Drew University

College of Liberal Arts

Climate change and the Asteraceae family: Changes in leaf length and area from 1886 to 2021 in Morris County, NJ

A Thesis in Biology

By

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Abstract

Climate change, specifically human-caused climate change, has presented ecosystems, and organisms within them, with unprecedented challenges. In this study, I investigated how leaf size has changed over time in response to climate change, with the hope to better understand one of climate change's impacts. I utilized herbarium specimens, ImageJ, and climate data to see how leaf size has changed over time in the plant family Asteraceae from 1886 to 2021. I analyzed 151 specimens from Drew University's Herbarium, the Virtual Chrysler Herbarium at Rutgers University, and my personal 2021 field collections. These 151 specimens were representative of 10 genera, *Ambrosia, Artemisia, Bidens, Cirsium, Eurybia, Euthamia, Eutrochium, Helenium, Solidago,* and *Symphyotrichum.* To measure leaf length and leaf area, I used ImageJ, software used in various fields of biology to collect precise size measurements. After collecting leaf size measurements for these specimens, I collected climate data from Weather Underground, NOAA, and the NJ State Climatologists Office, to analyze how the climate in New Jersey has changed over time, and relate this to how leaf length and area have changed over this 135 year period. A correlational analysis was performed to determine if leaf length or area varied as a function of year and three climate variables: annual mean maximum temperature, mean minimum temperature, and mean precipitation for all ten genera combined, for these genera individually, and over 30 year time periods. The results were not statistically significant for leaf length and leaf area when all genera were combined. One genus, *Euthamia*, showed a statistically significant increase in leaf area by year, but not leaf length, and this was consistent across all three climate variables. In contrast, from 1950 to 1980, *Ambrosia* and *Symphyotrichum* both showed a statistically significant decrease in leaf length and area as a function of the year, with *Symphyotrichum* showing a statistically significant decrease for both dependent variables as a

function of mean maximum temperature. Additional research is needed to further investigate some genera, specifically those that had a small representative sample in this study. Additional research is also needed to further examine all the ways plants are affected by anthropogenic climate change, and how they respond. My hypothesis was not supported by some of these findings, specifically an increase in *Euthamia* leaf area. On the other hand, my hypothesis was supported by other results, specifically a decrease in *Symphyotrichum* leaf length and area. These findings support the idea that different organisms, even those within the same family, are responding to climate change in different ways.

Table of Contents

Introduction

Overview

As anthropogenic climate change worsens, it is imperative that we understand the extent of its impacts. Changes in temperature and precipitation can greatly affect different organisms and ecosystems. In this study, I focused on how ten genera within the plant family Asteraceae have changed in response to changes in climate, specifically through changes in their leaf length and leaf area. To measure these changes in leaf size over time, I used specimens from the Drew University Herbarium and the Virtual Chrysler Herbarium at Rutgers University over a 135-year period. To measure leaf size for these specimens, I used ImageJ, open-source software for processing and analyzing digital images, coupled with digitized herbarium specimens that had a ruler present in their picture. After collecting the plant data, I focused on collecting the associated climate data by using a combination of resources, including Weather Underground, NOAA's Historical Database, and Historical Climate Tables from the NJ State Climatologist.

Climate Change: The Basics

From the beginning of time, our climate has fluctuated. There have been extreme highs and extreme lows, but modern climate change is different. We are here, humans are now in play. It is estimated that the Earth's climate has exceeded natural variability, and this has been the case since the 1980s (Karl & Trenberth 2003). Climate change, defined by NASA, is a long-term change in the average weather patterns that have come to define Earth's climates (NASA Global Climate Change 2022). Climate change is different from global warming, and climate is different from weather. Global warming is the long-term heating of Earth's climate system due to human activities. Climate change encompasses both human and naturally occurring warming on our planet (NASA Global Climate Change 2022). The difference between weather and climate is that weather refers to short-term conditions, while climate refers to long-term changes. Most of the warming since the 1850s can be attributed to human emissions (Ritchie & Roser 2020). Greenhouse gases, like carbon dioxide, methane, and nitrous oxide, are emitted from most human activities, including burning fossil fuels for transportation, electricity, and heat (Karl & Trenberth 2003). Anthropogenic greenhouse gas emissions are driven largely by economic and population growth. Our population is continuously growing, so these atmospheric concentrations of gases are also going to increase. We are not at our peak yet (Ritchie & Roser 2020). Greenhouse gases cause a greenhouse effect, trapping the Sun's heat in the Earth's atmosphere. This is what is causing global warming, and ultimately modern climate change.

The impacts of climate change are still being researched. Some impacts are already known, and some we are already experiencing. The more well-known impacts are sea-level rise, decreases in snow, melting glaciers and ice, increasing temperatures, and extreme weather events (Karl & Trenberth 2003). The combination of melting ice sheets and increasing ocean temperatures could inundate coasts globally, increasing the sea level for centuries (Karl & Trenberth 2003). Many places around the world, including New Jersey, are feeling the impacts of climate change. The New Jersey Department of Environmental Protection, a leader in assessing and responding to environmental and public safety risks in NJ, released two studies confirming increases in precipitation over the years. These studies indicated that there has been an increase in precipitation in New Jersey over the last 20 years, and they projected a more than 20% increase in precipitation from the baseline, set in 1999, by 2100 (NJDEP 2021). Since 1985, New Jersey's average annual temperature has increased by 3.5 degrees Fahrenheit, and NJ is warming faster than the rest of the Northeast region. In addition to these precipitation and temperature increases, New Jersey is also feeling the effects of climate change through sea-level rise, ocean acidification, and pressure on natural and human systems (NJDEP 2020).

The risks associated with climate change and increasing global average temperature are positively correlated. As global mean temperatures increase, risks facing species, ecosystems, agriculture, water, etc. increase. Between a 1 to 2 degrees Celsius increase above pre-industrial levels, risks increase significantly (Hare 2006). Ecosystems will suffer greatly, especially coral reefs and other vulnerable ecosystems. Above a 2 degrees Celsius increase, the risks increase, even more, involving large-scale extinctions, ecosystem collapses, food and water shortages, and socio-economic damage (Hare 2006). Global mean temperatures are expected to rise by up to 4 degrees Celsius by 2100, which would cause unprecedented changes and consequences for all living organisms (Thuiller 2007).

Climate change will also have many impacts on ecosystems, including affecting species' home ranges and phenologies. Increases in temperature and precipitation have influenced a shift of species' ranges. In the Northern Hemisphere, the range of terrestrial plants and animals has shifted about 6.1 kilometers northwards or 6.1 kilometers upwards per decade, on average (Thuiller 2007). Another example of this home range expansion was investigated in a study of 57 nonmigratory European butterflies. Nearly 63% had shifted their ranges to the north by 35 to 240 kilometers, and only two species shifted to the south (Parmesan 2006). Most recorded observations on the impact of climate change on ecosystems, by far, have involved species' phenologies (Parmesan 2006). Phenology is the study of periodic events in the life cycles of plants and animals, as influenced by the environment (Cleland et al. 2007). Phytoplankton bloom, a phenological event in water systems, has advanced by 19 days from 1962 to 2002 in a lake in the northwestern United States (Winder & Schindler 2004). Some examples of phenological events in plants are flowering times, leaf out, and fruiting. Plant phenology doesn't only affect the individual, it also impacts the fitness of all organisms that rely on them. Changes in plant phenology can negatively affect demography and agriculture, but understanding phenological drivers can help us learn more about species distributions, biogeochemistry, and ecosystem services (Stucky et al. 2018).

The History of Herbaria

Herbaria have an interesting history that goes back to the 16th century. Herbaria are dried pressed plant specimens and their associated data, photographs, and library material (Funk, 2003). The following background information was obtained using the information written by Verlinde in an article for the Bothell Herbarium at the University of Washington (Verlinde 2016). Luca Ghini, a Botany Professor at the University of Bologna, Italy, in the 1500s, is credited with preserving plants by using a press and binding them to a book. This practice then became popular throughout Europe, specimens were housed in personal collections and traded between botanists. Two centuries later, Carl Linnaeus found trouble with this practice because as his collections expanded, he found it difficult to catalog when binding his specimens within a book. Linnaeus came up with a system that mounted one specimen per large sheet of paper. After pressing and transferring the specimen to paper, they were stored in cabinets with other closely-related plants. In his methodology, Linnaeus used the same size papers for each specimen, creating a uniform collection. This methodology and the standards used by Linnaeus are still used today (Verlinde 2016).

Herbarium specimens have been useful resources in the past, and continue to be useful. They provide the comparative material that is essential for research in ecology, anatomy, biology, ethnobotany, and conservation biology (Funk 2003). From data collected in the early 2000s, there are 3,240 herbaria in the world, with 623 of them being in the United States (Funk 2003). In “100 Uses for an Herbarium (Well at Least 72)”, Funk (2003) details many ways in which an herbarium can be used, from basic research to education and outreach. Under the research category, herbarium specimens can be used to provide material for DNA analysis, provide information on species that are extinct in the wild, and provide material for making morphological measurements. They can also be used to document what plants grew where and near what other plants, provide information on the local uses of plants, and provide pollen for pollination and allergy studies (Funk 2003). They also can be used for educational purposes and for outreach. Herbaria can provide internship and job opportunities for students, promote appreciation of plant diversity, and provide material for courses like Forest Ecology or Plant Biology (Funk 2003). Both of these classes have been taught at Drew University in the past, and both utilized the Drew Herbarium, to some extent. Herbaria can also provide inspiration for artists, help establish new museums or exhibits, and help facilitate international collaborations (Funk 2003). From this vast list of potential uses, we can see just how important herbaria are for research, education, and outreach.

Herbarium specimens are being recognized and valued as a reliable source for a diversity of plant species (Willis et al. 2017). Herbarium specimens can offer phenological information, like leaf-out and flowering times. Plant phenology has been shown to be particularly sensitive and responsive to a warming climate. Experiments and studies regarding flowering times have been conducted worldwide (Menzel et al. 2006). Growing evidence supports the hypothesis that plants are flowering earlier due to increasing temperatures (Panchen et al. 2012). They can also be a tool to see how specific plant organs have changed over time, which is how I am utilizing them in this study. Herbarium specimens act as a view into the past, where we can reconstruct or view the composition of plants in a specific area. One way to reconstruct past ecosystems is through reconstructing the spread of invasive species. A 2003 study used herbarium specimens to reconstruct the spread of invasive wetland species in Southern Quebec (Delisle et al. 2003). Plant pressed specimens have also been used to look at herbivory over time. A 2018 study, using herbarium specimens ranging 112 years, found increasing herbivory over the past century, and these results are consistent amongst four different species with four distinct herbivore communities (Meineke et al. 2018).

As digitization improves worldwide, we will be able to use herbarium specimens as a research tool more readily and efficiently (Willis et al. 2017). The process of digitization for herbarium specimens consists of capturing and processing a digital image, transcribing the associated identification card, and georeferencing location information (Willis et al. 2017). Large-scale digitization processes are underway in the United States, Australia, Austria, Brazil, Canada, China, France, and South Africa (Willis et al. 2017). The widespread digitization of herbaria allows for novel research, large-scale collaboration, and education. As more herbaria become available online, more questions can be asked regarding phenology, morphology, herbivory, conservation, and the impacts of climate change.

Leaf Morphology Basics

Leaves are important organs for a plant. They are the primary sites of photosynthesis and transpiration, and they are also involved in defense. Photosynthesis is the process that plants use to capture radiant energy, or energy from the Sun, and convert it into biochemical energy, the energy that is stored in organic and living matter (Evans 2013). Transpiration is the exhalation of water vapor through the stomata, pore-like structures on a leaf. Defense is also an important function for a leaf. Plant structures are the first line of defense against herbivory. Structural defenses, which include morphological traits, deter insect pests. Some examples of structural defenses are cuticles, spines, and hardened leaves (War et al. 2012).

A leaf may seem simple in appearance, but it is a highly-efficient structure with several parts, including the blade, or lamina, petioles, stipules, a midrib, and a margin (Figure 1). The leaf blade is the expanded portion of the leaf (The William & Lynda Steere Herbarium). The petiole is the stalk that extends from the stem to the base of the leaf (Boundless Biology). Stipules are reduced leaf-like appendages inserted at the base of the petiole, and they are often variable in morphology (The William & Lynda Steere Herbarium). The midrib is the primary vein of the leaf, and the margin is the edge of the leaf (The William & Lynda Steere Herbarium).

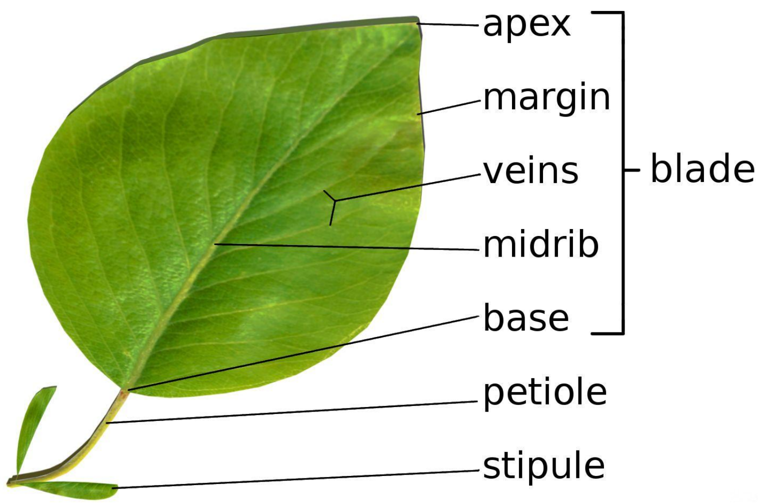

Figure 1. The structure of a leaf with several important parts labeled. This picture was obtained from a ThoughtCo article written by Regina Bailey titled Plant Leaves and Leaf Anatomy (https://www.thoughtco.com/plant-leaves-and-leaf-anatomy-373618).

