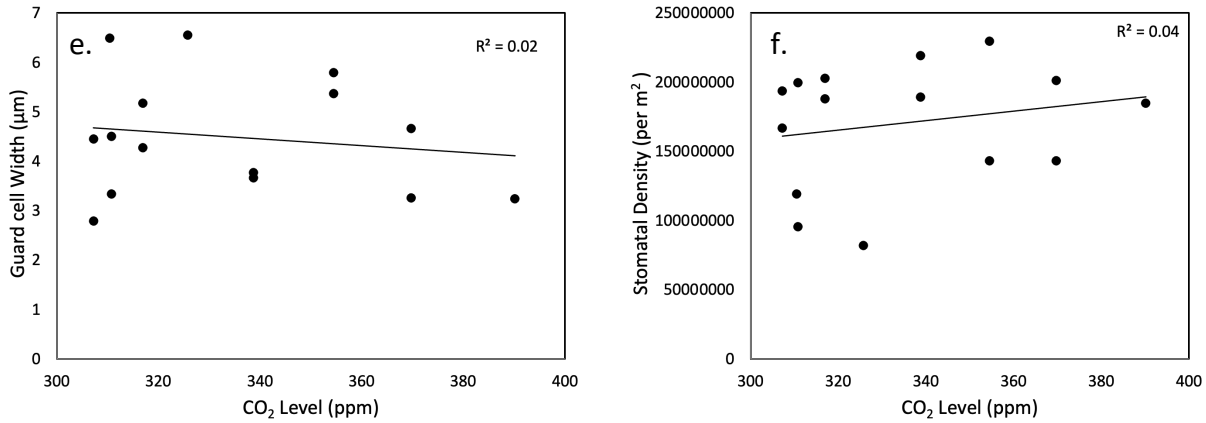


Figure 6 – Relationship between stomatal dimensions of *P. serotina* and atmospheric carbon dioxide levels. (a) Stomatal pore depth (μm). (b) Stomatal pore length (μm). (c) Guard cell width. (d) Guard cell length (μm). (e) Guard cell width (μm). (f) Stomatal density (stomata/m^2).

Stomatal Conductance

Graph on figure 7a exhibits the relationship between atmospheric carbon dioxide levels and stomatal conductance calculated directly from the measured values of stomatal pore depth. Graph on figure 7b uses the stomatal conductance values calculated using an average value of the all the *O. regalis* stomatal pore depth values. The graph on figure 7c on the second row uses the stomatal conductance values calculated using a predicted stomatal depth value. The predicted pore depth value was the width of the guard cells (Franks and Beerling, 2009). Although slightly different, none of the three graphs revealed a high level of correlation between the two variables.