The following information on leaf morphology was obtained from the University of Rochester's 'Description of Leaves' index. There are many technical terms to talk about leaf morphology, and they can be divided into several categories: arrangement, structure, margin, attachment, and shape. To describe the arrangement of leaves, they can be categorized as alternate, opposite, or whorled (Figure 2). An alternate arrangement is when leaves are singularly attached to the stem, ascending alternately along the stem, or stalk. An opposite arrangement is when leaves are attached to the stem in pairs and are opposite to each other. A whorled arrangement is when leaves are attached in groups of three or more at the same level.

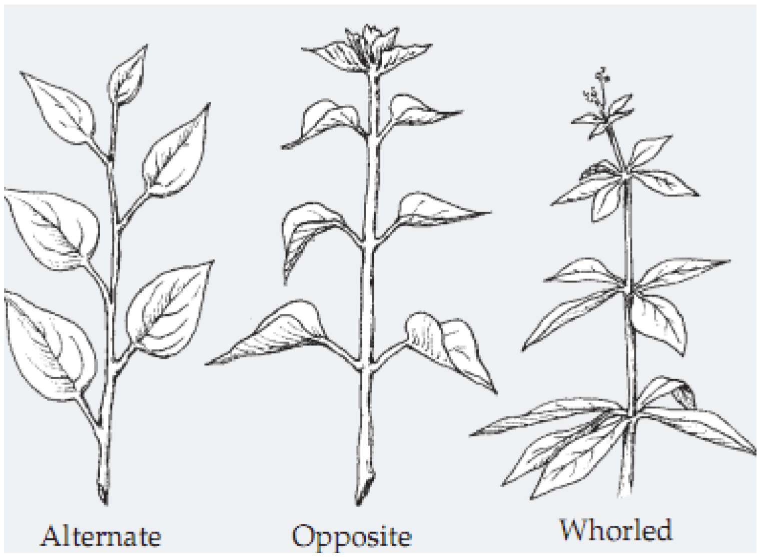

Figure 2. The three most common terms to characterize leaf arrangements, alternate, opposite, and whorled. This picture was obtained from Boundless Biology's 'Biology for Majors I' course (https://courses.lumenlearning.com/boundless-biology/chapter/leaves/)

To describe the general structure of a leaf there are several different classifications, including simple, lobed, dissected, and compound (Figure 3). Simple leaves are leaves that do not have partitions, lobes, or large teeth, and are usually convex, curved or rounded outwards. Lobed leaves have distinct protrusions, either pointed or rounded. There are two different types of lobed leaves, pinnately lobed and palmately lobed (Figure 4). Pinnately lobed leaves are arranged on either side of a center point, resembling a feather, while palmately lobed leaves spread radially from a point, resembling fingers on a hand. Dissected leaves are deeply and/or repeatedly cut into partitions, but not into individual leaflets. Compound leaves are divided into distinct leaflets, and they may be either pinnately or palmately compound.

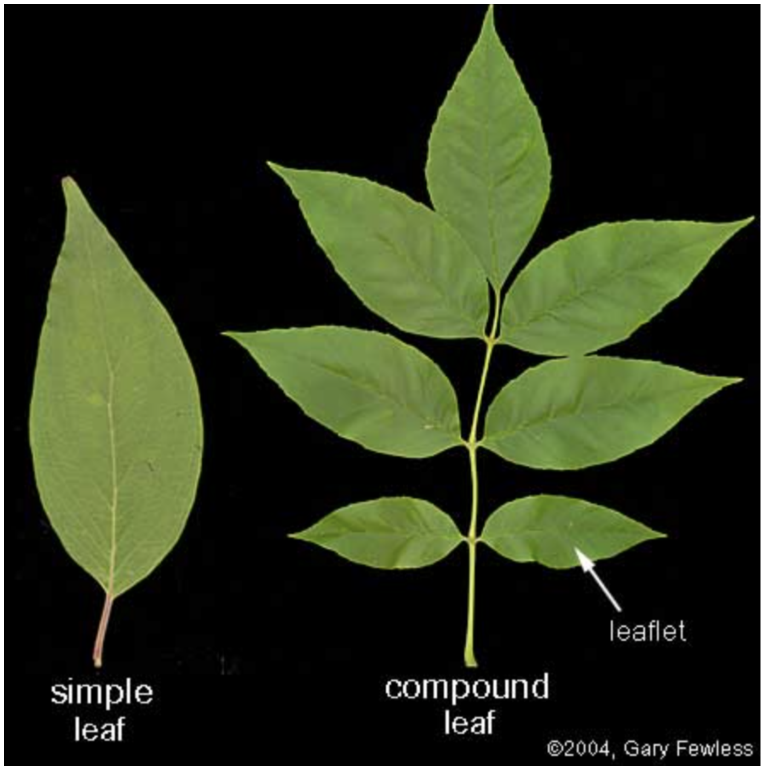

Figure 3. Two terms, simple and compound, describing the structure of leaves. This figure was obtained from the Cofrin Center for Biodiversity at the University of Wisconsin (https://www.uwgb.edu/biodiversity-old/herbarium/trees/simple_compound_leaves01.html)

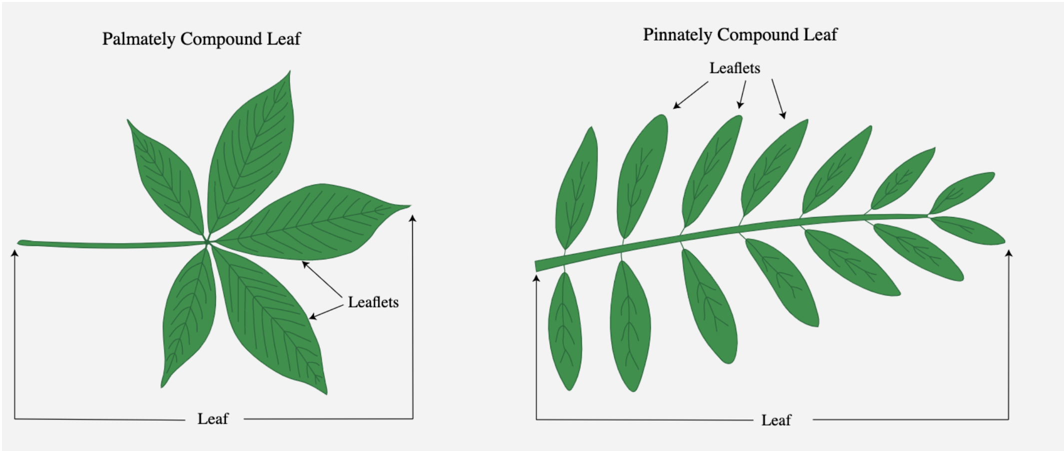

Figure 4. The differences between a palmately compound leaf and a pinnately compound leaf. This figure was obtained from the Indiana Nature Biology Glossary (https://www.indiananature.net/pages/glossary/p.php)

There are many different terms to describe the margin of the leaf, including entire, serrate, dentate, and crenate (Figure 5). Entire leaves have margins with no serrations or teeth. Serrate, dentate, and crenate leaves are considered toothed, or have different types of 'teeth'. Serrate margins are continuous, sharp, and resemble the blade of a saw. Dentate margins are generally outward-facing and continuous. Crenate is very similar to dentate, but crenate margins have more rounded teeth.

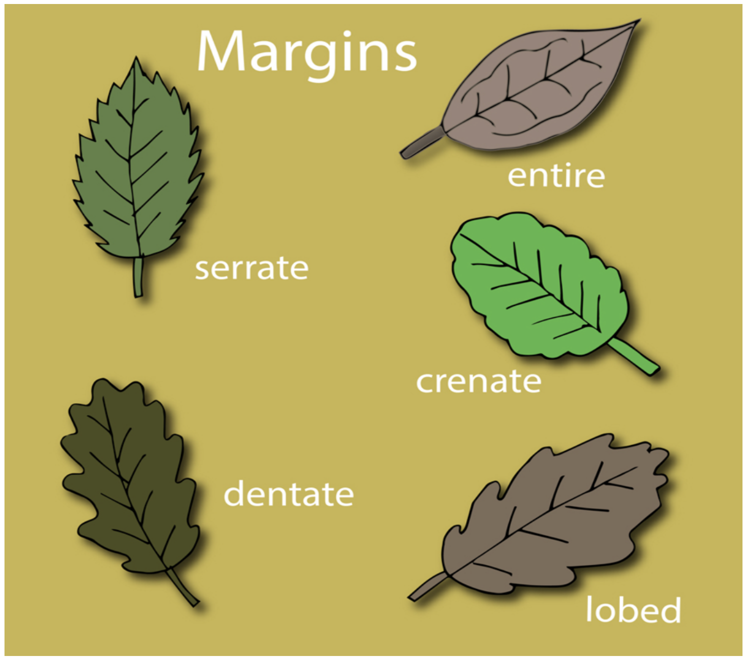

Figure 5. Terms to describe the margins of leaves. This figure was obtained from a blog post written by Nicole Elmer at the Biodiversity Center at the University of Texas at Austin (https://biodiversity.utexas.edu/news/entry/leaves).

There are two main ways to classify the attachment of leaves, petiolate or sessile (Figure 6). Petiolate leaves are attached from the leaf blade to the stem by a stalk, or petiole. Sessile leaves are connected straight to the plant stem.

Figure 6. Terms to describe the main two attachments to stems. This figure was obtained from the Laidback Gardener blog (https://laidbackgardener.blog/2018/01/08/plants-with-weird-foliage-perfoliation/).

There are many terms to describe the shape of a leaf (Figure 7). Ovate and obovate leaves are generally egg-shaped. Obovate leaves have the broader portion by the tip, while ovate leaves have the broader portion by the base. Elliptical leaves are shaped like an ellipse or a circle that has been stretched in one direction. Lanceolate leaves are wider at the base and taper towards both ends. Linear-shaped leaves refer to long and thin leaves, like grass. There are two different types of heart-shaped leaves, one with the lobes at the base of the leaf, cordate, and the other with the tip at the base of the leaf, obcordate. Understanding leaf morphology can be complicated and difficult, but some of these terms will come up later in this paper.

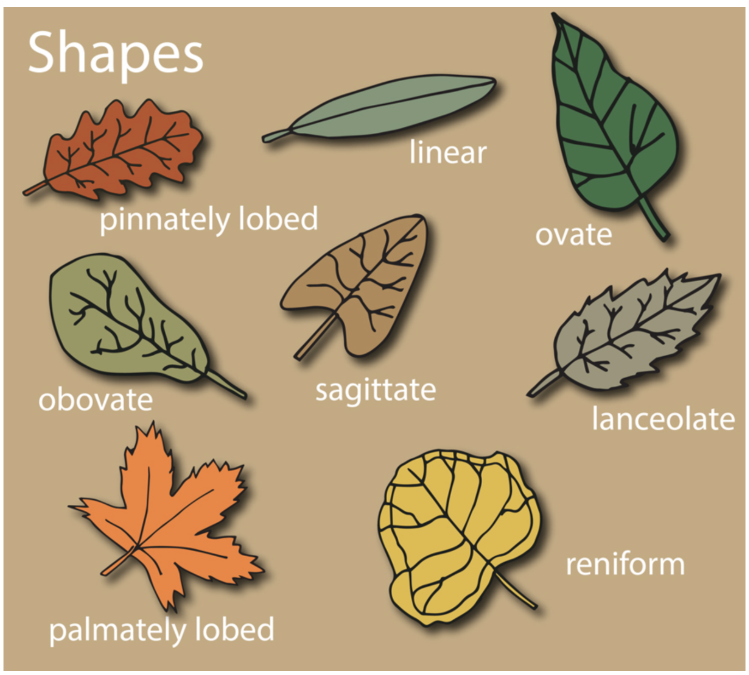

Figure 7. Some terms to describe the shape of leaves, and the difference between pinnately and palmately lobed leaves. This figure was obtained from a blog post written by Nicole Elmer at the Biodiversity Center at the University of Texas at Austin (https://biodiversity.utexas.edu/news/entry/leaves).

The Focal Family: Asteraceae

Taxonomy is the science of classifying organisms to construct systems with each organism placed into more and more inclusive groupings. The main taxonomic ranks are domain, kingdom, phylum, class, order, family, genus, and species. In this paper, I mainly focus on specimens within the Asteraceae family, previously known as Compositae. Plant families are separated and characterized by structural differences in flowers, fruits, and seeds. Some plant families can have hundreds or even thousands of members, while others can just have one or two members. Asteraceae is a family of flowering plants, also known as angiosperms. Angiosperms and gymnosperms are the two major groups of vascular seed plants, with angiosperms being bigger and more diverse. There are about 300,000 angiosperm species, and they represent about 80% of all living green plants currently (Britannica 2015). They have a wide variety of forms, ranging from herbaceous plants, like those in the family Asteraceae, to climbing vines (Britannica 2015). Some paleobotanists propose that the first members of Asteraceae may have evolved 50 million years ago, based on fossils that date to the Eocene Epoch, which lasted from about 56 to 33.9 million years ago (Britannica 2015). Other paleobotanists propose an earlier date for the evolution of the Asteraceae family, 83 million years ago.

Many genera within Asteraceae are known for their ornamental value (ex: *Tagetes,* marigolds), while other genera are known for being noxious weeds (ex: *Taraxacum,* dandelions), having large economic importance (ex: *Cynara,* artichoke), or for their production value (ex: *Helianthus,* sunflower*)* (Britannica 2015). The most distinctive feature of Asteraceae is its flowers. The flowers are grouped into compact heads that resemble individual flowers. The leaves are simple or occasionally compound, with an opposite or alternate arrangement (Britannica 2015). The members within Asteraceae produce an achene, which is a type of fruit that is characteristically dry, single-seeded, and does not open until maturity (Britannica 2015). Asteraceae is a large, diverse plant family, with an interesting history and interesting characteristics, including their unique flowers.

My Project

Climate change studies are becoming increasingly important as our climate continues to change, and its impacts continue to worsen. Understanding how ecosystems and organisms have changed or are changing is extremely important. In formulating this project, I knew I wanted to address a climate-related question using Drew's Herbarium. After some consideration, I decided on analyzing how leaf length and area have changed over time in response to changes in climate in 10 genera, *Ambrosia, Artemisia, Bidens, Cirsium, Eurybia, Euthamia, Eutrochium, Helenium, Solidago,* and *Symphyotrichum*. These genera are commonly known as ragweed, mugwort, beggartick, thistle, aster, goldentops, Joe-Pye weeds, sneezeweed, goldenrods, and asters, respectively. I hypothesized that leaf length and leaf area will decrease over time in response to increasing temperature and precipitation, due to the fact a shorter leaf size can better reduce excess water loss, preserve leaf water use efficiency, and maintain water transport. With this question and hypothesis in mind, I could consider several key questions, including how increased temperature and precipitation have impacted a plant family, how several genera have responded to changes in climate, and how different species and/or genera may respond to changes in climate differently.

Methods

Herbarium Cataloging

The Drew University Herbarium stores pressed botanical specimens that serve as a historical reference, a reference to identify recently collected specimens, an aid in teaching, and a research tool. Within this herbarium, there is a wide variety of specimens, totaling about 2,000. Most specimens are within the 1937 to 1984 range. From the 1980s on, there was no student or faculty member that actively used or contributed to the herbarium. There are specimens in this herbarium ranging from maples to asters to ivies, and there are both native and invasive specimens present. Within this herbarium, the location also varies. Some specimens were collected in Puerto Rico or in southern New Jersey, while others were collected in the Drew Forest. When I first decided to use this herbarium for my research project, it was unused for some time. It was unorganized, messy, and chaotic, but there were some benefits to this. I could organize it and utilize it in a way that makes sense for this project, but also in a way that allows students to use it for future research.

After looking through Drew's Herbarium, and organizing it in alphabetical order by family name, I selected a family to study based on the abundance of that specific family in Drew's Herbarium. I selected Asteraceae. Asteraceae is one of the largest plant families, with more than 1,260 genera (Hosch et al. 2008). This family, formerly known as Compositae, can be found all over the world. Members of this family are known for several different reasons ranging from ornamental wildflowers to food crops (Asteraceae). At Drew's Herbarium, Asteraceae was by far the most abundant family, with 217 specimens collected from 1937 to 1984. On most herbarium specimens at the Drew University Herbarium, there was an identification card attached, which included the specimen type, family name, previous catalog #, year collected, the person who collected this specimen, and where this specimen was collected. For each specimen in the Aster family, I placed all of this information into an Excel sheet. I also noted if there were any leaves, flowers, or fruits present on the particular specimen, and assigned it a new, unique catalog number (ex: EC0001). I then refined the dataset to include specimens only in Morris County and the surrounding counties. The specific genera in Asteraceae had to have 4 or more sample representatives to be included for later statistical analysis. This refining process led to a total of 98 specimens, in 10 different genera. The 10 genera included *Ambrosia*, *Artemisia*, *Bidens*, *Cirsium*, *Eurybia*, *Euthamia*, *Eutrochium*, *Helenium*, *Solidago*, and *Symphyotrichum*.

As I began working with the Drew University herbarium specimens, I learned that some old university herbarium specimens, specifically those from the 1800s to early 1900s, were treated with mercuric chloride as a preservative. This practice ceased in the 1960s (Webber et al. 2011). Mercuric chloride-treated samples may be a source of mercury exposure and contamination, which could be very hazardous to one's health. Extended exposure to mercuric chloride could cause gastrointestinal and behavioral effects, as well as central nervous system and kidney effects (Webber et al. 2011). I carefully handled these possibly treated specimens by wearing disposable gloves whenever handling pressings, closing the herbarium specimen cabinet immediately after use, not breathing the air in the cabinet directly, and keeping the workspace well-ventilated.

To add a 2021 component to my data, I went to the Great Swamp Watershed Association's Conservation Management Area located in Harding NJ. I collected 10 specimens, 8 from *Solidago* and 2 from *Symphyotrichum*. This collection occurred in July when Asters are not in flower, and again in October during their flowering time. All other Asteraceae specimens at Drew University were collected during their flowering time, August to October. These specimens were then pressed using a plant press, which contained a wooden frame, corrugated cardboard, blotter paper, and newspaper. The specimens were pressed for a week and then transferred over to presentation paper. A specific identification card was created for each specimen collected and pressed, which included specimen type, family name, EC catalog #, date collected, the person who collected the specimen, and where the specimen was collected. All of this information was then added to the master herbarium spreadsheet, mentioned previously.

To further increase my sample size from 108 specimens to 151 specimens, I included specimens from the Virtual Chrysler Herbarium at Rutgers University. I refined this data in the same way as before, I searched for specimens in the Asteraceae family collected in Morris County. This led to my final dataset (Table 1).

Genus	Number of Specimens
Ambrosia	6
Artemisia	5
Bidens	12
Cirsium	6
Eurybia	9
Euthamia	6
Eutrochium	6
Helenium	4
Solidago	46
Symphyotrichum	49

Table 1. The number of specimens used in this study within 10 genera located in Morris County. Specimens were collected using a combination of field sampling, Drew's Herbarium, and the Virtual Chrysler Herbarium.

Leaf Size Measurements

To perform the leaf length and area measurements, I used a program called ImageJ (https://imagej.nih.gov/ij/download.html). ImageJ is open-source Java-based software for processing and analyzing scientific images (Abràmoff et al. 2004). ImageJ can be used in a variety of fields in biology, ranging from molecular biology to ecology. In ecology, ImageJ can be used to analyze many different things, including the size of fish eggs (Is-haak et al. 2016) and local vegetation cover (Ricotta et al. 2014). ImageJ can also be used to measure leaf size and leaf area (Gao et al. 2011, Li et al. 2020, Stropp et al. 2017).

Before performing any length or area measurements, the image type was changed from RGB to 8-bit. This conversion from RGB to 8-bit made it easier for the software to analyze the pixels, which helped in calculating length and area using this program. Another task that had to be accomplished before performing any measurements, was setting the ruler to scale. Without setting the scale to centimeters or millimeters, the program would give back measurements in pixels, which is not helpful for this study.

I selected three mature leaves in the specimen photo. I did not choose the most basal or terminal leaves as these leaves may be at abnormal stages in their maturity, especially those near the tip. I selected one leaf from the bottom, one leaf from the middle, and one leaf from the top. I made sure that nothing was obscuring the view for any of the selected leaves. If there was an obstructed view for any of these leaves, I moved either one leaf to the top, to the bottom, or to the side.

To begin leaf length measurements, I used the straight line tool and drew a line from the petiole to the tip of the leaf, then clicked measure. For the next two leaf measurements, I drew a line from the petiole to the tip and used Command M as a shortcut. The Results window showed

3 length measurements, which were then averaged for a final leaf length for this specific specimen.

To begin leaf area measurements, I clicked on the rectangle tool and placed a rectangle around the leaf of interest. I duplicated this image so that the specific area came into a separate window. Then, I adjusted this image using a technique called thresholding. Thresholding is an image processing technique for dividing an image into two classes of pixels typically called foreground and background. By using the rectangle tool, I highlighted pixels in the image of interest, which designated all pixels as either part of the area of the leaf or not. This thresholding process makes it easier for the program to calculate area. The program analyzed the particles and displayed area results for one leaf. I repeated this process for the other two leaves, which were then averaged together for a final leaf area for this specific specimen.

For a more technical overview of how to use ImageJ for leaf length and area measurements, including pictures of the program and tools, please refer to Appendix A.

Climate data

I obtained climate data, specifically monthly maximum and minimum temperatures, and monthly precipitation data, by using a combination of Weather Underground's data archive, NOAA's historical database, and the New Jersey State Climatologist's historical monthly climate tables. To use Weather Underground (https://www.wunderground.com/history), I searched for historical data by typing in the zip code for Madison, NJ, and putting in the appropriate date. The closest weather station to Madison is the Newark Liberty International Airport Station. Weather Underground provided temperature data (maximum temperature, average temperature, minimum temperature), dew point, precipitation (in inches), wind and gust wind speeds, and sea level pressure. I was only interested in temperature and precipitation data for this study. For certain years, specifically more recent years, Weather Underground also provided daily observations for each day of the month. To further look at maximum and minimum temperatures and precipitation in New Jersey as a whole, I reviewed the Historical Monthly Climate Tables from the Office of the New Jersey State Climatologist (https://climate.rutgers.edu/stateclim_v1/nclimdiv/).

I utilized NOAA's historical database by using their Climate Data Online Search tool (https://www.ncdc.noaa.gov/cdo-web/search). I selected the global summary of the month, then selected the specific date range. I searched for the closest station, which was Newark Liberty International Airport Station. After filling in these search parameters, I then selected the correct data set and added it to my cart. I reviewed the order and submitted my data request. I then received an email with a PDF from NOAA detailing all of the data I requested. This PDF contained temperature data (average temperature, average maximum temperature, average minimum temperature) and precipitation data (greatest observed, total fall per month, number of days).

Results

Herbarium specimens, from 1886 to 2020, and field specimens, collected in 2021, were studied and analyzed. All specimens were collected in Morris County, NJ. Drew University's This region has a fairly moderate climate with cold winters and warm, humid summers. Morris County, NJ receives higher than national average precipitation. Annual mean maximum and minimum temperatures and mean annual precipitation data were compiled from 1886 to 2021. A correlational analysis was performed to determine if these climate parameters varied as a function of year. Maximum (Pearson correlation: $r(n = 23) = 0.44$, $b = 0.02$, $p = 0.04$) and minimum temperatures (Pearson correlation: $r(n = 23) = 0.56$, $b = 0.03$, $p = 0.01$) were significantly correlated with the year (Figure 8). Precipitation was not significantly correlated with year (Figure 9; Pearson correlation: $r(n = 23) = 0.10$, $p = 0.65$). Leaf length (Figure 10) and leaf area (Figure 11) of these specimens were then measured using ImageJ. Descriptive statistics of leaf length and leaf area are provided in Table 2.

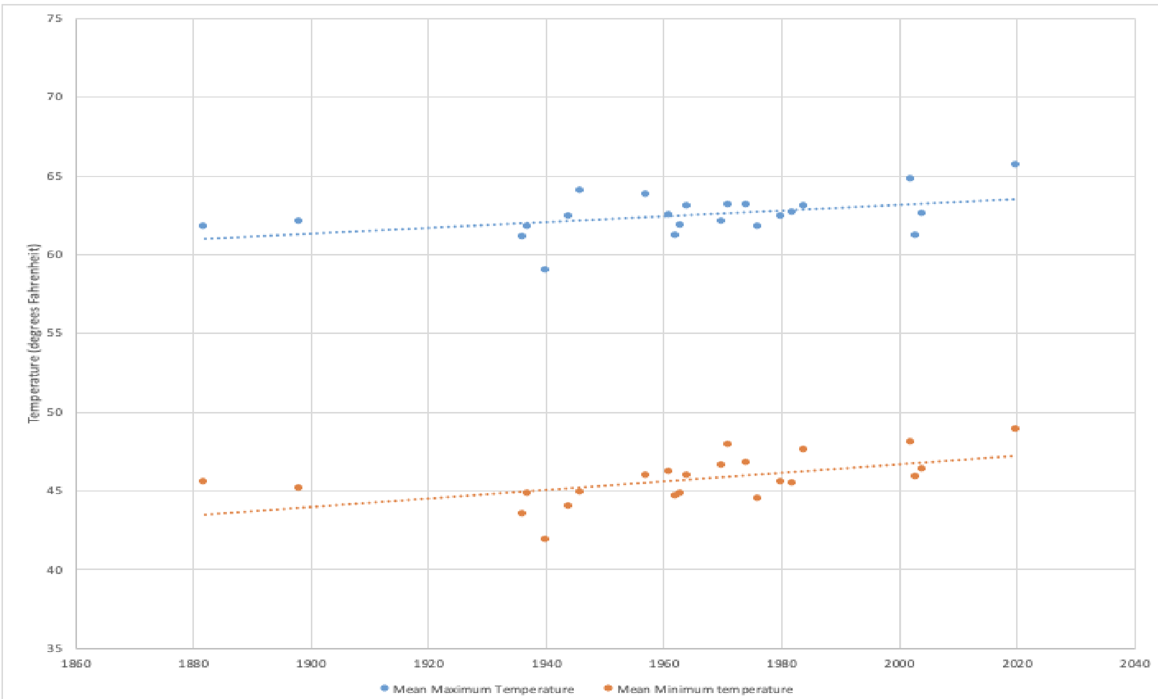

Figure 8. Annual mean maximum and minimum temperatures in degrees Fahrenheit from 1882 to 2020.