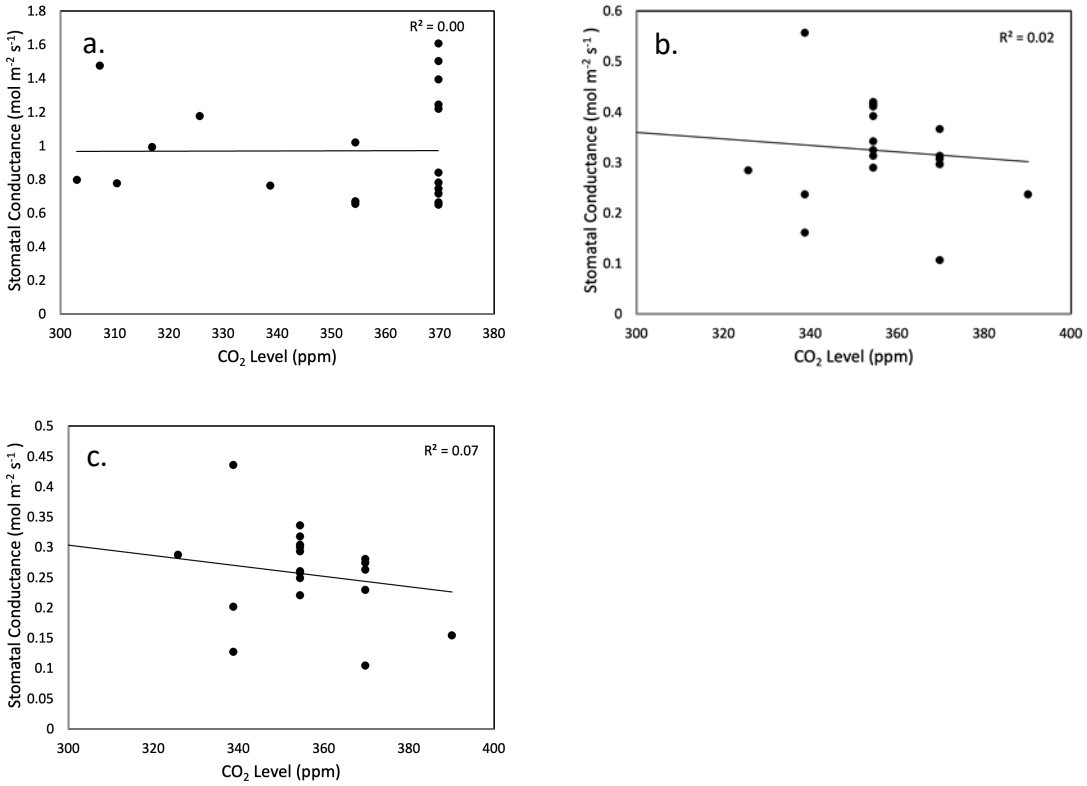


Figure 7 – The relationship between atmospheric carbon dioxide levels and the stomatal conductance of *O. regalis* calculated with various stomatal pore depths. (a) Stomatal conductance was calculated with measured values of stomatal pore depth. (b) Stomatal conductance was calculated from the mean value of stomatal depths of *O. regalis*. (c) Stomatal conductance was calculated from a predicted stomatal depth value, which was width of the guard cell.

The relationship between atmospheric carbon level and stomatal conductance calculated from predicted stomatal pore depth (w) expressed the highest level of correlation (Figure 8).

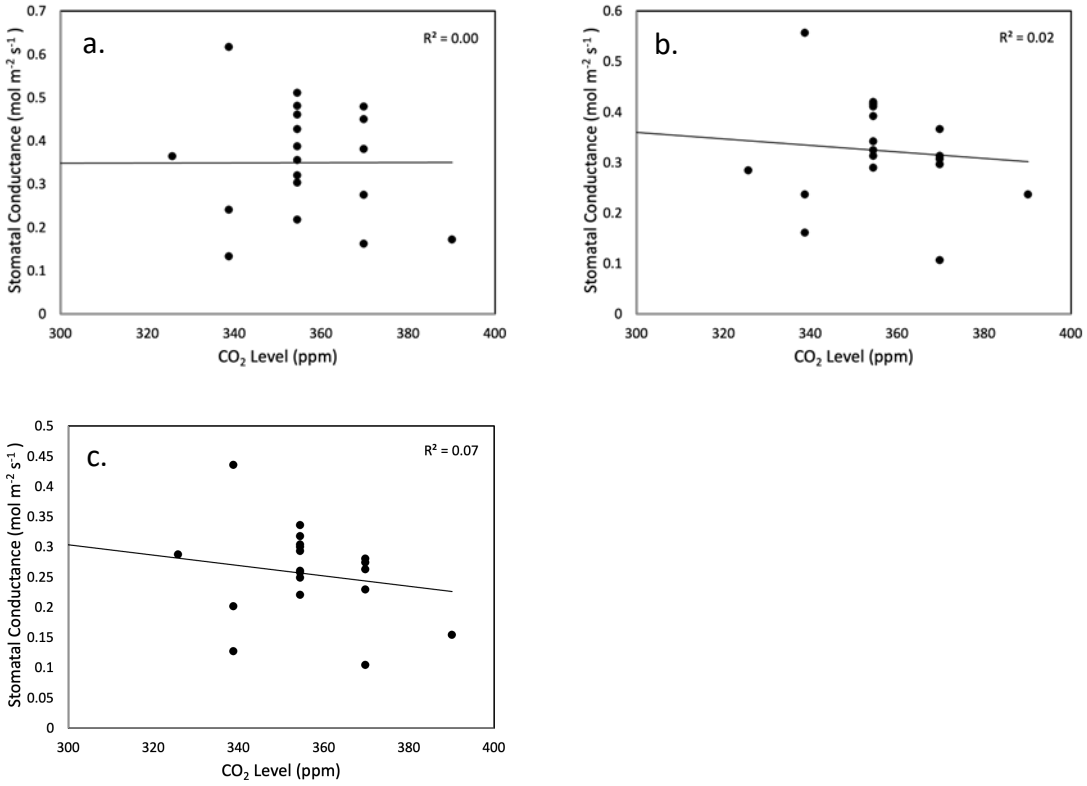


Figure 8 - The relationship between atmospheric carbon dioxide levels and the stomatal conductance of *P. taeda* calculated with various stomatal pore depths. (a) Stomatal conductance was calculated with measured values of stomatal pore depth. (b) Stomatal conductance was calculated from the mean value of stomatal depths of *P. taeda*. (c) Stomatal conductance was calculated from a predicted stomatal depth value, which was width of the guard cell.

Amongst the *R. trivialis*, the relationship between atmospheric carbon dioxide levels and stomatal conductance calculated from average pore depth expressed the highest level of correlation (**Error! Reference source not found.** 9). That of stomatal conductance calculated from predicted pore depth (w) had the lowest.