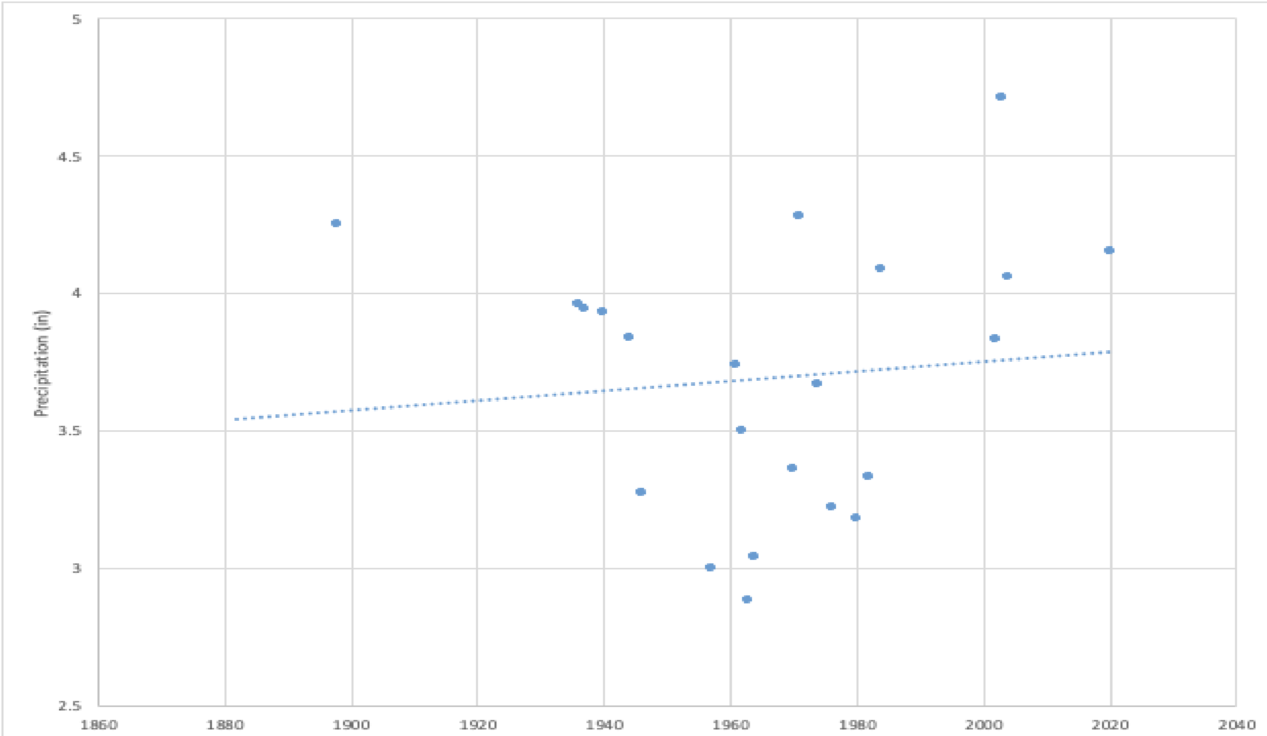

Figure 9. Annual mean precipitation data in inches from 1898 to 2020.

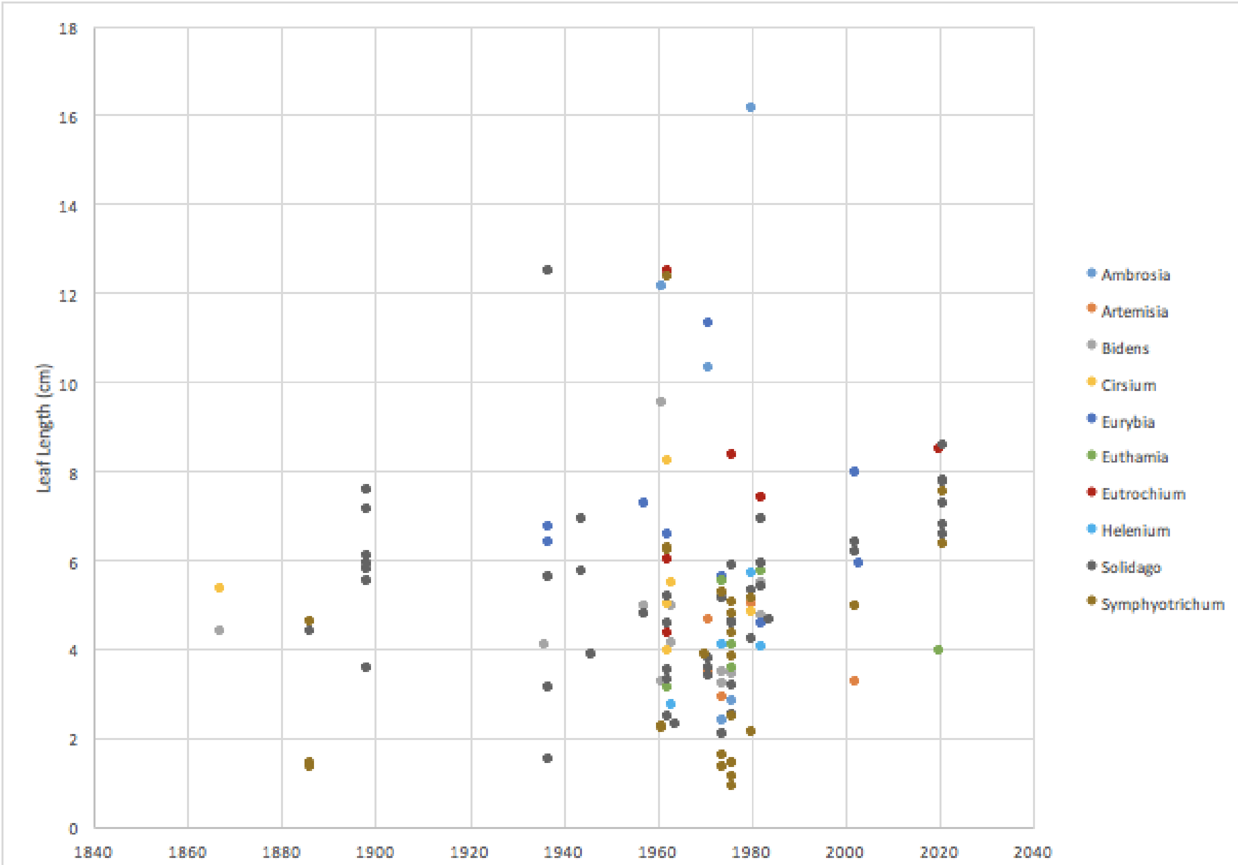

Figure 10. Leaf length measurements from 1886 to 2021 for 10 genera within the Asteraceae family using ImageJ software.

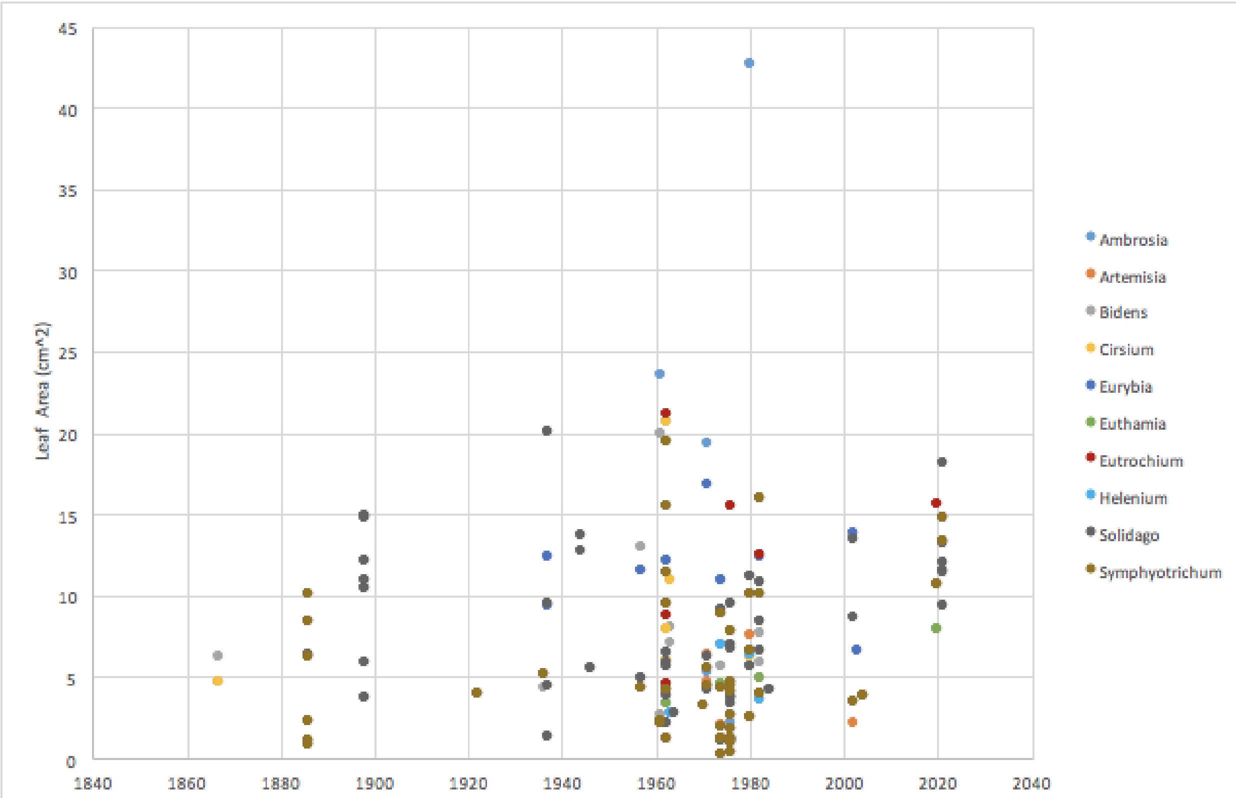

Figure 11. Leaf area measurements for genera *Ambrosia, Artemisia, Bidens, Cirsium, Eurybia, Euthamia, Eutrochium, Hemenium, Solidago, Symphyotrichum* over a 135 year period.

Genus		N	Minimum	Maximum	Mean	Std. Deviation
Ambrosia	Length	7	2.375	16.147	7.16929	5.607129
	Area	7	1.099	42.619	13.72543	15.627202
Artemisia	Length	4	3.242	4.998	4.08600	.860526
	Area	4	2.131	7.543	5.18100	2.358796
Bidens	Length	12	3.235	9.539	4.63725	1.713156
	Area	12	2.643	19.900	7.39375	4.758828
Cirsium	Length	6	3.951	8.217	5.47233	1.449491
	Area	6	4.729	20.615	9.42000	5.883769
Eurybia	Length	9	4.576	11.321	6.93033	1.911523
	Area	9	6.635	16.808	11.77522	2.805353
Euthamia	Length	6	3.147	5.719	4.32583	1.052227
	Area	6	3.420	7.906	4.73500	1.665544
Eutrochium	Length	6	4.348	12.492	7.84633	2.758344
	Area	6	4.624	21.094	13.01500	5.778537
Helenium	Length	4	2.733	5.696	4.13075	1.213685
	Area	4	2.799	6.933	4.94575	2.041145
Solidago	Length	40	1.518	12.481	4.87160	1.937105
	Area	40	1.045	20.096	7.57150	4.248625
Symphyotrichum	Length	47	.919	12.341	3.89189	2.228327
	Area	47	.294	19.441	4.94862	4.205269

Table 2. Descriptive statistics for leaf length and leaf area for each genus.

Changes in Leaf Area and Length as a Function of Year

A correlational analysis was performed to determine if the leaf length or area varied as a function of year for all ten genera combined. The results were not statistically significant for both leaf length (Pearson correlation: $r(n = 141) = 0.93$, $p = 0.45$) and leaf area (Pearson correlation: $r(n = 141) = -0.01$, $p = 0.46$). Correlational analyses were then performed for each genus. One genus, *Euthamia*, showed a statistically significant increase in leaf area, but not leaf length (Pearson correlation: $r(n = 5)= 0.96$, $b = 0.08$, $p < 0.005$), which was consistent with the correlations against climate variables. According to the results of the correlation analysis, for *Euthamia*, the leaf area increased over time but the leaf length did not change systematically over time (Figure 20). For the other genera, there were no statistically significant correlations with leaf area or leaf length over time, except for *Solidago*. *Solidago,* showed a negative trend between year and leaf area (Figure 12; Pearson correlation: $r(n = 40) = -0.26$, $p = 0.05$).

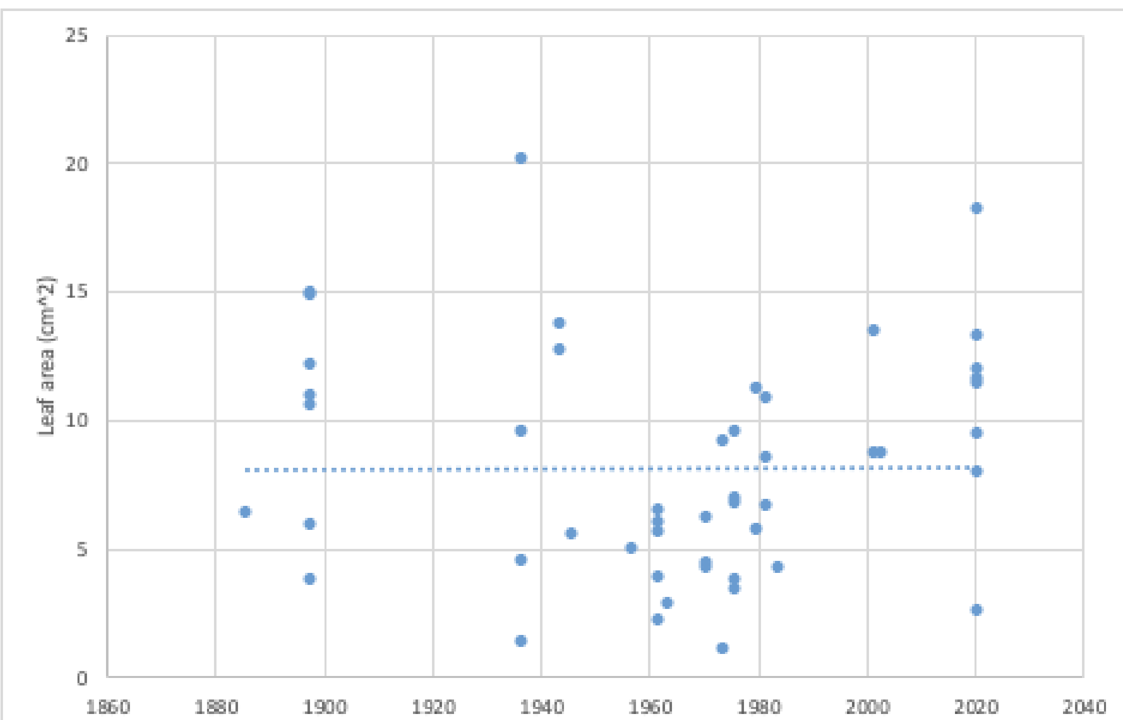

Figure 12. Leaf area measurements for the genus *Solidago* from 1886 to 2021.

Correlational analyses were then performed for 30-year time periods to determine if there were any differences depending on the time period. The 30 years analysis served to identify if there was a 'seesaw pattern' amongst the specimens, if for one period there was an increase, while in the next time period there was a decrease. This chunked analysis also served to analyze possible trends on a smaller scale, a more restricted time period. This analysis was performed from my own curiosity, to see if shorter term changes in climate may have an impact on plant responses. I could not find another study that broke down their analysis in this way. For periods 1860-1890, 1890-1920, 1920-1950, and 2010-2040, there were not enough specimens present to analyze, or all of the specimens were collected in the same year. From 1950-1980, *Ambrosia* showed a statistically significant decrease in leaf length (Figure 13; Pearson correlation: $r(n = 6) = -0.848$, $b = -1.57$, $p = 0.02$) and a statistically significant increase in area (Figure 14; Pearson correlation: $r(n = 6) = -0.84$, $b = -0.68$ $p = 0.02$). *Symphyotrichum* also showed a statistically significant decrease for both leaf area (Figure 22; Pearson correlation: $r(n = 30) = -0.47$, $b = -0.28$, $p = 0.01$) and leaf length (Pearson correlation: $r(n = 30) = -0.45$, $b = -0.14$, $p = 0.01$). For the period 1980-2010, *Solidago* and *Symphyotrichum* did not show any statistically significant changes in leaf length or area.

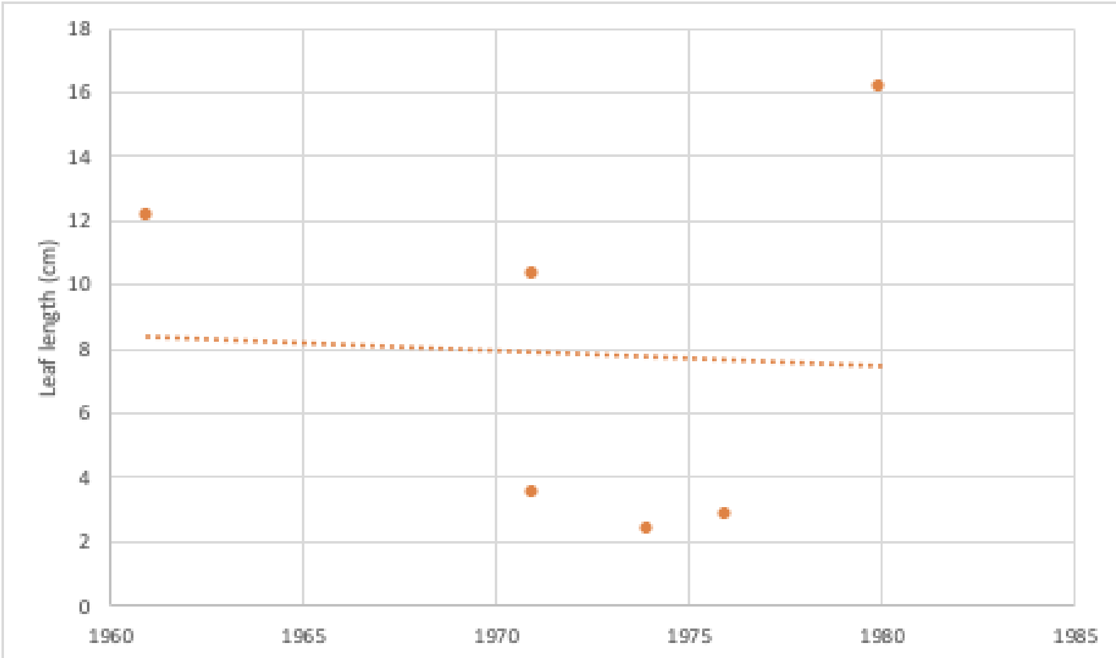

Figure 13. Leaf length measurements for *Ambrosia* in a 30-year period from 1950 to 1980.

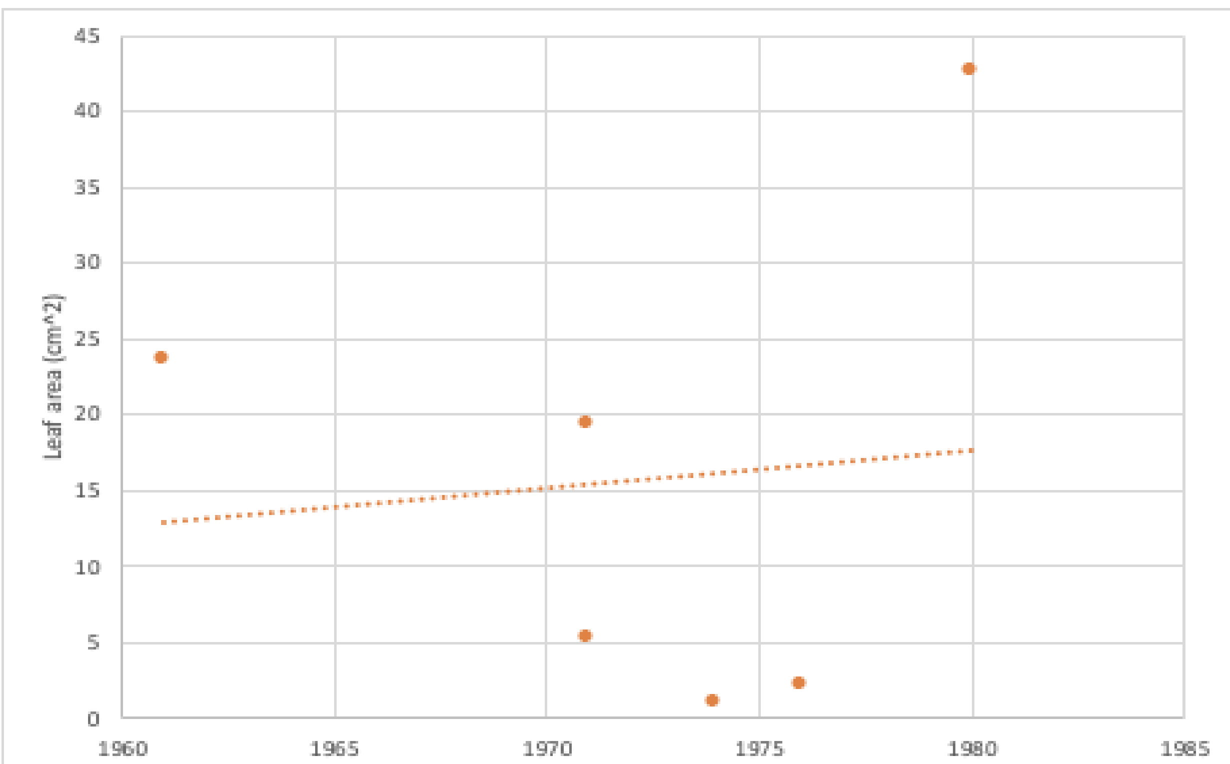

Figure 14. Leaf area measurements for *Ambrosia* from 1950 to 1980.

Changes in Leaf Area and Length as a Function of Climate

A correlational analysis was performed for all specimens and by genus to determine if leaf size varied as a function of three climate variables, annual mean maximum temperature, annual mean minimum temperature, and annual mean precipitation. Mean maximum temperature seems to be the best indicator for change over time, and those results will be emphasized in this section. The results for all specimens taken together were not statistically significant for both leaf length and leaf area. When conducted separately by genus, *Euthamia,* showed a statistically significant increase in leaf area across all three variables, mean maximum temperature (Figure 15; Pearson correlation: $r(n = 6) = 0.98$, $b = 1.01$, $p = < 0.005$), mean minimum temperature (Pearson correlation: $r(\mathrm{n} = 6) = 0.94$, $b = 0.90$, $p < 0.005$), and mean precipitation (Pearson correlation: $r(n = 6) = 0.86$, $b = 4.03$, $p = 0.01$). *Artemisia* showed a statistically significant decrease in leaf area as a function of mean maximum temperature (Figure 16; Pearson correlation: $r(n = 4) = -0.95$, $b = -2.04$, $p = 0.03$). From further analysis of *Solidago*, there was no trend, as there was by year, or significant correlation as a function of the three climate variables, mean maximum temperature, mean minimum temperature, and precipitation.

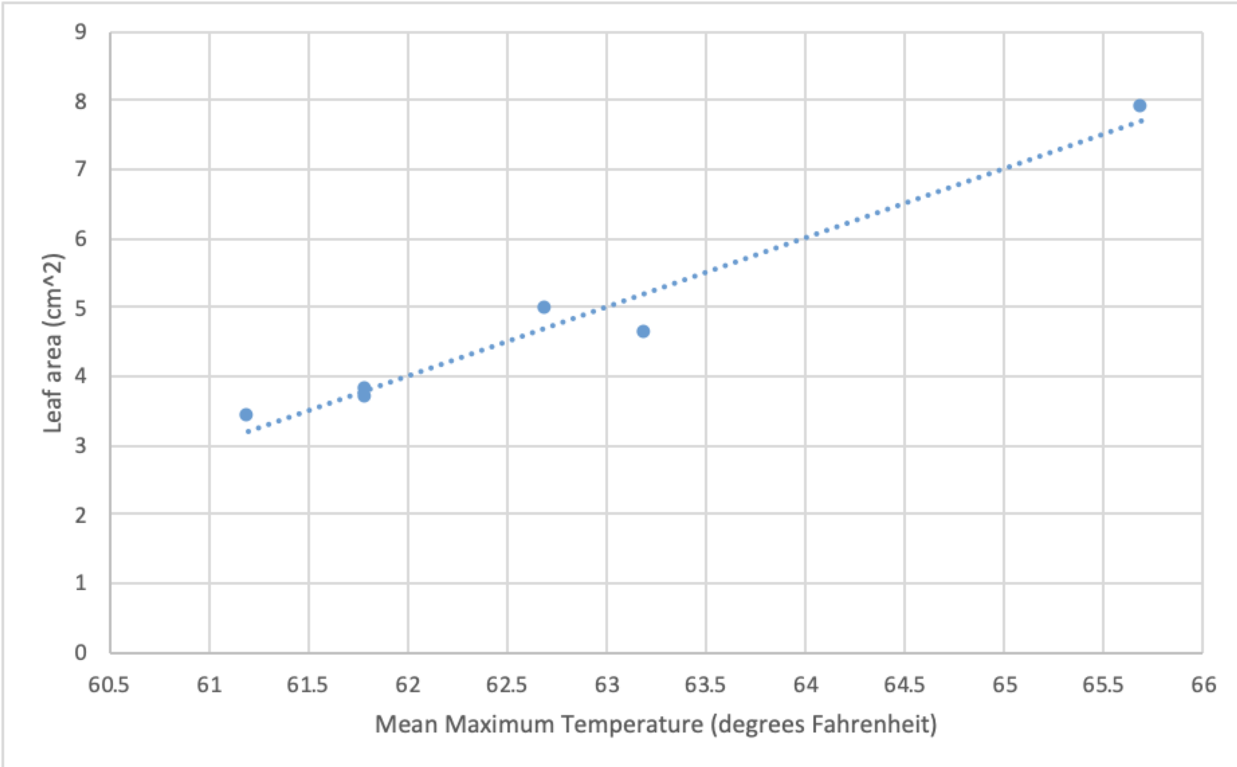

Figure 15. Leaf area measurements for *Euthamia* in relation to annual mean maximum temperatures.