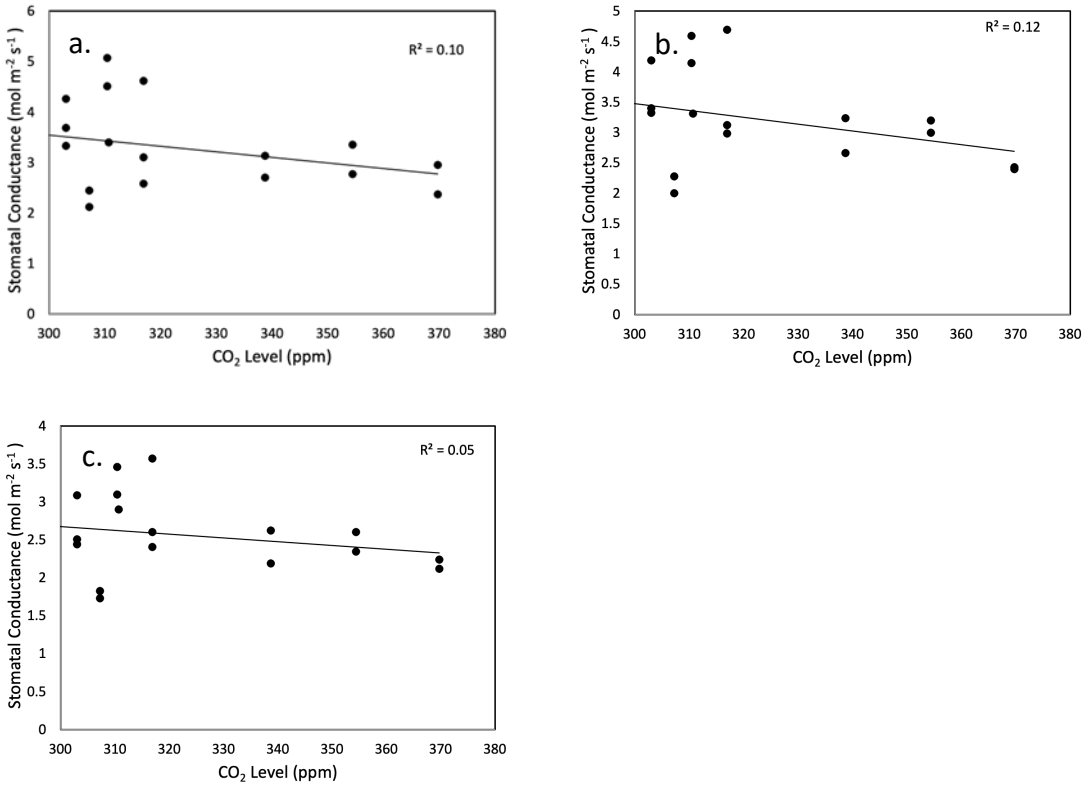


Figure 9 – The relationship between atmospheric carbon dioxide levels and the stomatal conductance of *R. trivialis* calculated with various stomatal pore depths. (a) Stomatal conductance was calculated with measured values of stomatal pore depth. (b) Stomatal conductance was calculated from the mean value of stomatal depths of *R. trivialis*. (c) Stomatal conductance was calculated from a predicted stomatal depth value, which was width of the guard cell.

Amongst *P. serotina*, all three calculations of stomata had an equally low level of correlation between atmospheric carbon dioxide levels and stomatal conductance (Figure 10).

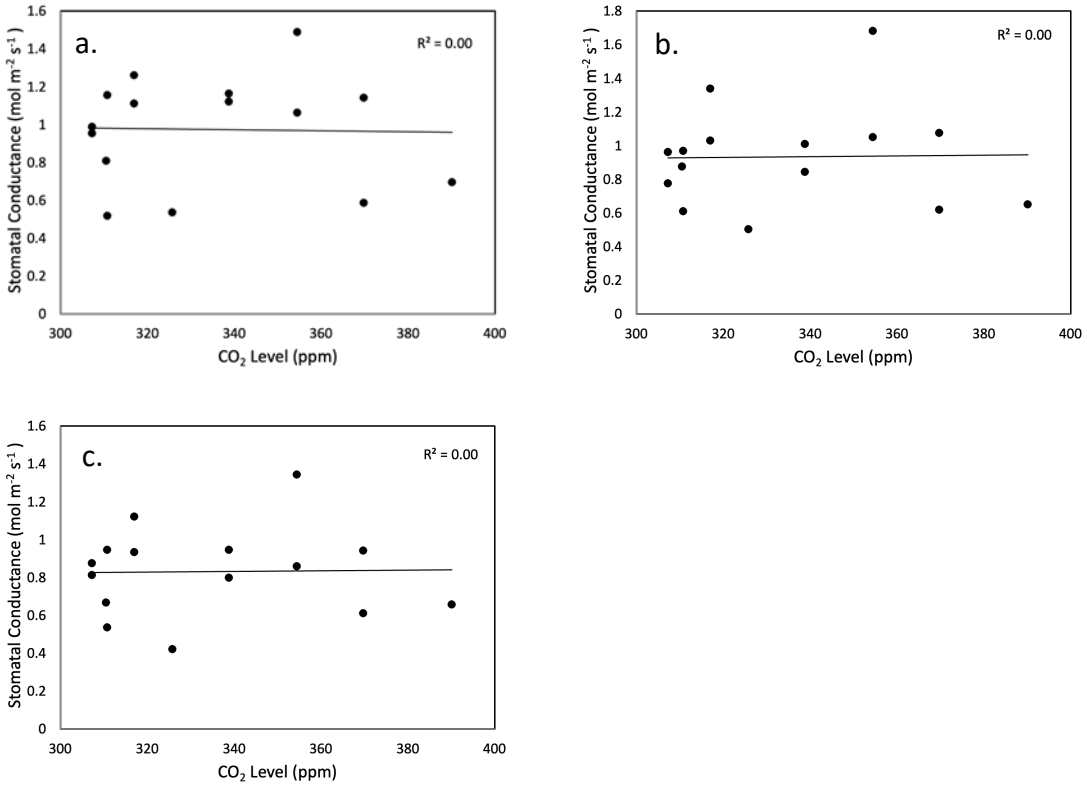


Figure 10 – The relationship between atmospheric carbon dioxide levels and the stomatal conductance of *P. serotina* calculated with various stomatal pore depths. (a) Stomatal conductance was calculated with measured values of stomatal pore depth. (b) Stomatal conductance was calculated from the mean value of stomatal depths of *P. serotina*. (c) Stomatal conductance was calculated from a predicted stomatal depth value, which was width of the guard cell.

Statistical Analysis of Stomatal Dimensions

The Jarque-Bera test was used to determine the normality of the stomatal dimensions. If the p-value was lower than 0.05, the null hypothesis was rejected, or the distribution did not assume a normal curve. Table 4 lists the p-value of each of the stomatal dimension in each of the species. None of the p-values were statistically

significant and we fail to reject the null hypothesis that the dataset is normally distributed.

Table 4 – List of p-values for each stomatal dimension of each of the four species. Asterisk indicates that the value did not originally assume normality, thus they were adjusted to fit the normal curve. The new p-values are shown for those dimensions.

	O. regalis	*P. taeda*	*R. trivialis*	*P. serotina*
Stomatal pore depth	0.63	0.47	0.59	0.92
Stomatal pore length	0.27*	0.051	0.71	0.8
Stomatal pore width	0.53	0.92	0.98	0.58
Guard cell length	0.11*	0.87	0.89	0.58
Guard cell width	0.84	0.72	0.81	0.64
Stomatal density	0.40	0.30*	0.32	0.43
Stomatal conductance	0.31	0.90	0.39	0.80

CHAPTER FOUR

Discussion

My data and analyses reveal the changes in stomatal dimensions and stomatal conductance over the change in atmospheric carbon dioxide levels for approximately the past hundred years. The differences in, the degree of physiological changes between the four species reveal the effects of rise in carbon dioxide levels on different phylogenies of vascular plants. It also provides a way of estimating atmospheric carbon dioxide levels of the past and potential responses in physiology of past vascular plants.

Overall Characteristics of Stomatal Dimensions

The calculation of the average stomatal dimensions revealed the general differences in stomatal sizes and dimensions between the four species and allowed for comparisons between them. *O. regalis* exhibited the biggest stomatal dimensions, with the highest mean stomatal pore depth, mean stomatal pore length, mean stomatal pore width, mean guard cell width, and mean guard cell length. Compared to other studies, the values of stomatal pore length I have collected were smaller (26.6 μm) (Table 1). McElwain et al. reported mean stomatal pore value of 32.7 μm and Elliott-Kingston et al reported 29.8 μm (Elliott-Kingston et al., 2016; McElwain et al., 2016). Furthermore, the mean stomatal depth value I had (3.24 μm) was also smaller than the value reported by McElwain et al., which was 17.02 μm (McElwain et al., 2016). Having bigger stomatal area indicates a greater area for gas exchange between the plant and the atmosphere. Whilst increasing the intake of carbon dioxide, it also increases the rate of transpiration,

or losing water to the environment. This increased rate of water loss is compensated by having less stomata open to the environment. This compensation is reflected in *O. regalis*, as it exhibited the lowest stomatal density out of the four species (Table 1). On the other hand, *R. trivialis* had the lowest mean pore length, mean guard cell length, and mean guard cell width. Overall, smaller stomatal dimensions would constitute a smaller area for gas exchange, which means lower carbon dioxide intake and greater water conservation. To achieve a viable balance between carbon dioxide and water exchange, a general species of plants would increase stomatal density to increase the frequency of stomatal openings (Franks and Beerling, 2009). This is again reflected by *R. trivialis* having the greatest stomatal density out of the four species (Table 1). It was found (by Franks and Beerling?) that plants decreased stomatal size and increased stomatal density in response to rising atmospheric carbon dioxide levels within a theoretical maximum because small stomata had faster response times. My results reflect this, as stomatal dimensions of *R. trivialis* experienced a negative correlation with increasing atmospheric carbon dioxide levels while its stomatal density exhibited a positive correlation.