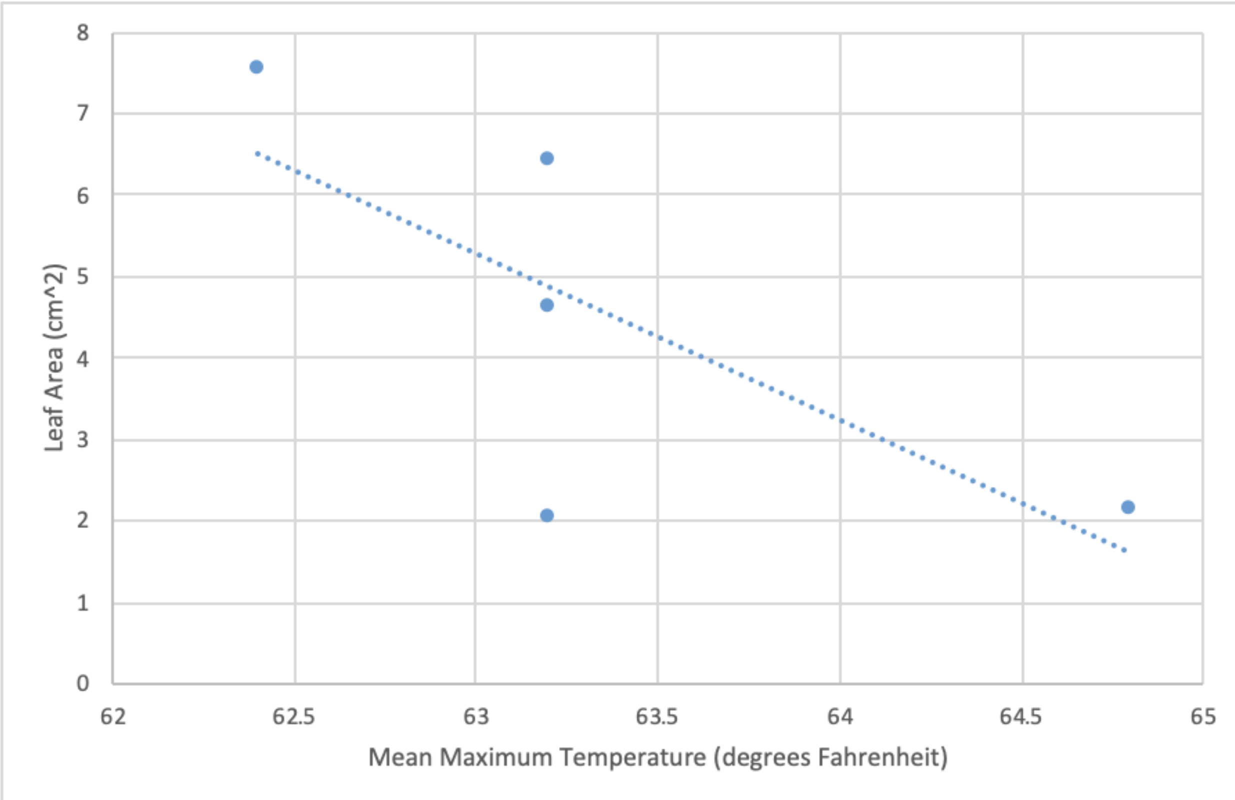

Figure 16. Leaf area measurements for the genus *Artemisia* as a function of mean maximum temperature.

Correlational analyses were then performed for 30-year time periods to determine any differences depending on the time period. For the period 1950-1980, two genera, *Euthamia* and *Symphyotrichum,* showed statistically significant results as a function of temperature. *Euthamia* showed a statistically significant increase in leaf length (Figure 17; Pearson correlation: $r(n = 4) = 0.98$, $p = 0.01$) and leaf area (Figure 15; Pearson correlation: $r(n = 4) = 0.99$, $b = 0.76$, $b = 1.37$, $p < .005$) as a function of mean maximum temperature, and a statistically significant increase in leaf area as a function of mean minimum temperature (Pearson correlation: $r(n = 4) = 0.93$, $b = 1.09$, $p = 0.04$). *Symphyotrichum* showed a statistically significant decrease in leaf length (Figure 18; Pearson correlation: $r(n = 30) = -0.31$, $b = -1.17$, $p = 0.05$) and leaf area

(Figure 19; Pearson correlation: $r(n = 30) = -0.33$, $b = -2.18$, $p = 0.04$) as a function of mean maximum temperature.

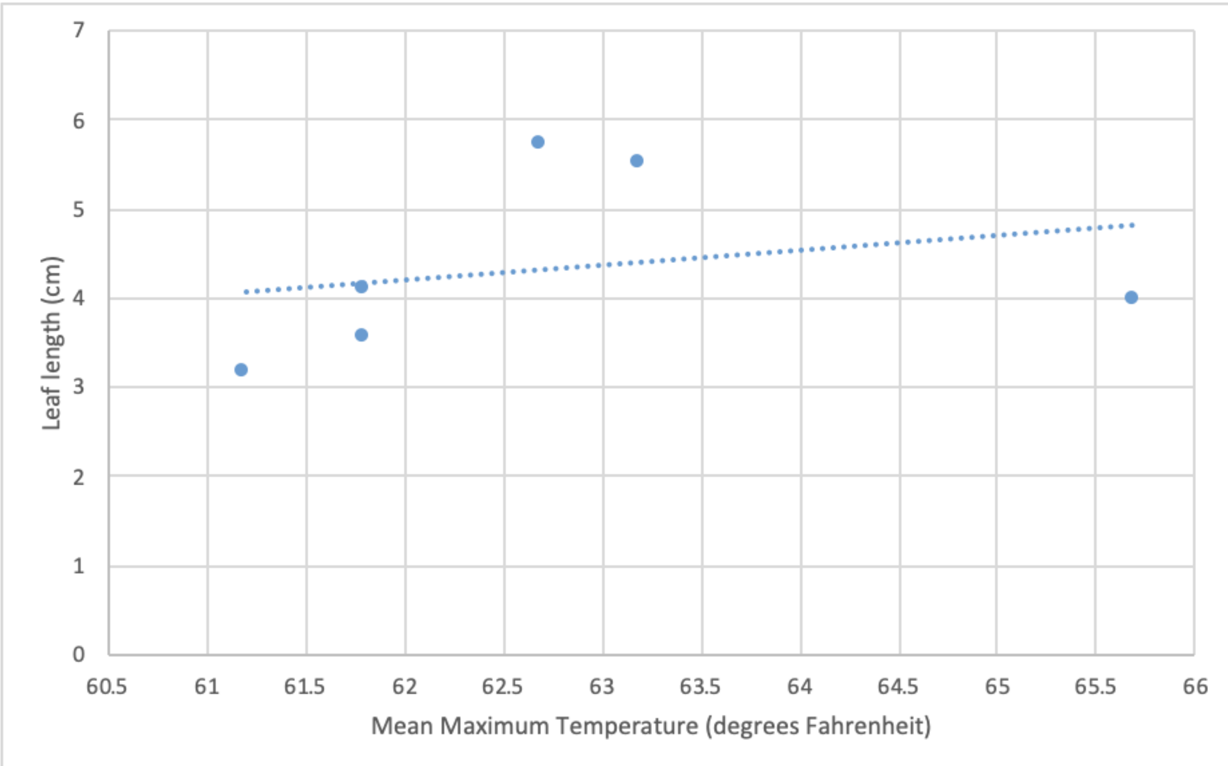

Figure 17. *Euthamia* leaf length measurements in relation to a climate variable, mean maximum temperature.

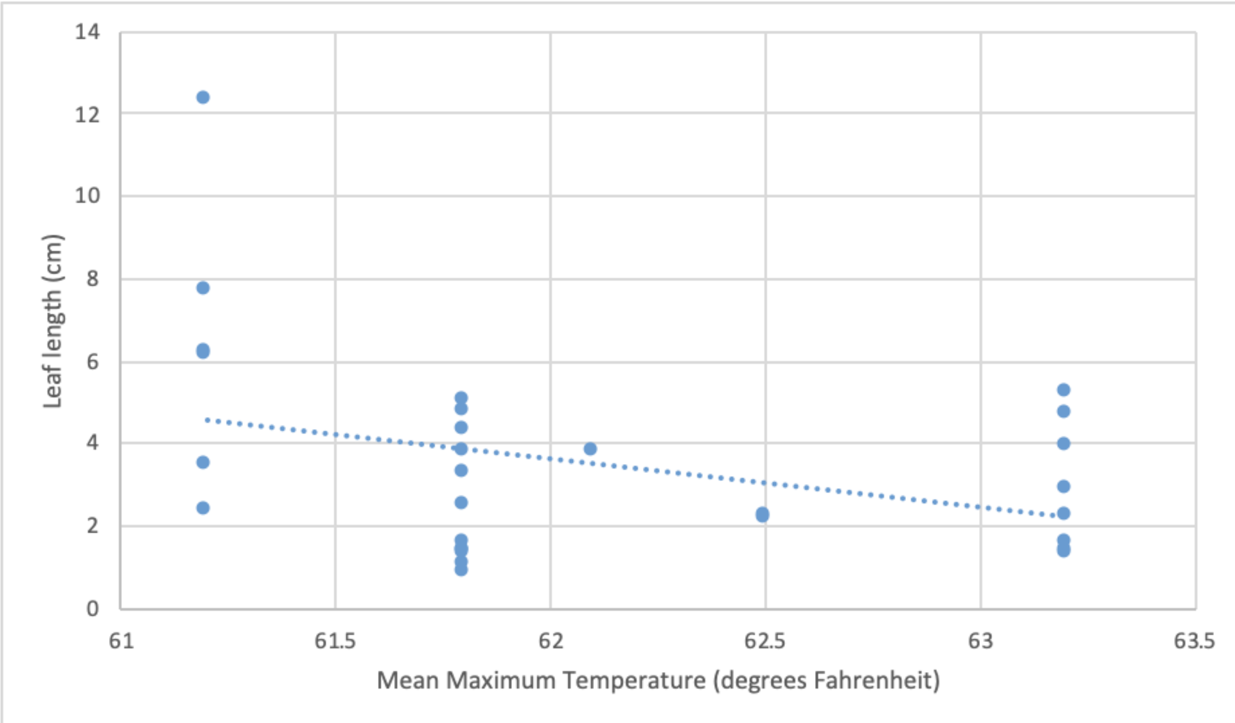

Figure 18. *Symphyotrichum* leaf length measurements in relation to a climate variable, mean maximum temperature.

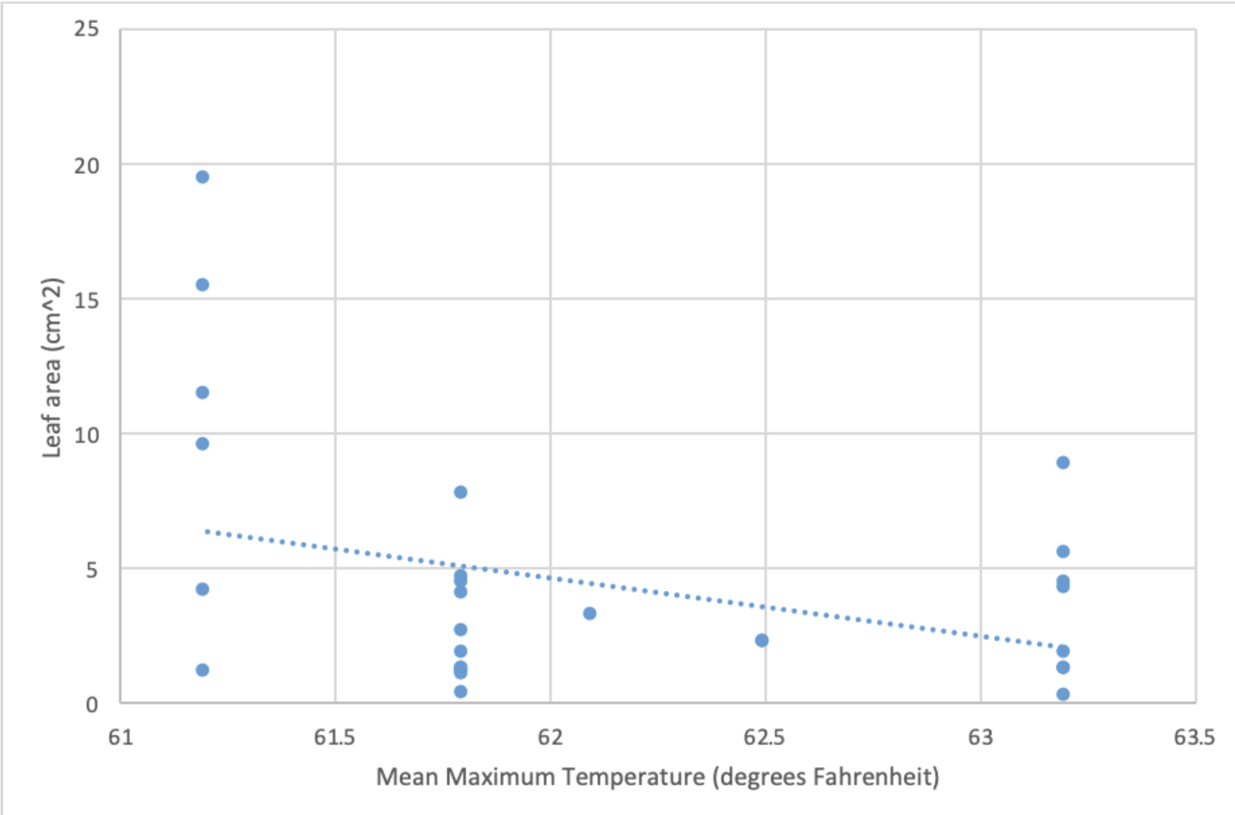

Figure 19. Leaf area measurements for one genus, *Symphyotrichum,* as a function of one climate variable, mean maximum temperature.