Regulation of Stomatal Density in Response to Atmospheric Carbon Dioxide Levels

A correlation between genome size and guard cell size has been identified in ferns (monilophytes), gymnosperms, and angiosperms in varying levels (Henry et al., 2014) (McElwain and Steinthorsdottir, 2017). As greater cell size is related to lesser stomatal density, genome size has been found to be negatively correlated with stomatal density (Lattier et al., 2019). This indicates an underlying mechanism between the genome and

the expression of stomatal dimension. The most common mechanism of altering the genome size is polyploidization, which results from genome duplication events (Corneillie et al., 2019). There are two known types of polyploids: autopolyploids and allopolyploids. Autopolyploidy is the results of the duplication of diploid genome, which is an intraspecies event. Allopolyploidy is an interspecies event that results from the hybridization of two haploid genomes (Corneillie et al., 2019). Polyploidy is much more common in angiosperms than gymnosperms, with most of the angiosperm species being the result of allopolyploidy (Leitch and Bennett, 1997). While approximately 80% of angiosperms are polyploids, the frequency of polyploidy in gymnosperms may be as low as 5% (Ahuja, 2005). Altering the genome size and, thus, stomatal dimensions through polyploidization would be a process that spans over generations of evolutionary changes. Therefore, changing the frequency of stomatal expression, or stomatal density, is a more plausible mechanism in adjusting to changing atmospheric carbon dioxide levels. This is reflected in my data, as the two angiosperm species exhibited greater stomatal density than *O. regalis* and *P. taeda*. This may indicate polyploidy in *R. trivialis* and *P. serotina.*

Intercellular carbon dioxide of leaves (C_i) controls stomatal development in plants (Engineer et al., 2016). Disruption of the response between C_i and stomatal expression was observed when a cell wall wax biosynthesis mutant *hic* was introduced (Engineer et al., 2016). *Hic* codes for 3-keto acyl coenzyme A synthase, which is involved in the synthesis of very-long-fatty acid chains. In the *hic* mutant, more stomata were produced at elevated CO_2 levels. This suggests that cuticular waxes may play a role in the transportation of signaling molecules for the expression of stomatal density in response to

changing CO_2 levels. Abscisic acid (ABA) is a hormone that also plays a role in stomatal response to atmospheric carbon dioxide levels. Although ABA does not directly impact the stomatal response, the disruption of ABA receptors has been shown to slow the response. Another process in which plants alter the frequency of stomata in response to changing atmospheric carbon dioxide levels involves epidermal patterning factor gene (EPF2), ERECTA receptor kinase, and CRSP protease. Although the precise mechanisms are unknown, ERECTA receptor kinase and regulation of EPF2 expression have been shown to play a role in affecting overall plant water use efficiency by regulating stomatal index, which is the ratio between stomatal cells and all epidermal cells (Engineer et al., 2016). Mutations in the EPF2 genes have shown an inverse expression of stomatal density with increasing CO_2 levels. Although the mechanism involving *hic* and the one involving EPF2 are not yet precisely known, they are both mechanisms involving a signaling molecule and a receptor. This cell-to-cell signaling pathway involved in the expression of stomatal density in response to atmospheric carbon dioxide level is a quicker response than the alteration of genomic sizes to change the stomatal dimensions. Thus, this also suggests that changing stomatal density is a more plausible mechanism in adjusting stomatal conductance to increasing atmospheric carbon dioxide levels. Although this mechanism was heavily studied in *Arbidopsis thaliana,* an angiosperm, a similar signaling mechanism involving the ERECTA receptor kinase has been identified in moss species and the one involving ABA has been suggested to also be in ferns (Chater et al., 2013).

Species Reponses to Increased CO_2

My finding that only one of four species studied, *R. trivialis*, showed a significant response to increasing CO_2 over the study period is contradictory to the findings of Wagner et at. (2005) which found a continual decrease in mean stomatal index of *O. regalis* as atmospheric CO_2 levels increased from 310 to 370 ppmv (Chater et al., 2013). Of the three species that did not show changes, there are morphological factors that could be contributing to their CO_2 insensitivity.

With the greatest stomatal pore and guard cell dimensions, *O. regalis,* of the four species studied, was predicted to have greater plasticity with regard to modification of stomatal size. With larger stomatal pore length and width, a higher g_{max} values would be expected with a positive feedback to further stomatal higher plasticity (Franks and Beerling, 2009; McElwain et al., 2016) . Furthermore, plant fossil and herbarium specimens of *O. regalis* are observed to exhibit a decrease in the mean percentage stomatal index, the ratio of stomatal density to epidermal cell density, to increasing atmospheric carbon dioxide (Wagner et al., 2005). However, in my study neither the stomatal dimension nor the stomatal density had significant correlation against atmospheric carbon dioxide levels. The lack of response to CO_2 by *O. regalis* may be related to water supply to leaves. Ferns, unlike angiosperms, do not have a complex vein system nor a sufficiently high g_{max} to for it to support a wide ecophysiological niche space and confer greater plasticity. It was found that ferns, on average, had a much lower minimum-maximum range of vein density than angiosperms across many species (Boyce et al., 2009). Also, *O. regalis* is restricted in its distribution to areas with high perennial

precipitation and high water availability (Landi and Angiolini, 2008). This indicates high water sensitivity of *O. regalis*, which can be another factor contributing to its insensitivity to increasing atmospheric carbon dioxide levels. For the uptake of carbon dioxide to be possible, the stomata must be open to the environment, which simultaneously leads to the loss of water from the plant (Klein and Ramon, 2019). If a plant is sensitive to water availability, it would not exhibit a significant increase in stomatal conductance in response to increasing atmospheric carbon dioxide levels, for the rate of water loss would also rise. Thus, *O. regalis*' water sensitivity may be another factor contributing to its insensitivity to increasing atmospheric carbon dioxide levels. An alternative argument that water-sensitive species are more likely to show changes to increasing carbon dioxide by downregulating stomatal dimensions, and thus stomatal conductance, can be made However, some findings reveal that ferns are more conservative in water usage than angiosperms, meaning that would close their stomata earlier than angiosperms to water stress (Brodribb and McAdam, 2017). While this would minimize water loss, it would also decrease photosynthetic capabilities. With a lower ecophysioloigcal niche and lower photosynthetic capabilities, ferns would exhibit less opportunity to segregate resource use to enhance plasticity (McElwain et al., 2016).

The low correlation both the stomatal dimensions and the stomatal conductance of *P. taeda* had with increasing atmospheric carbon dioxide level corresponds with a prior study, where gymnosperms were shown to express lower levels of plasticity in response to changing carbon dioxide levels relative to angiosperms (McElwain et al., 2016). The same study also showed that lower levels of stomatal variability observed in *P. taeda* are

also attributed to gymnosperms generally having a lower vein density than angiosperms. Because having a smaller vein density narrows the ecophysiological niche space, it confers less plasticity exhibited by the plant. Furthermore, while genome duplication is argued to increase the vein density and maximal stomatal conductance by altering cell size, it was found that genome duplication was common in angiosperms but not gymnosperms. In a different study, it was revealed that genome size was positively associated with guard cell size in 101 angiosperm species (McElwain and Steinthorsdottir, 2017). Thus, it can be argued that this is a factor contributing to its insensitivity to increasing atmospheric carbon dioxide levels. However, this is not without any contradictions, as it was also found in the same study that an increase in guard cell size in association with increasing ploidy has also been identified in the fossil specimen of *Ginkgo biloba*, a gymnosperm species.

Out of the four species, *R. trivialis* is the only one that showed a significant response to increasing atmospheric carbon dioxide levels, specifically in its stomatal pore length, stomatal pore width, guard cell length, guard cell width (Table 1). The high plasticity usually observed in angiosperms could be found in the high level of negative correlation between increasing atmospheric carbon dioxide levels and various stomatal dimensions of *R. trivialis* (McElwain et al., 2016). In particular, strong negative correlation between stomatal dimensions would ultimately result in a decrease in stomatal conductance reducing exposure to the dry atmosphere and also decreasing for carbon dioxide intake (Franks and Beerling, 2009). This change is contradictory to prior findings that changes in the dimension of the guard cell and stomatal pore are usually a result of

genomic alterations, which is an evolutionary mechanism that occurs over generations (McElwain and Steinthorsdottir, 2017). In *R. trivialis*, both changes in stomatal density and the dimensions of the guard cell were observed, which suggest both genomic and expressional changes were made in response to increasing carbon dioxide levels. However, the stomatal conductance of *R. trivialis* still experienced a high negative correlation despite having the highest positive correlation of stomatal density with increasing carbon dioxide levels out of the four species. This could mean the genomic changes that led to the gradual decline in the stomatal pore length, stomatal pore width, guard cell length, guard cell width of *R. trivialis* was a more significant response than the change in stomatal density. Furthermore, the decrease in stomatal size and the increase in stomatal density expressed in *R. trivialis* confirm other studies that identify the theoretic maximum stomatal size given a specific density and the theoretical maximum stomatal density given a specific stomatal size (Franks and Beerling, 2009).