There were two genera that specifically stood out, in terms of substantial correlations and/or decent sample size, as a function of both year and climate variables. To fully visualize these relationships together, Figures 20 & 21 show leaf area measurements for *Euthamia* and *Symphyotrichum*, respectively, and mean temperatures as a function of year. *Symphyotrichum* also showed a decrease in leaf length as a function of mean maximum temperature and year (Figure 22).

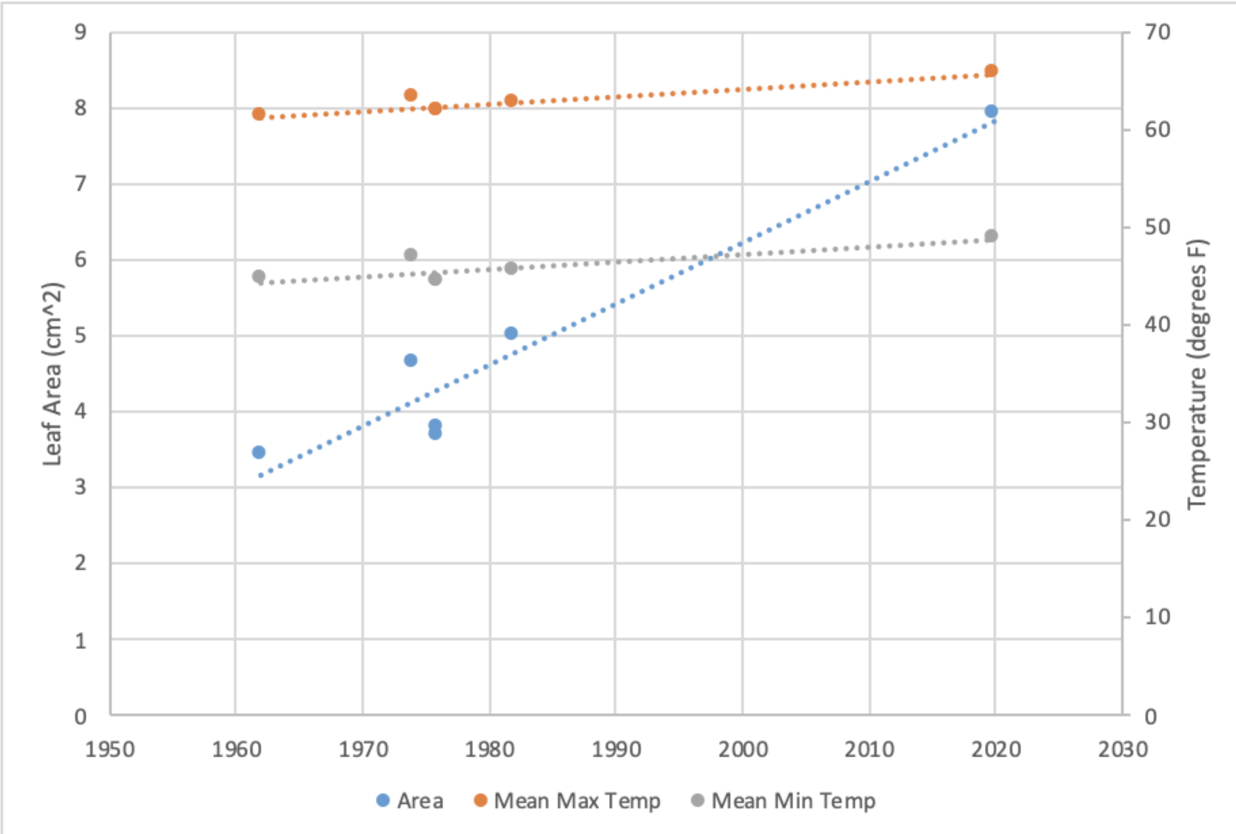

Figure 20. Leaf area measurements for *Euthamia* and mean temperatures as a function of year.

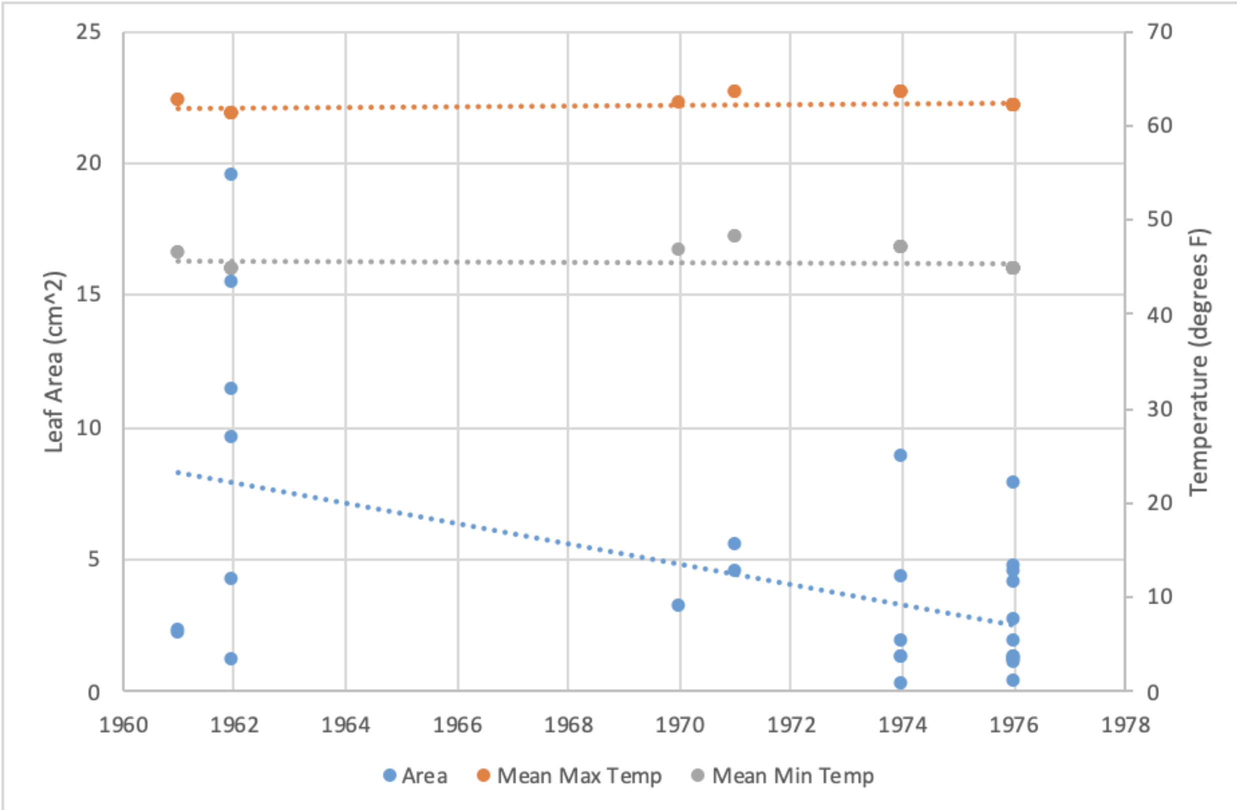

Figure 21. Leaf area measurements for *Symphyotrichum* and mean temperatures as a function of year.

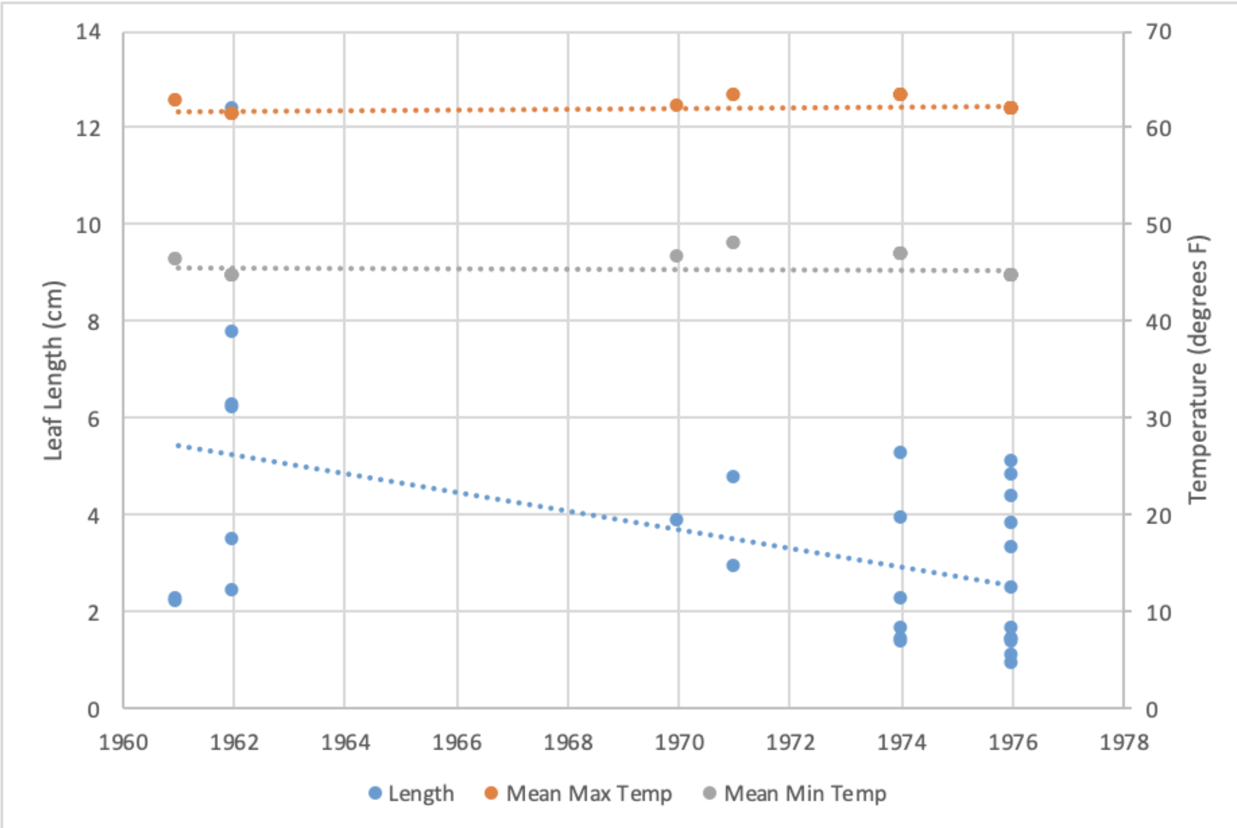

Figure 22. Leaf length measurements for *Symphyotrichum* and mean temperatures as a function of year.

In this study, correlations between year and length, year and area, length and climate, and area and climate were performed for each genera. From running several tests on the same sample set, I acknowledge that there is an increase in the likelihood of getting significant results by chance, but these results still highlight some interesting patterns that are worthy of future research. Had I corrected for multiple correlations by subgroup, most of the results would not have been statistically significant, excluding the correlations for the genus *Euthamia*.

Discussion

Change in Leaf Size as a Function of Time or as a Function of Climate

In my statistical analysis, I ran several tests against two different general independent variables, year and climate. For by-year analyses, there were several correlations or trends for different genera, including *Euthamia* and *Solidago*, and *Ambrosia* and *Symphyotrichum* from 1950 to 1980. To better test my hypothesis I ran the same tests for all specimens, by genera, and in chunked time periods for three climate variables, annual maximum temperature, minimum temperature and mean precipitation. Here, there were also some correlations that supported increases or decreases in leaf size that may have been due to changes in climate over time, specifically for the genera *Euthamia* and *Symphyotrichum*. Out of the three climate variables, the maximum temperature may be the dictating variable in measuring the impact of climate on leaf size over time. In two studies, the authors explore the negative correlation between leaf width and mean maximum temperature (Hill et al. 2014) and latitude (Guerin et al. 2012). Leaf width varies with environmental and climatic gradients, with leaves being narrower in hotter regions and higher elevations. Narrower leaves have a thin boundary layer that helps increase heat loss, which is favorable in hotter environments (Hill et al. 2014). More studies are needed to investigate the relationship between size, specifically area, and maximum temperature. Studying changes in climate over time and using this as a basis for analysis can be challenging, considering the general warming trend is not perfectly linear. Even though climate change studies can be tough, they are extremely important to help us understand its impacts on our surrounding world.