Although not as drastic as *R. trivialis*, *P. serotina,* stomatal pore length, guard cell length, and guard cell width also had a high negative correlation with atmospheric carbon dioxide concentration. This confirms findings that angiosperms tend to express greater plasticity with higher vein density and g_{max} (McElwain et al., 2016) thus reinforces the conclusion that *O. regalis* and *P. taeda*, lack similar genomic structure. *R. trivialis* is considered an evergreen perennial shrub while *P. serotina* is considered a deciduous tree. This distinction suggests why the two species showed different levels of correlation. Other studies have found that evergreen broadleaf angiosperms showed a higher stomatal conductance than broadleaf angiosperms, which granted them more leeway for change

(Klein and Ramon, 2019). Thus, it was also revealed that evergreen broadleaf angiosperms exhibited a stronger downregulation of stomatal conductance, while deciduous angiosperms exhibited a milder downregulation.

Stomatal Conductance

Stomatal depth values of a fully opened stomata were estimated to be equal to the guard cell width, as it was assumed that a guard cell would have a circular cross section, which meant the diameter across the width of the guard cell would be equal to the vertical diameter spanning the depth of the stomatal pore (Ibid, chapter 2). Furthermore, if the guard cell width values weren't available, it would be estimated to be $¼^{th}$ of the guard cell length for non-grass species and $1/8^{th}$ for grass species. This different estimation was seen as significant, as changing the stomatal depth values significantly altered the calculated stomatal conductance values (Figure 11). Another morphological analysis by Wagner et al. (2005) used herbarium specimen to measure stomatal index. Although they have also run into problems with unequal distribution of samples studied, they have gathered accurate data as well. The graphical model of prehistoric carbon dioxide they created with herbarium specimen aligned with calculations made from fossil specimens, considering the herbarium model highly accurate. The calculations made using the different stomatal pore depth values did not reveal any significance, as the level of correlation between maximum stomatal conductance and atmospheric carbon dioxide level remained low in four species (Figure 6, Figure 7, Figure 8, Figure 9).

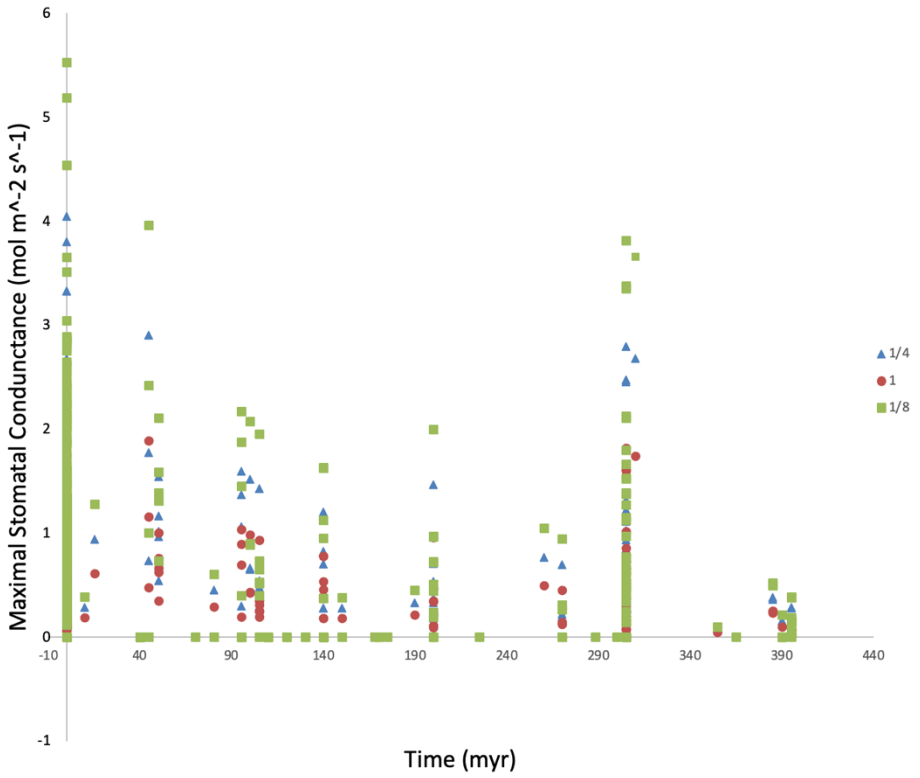


Figure 11 – The difference in stomatal conductance values with different predicted stomatal depth values. The green line represents stomatal conductance calculated from stomatal depth value predicted as the guard cell width. The blue line represents stomatal conductance calculated from stomatal depth value predicted as the one-fourth of the guard cell width. The red line represents stomatal conductance calculated from stomatal depth value predicted as one-eighth of the guard cell width.

Out of the three species, *R. trivialis* had the highest mean stomatal conductance in all three calculations. Again, *R. trivialis* possessed the highest stomatal density while having a lowest stomatal pore length, stomatal pore width, guard cell length, guard cell width. This reflects the finding that some species have the tendency of lowering stomatal size and increasing stomatal density in order to raise their stomatal conductance (McElwain et al., 2016). After *R. trivialis*, *O. regalis* had the next highest mean stomatal

conductance value when stomatal conductance was calculated using measured stomatal pore depth and average pore depth. However, when stomatal conductance was calculated using estimated stomatal pore depth, *P. serotina* had the second highest mean stomatal conductance. Overall, the angiosperms had a higher stomatal conductance than the gymnosperms and exhibited greater responses to changing atmospheric carbon dioxide levels, which correlates with other studies (Klein and Ramon, 2019). Evolution of high vein density in angiosperms was predicted to have led to greater plasticity, resulting in a more flexible stomatal conductance value. Higher vein density would have been found to greatly increase the operational stomatal conductance, which allows plants to operate at a greater range of ecophysiological niche (McElwain et al., 2016). Thus, with the recent rise in atmospheric carbon dioxide levels, angiosperms would be predicted to experience a more significant decline in stomatal conductance. Furthermore, having a more complex hydraulic architecture, or vascularization, within plant leaves would help supply more water to the stomata, increasing the photosynthetic capacity of the leaf. This enabled angiosperms to outcompete with gymnosperms with lower vein densities. With higher plasticity and photosynthetic capacity, angiosperms would respond more sensitively to the increasing atmospheric carbon dioxide levels, which also aligns with my findings.

Herbarium Specimens as Physiological Proxies

Although all the guard cell dimensions and stomatal pore length of *R. trivialis* were correlated with increasing atmospheric CO_2, stomatal pore depth did not. In fact, none of the four species exhibited any significant changes in their pore depth. This could indicate a potential flaw in the use of herbarium specimen. As collected specimen are

pressed and dried under high enough pressure to avoid dampness and molding (Seshagirirao et al.), the physiology of the plant may be distorted on a microscopic level. However, other studies have not specifically studied this confounding factor, particularly with herbarium specimens, though some have pointed at some inaccuracies (Wagner et al., 2005). Another factor that may introduce inaccurate stomatal pore depth value is the stage of development the specimen was in prior to collection and whether the specimen was senesced prior to collection. However, no studies found have acknowledged when the herbarium specimen were collected or what state the plant was in prior to collection. Other studies that reported stomatal pore depth values did not point out any potential source of variation from the stomatal pore depth measurements. The scarcity of studies that report stomatal pore depth values indicate the difficulty of obtaining such values. The use of the 3D laser microscope could have also introduced some inaccuracies in the stomatal dimensions. As the microscope does not account for the angles the stomata are in, the measurements could have been altered by curvature of the leaves or the angles the stomatal were facing.

Historical Perspectives and Proxies of Past and Current Changes in Atmospheric CO_2

The results produced here point to a mixed result of using historical specimens to assess past atmospheric carbon dioxide levels. My study confirmed general trends in the plasticity of stomatal conductance in response to rising atmospheric carbon dioxide levels in ferns, angiosperms, and gymnosperms from other different studies. It also revealed differences in the response within the angiosperms, and how evergreen angiosperms

expressed more plasticity than deciduous angiosperms (McElwain et al., 2016). However, my findings did not express correlation between *O. regalis* and atmospheric carbon dioxide levels, which other studies have found (Wagner et al., 2005). This presents some variability in the use of herbarium specimen to estimate stomatal conductance values in response to current rises in atmospheric carbon dioxide levels. Furthermore, the lack of response in stomatal pore depth values in all four species indicate some inaccuracies in the stomatal pore depth measurement from herbarium specimens. The process of collecting and preparing the herbarium specimen is an indication of such errors. Ultimately, this reveals that some element of stomatal dimension, such as stomatal density and 2 dimensional measurements, may provide accurate response of stomata while other aspects such as stomatal pore depth may introduce some inaccuracies.

Conclusion

I have argued throughout this work that the rise in atmospheric carbon dioxide levels will lower the stomatal conductance of vascular plants by altering the stomatal physiology. The specimens available from the Baylor herbarium have been scanned under the 3D laser microscope to scan for the stomatal dimensions. My findings reveal the responses in four species of vascular plants. *O. regalis* and *P. taeda* was found to be less sensitive to the changes in atmospheric carbon dioxide level relative to the angiosperms, which could be the results of less vein density and higher water sensitivity. *R. trivalis* on the other hand exhibited significant negative correlation between stomatal size and atmospheric carbon dioxide levels and a significant positive correlation between

stomatal density and atmospheric carbon dioxide levels. The higher level of sensitivity can be concluded to have been the impact of greater plasticity and the tendency for plants to decrease stomatal size and increase stomatal density for a quicker response in the stomata. The response in *P. serotina* confirms the higher level of change in angiosperms relative to ferns and gymnosperms but reveal the differences in response between evergreens and deciduous angiosperms. Despite the differences in the sensitivity of stomatal dimensions between the species, none of them exhibited a high level of correlation in its stomatal conductance.

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