***Euthamia:* Increase in leaf area overall**

One genus, *Euthamia,* showed a statistically significant increase in leaf area overall, from 1886 to 2021. *Euthamia* had a small sample size, $n = 5$, but the correlation was strong for an increase in leaf area ($r > 0.9$) as a function of year and all three climate variables. More length and area measurements should be taken for this genus, but this research can show an initial indication of an increase in leaf area over time. A larger leaf may be favored in a warmer environment because they allow for greater transpiration rates (Fleming & McCormack 2012). As temperature and precipitation increase, I hypothesized that leaf length and leaf area would decrease due to the fact a smaller leaf can better reduce excess water loss and maintain water transport. My hypothesis was not supported in this case. I have to further look at factors that could potentially have an impact on leaf area over time, including increasing CO2 levels and soil quality.

Before I look at potential environmental factors that could have influenced the growth of specimens within this genus, I first would like to analyze their general morphology and distribution. Species within this genus are native to North America and Mexico and have been introduced in Europe and Asia (Flora of North America 2020). *Euthamia* was originally included in *Solidago*, but further analysis of their leaf arrangements, leaves, and DNA, warranted that *Euthamia* should be treated as distinct from *Solidago*. Leaves within *Euthamia* are alternate and sessile, with varying shapes and entire margins (Flora of North America 2020).

The global environment is changing with increasing temperatures and increasing atmospheric carbon dioxide levels (Morrison & Lawlor 1999). While plants may benefit from elevated carbon dioxide levels, they could suffer from drought and heat stress, indicating that increasing global CO_2 levels could have both positive and negative impacts on plants (Qaderi et al. 2006). The continuously increasing carbon dioxide levels are affecting different ecosystems and organisms in different ways. From increasing temperature and CO_2 in three herbaceous plants native to Britain, *Sarracenia minor*, *Lotus corniculatus*, and *Persoonia media*, there was an increase in leaf size, mainly due to increased cell expansion and increased cell numbers per leaf (Morrison & Lawlor 1999). The response to increasing CO_2 levels can differ for each plant, this can depend on the plant's age (Radoglou & Jarvis 1990), and metabolism (Long 1991). Elevated CO_2 is often reported to increase whole plant leaf area, but plant responses to carbon dioxide are not easily predictable and critically depend on other environmental factors and on the species (Ackerly et al. 1992). Leaf size, in the case of this study, could potentially have been impacted by increasing CO_2 levels over time, but this is hard to pinpoint. With increasing temperatures over time, carbon dioxide levels have also been increasing. Was this leaf area increase in *Euthamia* due to temperature or due to CO_2 levels?

Ecologists have recognized that plants with nutrient-deficient soils tend to have smaller leaves (McDonald et al. 2003). This would also be conversely true, plants with nutrient-rich soils tend to have larger leaves. The excess nutrients present in this type of soil would allow for more growth to happen, there is no limiting factor. In nutrient-rich soils, plants tend to produce more nutrient-rich litter, which in turn sustains high levels of soil fertility and can do so for an extended period of time (Ordoñez et al. 2009). Predicting the effects of changes in nutrient availability on plant productivity is one of the greatest uncertainties of future climate change predictions. For instance, global change is very likely to influence soil nutrient availability on relatively small time scales (3–5 years) and plant species composition at longer time scales (Ordoñez et al. 2009). *Euthamia* specimens may have grown in a high nutrient environment, compared to other specimens collected.

In addition to environmental factors, genetic factors may also affect leaf size. In a fixed environment, the final size of leaves is constant, which implies that plant organ growth is tightly controlled by genetic factors (Gonzalez et al. 2010). Several genes have been described that when down-regulated or abnormally expressed can increase leaf size (Gonzalez et al. 2010). In a 2009 study, Gonzalez et al. investigated the genes leading to the formation of larger leaves in *Arabidopsis thaliana,* a small flowering plant native to Eurasia and Africa (Gonzalez et al. 2009). There were genes present, including those in transcriptional regulation, protein synthesis and modification, hormonal regulation, and cell wall extension that led to the formation of larger leaves (Gonzalez et al. 2009). In this specific study, the focus was on biomass production, but I think that on an individual level, some organisms may be genetically predisposed to larger or short leaves, depending on what genes are overexpressed, mutated, or underexpressed. For this study, I am less concerned with the genetic factors, but I wanted to consider them as a possibility for an increase in leaf size, a result that I did not necessarily anticipate. In *Euthamia*, there may be genes that are favored in certain environmental conditions, which led to the increase in leaf size in this study.

***Symphyotrichum, Ambrosia, Solidago:* Decrease in leaf length and/or area**

Three genera, *Symphyotrichum, Ambrosia,* and *Solidago,* showed a decrease or trended downwards in regards to leaf length and/or area over time. Specifically, *Symphyotrichum* and *Ambrosia* showed a statistically significant decrease from 1950 to 1980 for both leaf length and leaf area. In relation to climate, *Symphyotrichum* showed a statistically significant decrease in leaf length and leaf area as a function of mean maximum temperature, while *Ambrosia* did not show significant results as a function of climate. More length and area measurements should be taken for this genus, but this research can show an initial indication of a decrease in leaf length and an increase in leaf area over time, but may not be indicative of changing as the climate changes. Also, with *Ambrosia* leaf area, there was one outlier that may have skewed the data. Without this point, it looks like there would be a decrease in leaf area over time, and that just shows how important having a larger sample size is. *Solidago* showed a downwards trend, not a statistically significant decrease, for only leaf area over 135 years. This genus had an *r*-value between 0.5 and 0.1, which is a strong effect. With more measurements, there may be a statistically significant decrease in leaf area. From further analysis against three climate variables, *Solidago* did not show the same trend. From this, we can infer that this genus may not be as tied to changes in climate. There are many other variables to be accounted for and many other environmental pressures that may influence a decrease in leaf area. As temperature and precipitation increase, I hypothesized that leaf length and leaf area would decrease due to the fact a smaller leaf can better reduce excess water loss and maintain water transport. Leaf size influences a range of physiological processes, including transpiration, and varies with many environmental factors, including water availability, nutrient levels, and other stressors (Yates et al. 2010, Li et al. 2015). Leaf size is also controlled by heredity, which can be seen amongst several of the same species with different leaf sizes in the same environment (Parkhurst & Loucks 1972).

From herbarium studies, not all environmental factors are known or accounted for in the identification card. Since I am focusing on a genus level, there may be several differences between the morphology of *Symphyotrichum, Ambrosia,* and *Solidago. Symphyotrichum*, a genus with over 100 species, is mostly native to North America, with a few species being endemic or introduced to the West Indies, South America, and Europe (Flora of North America 2020). *Symphyotrichum*'s taxonomy can be difficult, which makes it challenging to make conclusions about this genus. Species within this genus are usually heterophyllous, having more than one type of leaf on the same plant. Individuals often vary in leaf shape and can vary considerably in plant size and genetic diversity (Flora of North America 2020). *Ambrosia,* more commonly known as ragweeds, are distributed in the tropical and subtropical regions of the Americas. Several species have been introduced to nonnative locations and have become problematic invasives. The leaves can be arranged alternately, oppositely, or both (Flora of North America 2020). The leaf blades come in different shapes, ranging from lanceolate to ovate, and they are often either palmately or pinnately lobed (JSTOR Global Plants 2005). Ragweed pollen is a common allergen, causing about half of all cases of pollen allergies in North America (Taramarcaz et al. 2005). *Solidago,* more commonly known as the goldenrods, is a genus of about 100 to 120 different species. They are mostly native to North America, with a few in South America and Eurasia (Flora of North America 2021). All *Solidago* species are herbaceous perennials and can be difficult to distinguish from each other due to their similar flowers. Leaf shape and arrangement can differ from species to species as well. The leaf margins are often entire, but some species have serrate margins. In some species, the basal, or most bottom, leaves are shed before flowering (Flora of North America 2021). Focusing on the genus level may prove to be challenging due to the vast differences between species. I hoped to account for this in my methods by measuring three different leaves on a single plant and averaging them. This protocol will hopefully correct for any species-level differences amongst the specimens.

Leaf size varies with different environmental factors. For the three genera discussed above, leaf size may be impacted by soil nutrient levels, herbivory, and/or exposure to sunlight during development. For example, many researchers have studied and recognized that plants with nutrient-deficient soils tend to have smaller leaves (McDonald et al. 2003). In Australia, total soil phosphorus was seen as one of the most important factors affecting and shaping vegetation structure (Beadle 1966). More studies have to further look at the impact of the functional basis of leaf size reduction to lower total soil phosphorus.

Why do leaves even have different leaf sizes and shapes in the first place? This comes from selective pressures of the surrounding environment, including the impact of herbivores (Brown & Lawton 1991). Over long periods, this selective pressure of herbivory could have affected different species and genera differently. Herbivory has a potential influence on the timing of phenological events and leaf expansion (Nayak & Ishida 2004). Plants could have adapted several ways to combat herbivory, including mimicry, crypsis, physical barriers, differing leaf morphology to reduce recognition by herbivores, dividing and dissecting leaves to reduce herbivore efficiency, and differing juvenile and adult leaves (Brown & Lawton 1991). *Symphyotrichum, Ambrosia,* and *Solidago* could have adapted to herbivory in the same way, reducing leaf size to avoid herbivores. Additionally, herbivory has been increasing over time. According to a 2018 study, herbarium specimens collected in the early 2000s were 23% more likely to be damaged by herbivores than specimens collected in the early 1900s (Meineke 2018). Furthermore, herbivory was greater following warmer winters, suggesting that climate change may drive increasing herbivory frequency (Meineke 2018). Within this current study, from 1886 to 2021, annual minimum and maximum temperatures increased, and this trend will continue as time goes on. The increased herbivory following warm periods could have affected leaf size, by pressuring these genera to decrease leaf area or differ leaf morphology.

At any stage in the development of a shoot, leaves are morphologically and functionally different as a result of their microenvironment and leaf form differences (James & Bell 2000). The amount of sunlight a leaf receives and absorbs could also have an impact on its size. Plants that are grown in low-light conditions, or shade, often show morphological differences compared with species grown in high-light conditions, or full sunlight (Adds et al. 2004). These leaf size differences may be prevalent on the same plant, but they also may be prevalent on ecotypes, another member of the same population adapted to the same environmental conditions (Young & Smith 1980). Shade leaves tend to be larger than sun leaves, providing a larger area for trapping light energy for photosynthesis in a low-light environment (Adds et al. 2004). For the specimens in this study, the identification card did not identify if they were in an area of sunlight, or a shaded area. This could potentially cause variation between leaf sizes in the same genus, which could have affected the leaf length and area measurements. Many potential factors could have impacted leaf size in these three genera and they could have been impacted by different factors, a combination of factors, or differently by each factor.

In my hypothesis, I suggested that a decrease in leaf size would be favorable due to the fact it could better reduce water loss and maintain water transport. Leaf size tends to decrease with decreasing water availability, making smaller leaves advantageous in hot and dry conditions (Wang et al. 2019). A large portion of the variation in leaf size contributes to water balance. Leaves are a critical factor in the water transport system, especially in hot and dry environments (Wang et al. 2019). Plants can survive and function under extremely variable conditions, and this could not be achieved without strong water transport regulatory mechanisms. Stomata allow plants to control water transport and loss under drought conditions (Martinez-Vilalta et al. 2014). Plants have been characterized into two categories based on the ability of the stomata to regulate leaf water potential, isohydric and anisohydric species. Anisohydric species have less stomatal control, and track changes in environmental fluctuations, while isohydric species have more stomatal control and stay relatively stable as environmental conditions change (Martinez-Vilalta et al. 2014). Both anisohydric and isohydric behaviors have been observed in numerous plant groups and within individual species, suggesting that environmental impacts and relations influence these differences in behavior (Sade et al. 2012). In the genera I studied, isohydric and anisohydric species were present, and it would be hard to track if this factor had any impact on the results. It would be interesting for future research to look further into the impact of anisohydric and isohydric species, and their responses to a changing environment. In New Jersey, the maximum and minimum annual temperatures are increasing over time. The frequency of droughts and daily temperature extremes are only going to become more frequent and widespread as temperatures continue to increase (NJDEP 2020). These stressors on plants could over time pressure them to change their leaf size to better adapt to hotter environments.

The Other 6 Genera: *Artemisia, Bidens, Cirsium, Eurybia, Eutrochium, Helenium*

Six genera, *Artemisia, Bidens, Cirsium, Eurybia, Eutrochium, and Helenium,* did not show any statistically significant increase or decrease in leaf length or area over time. But, *Artemisia* did show a significant decrease as a function of climate, specifically as a function of mean maximum temperature. This result seemed a bit awkward, that leaf area was changing as mean maximum temperature increased, but not over time. Further research is needed, with a larger sample size, to investigate this finding. *Bidens, Cirsium, Eurybia, Eutrochium, and Helenium* may be reacting to climate change in different ways, rather than by decreasing or increasing leaf size. This is still a result, and I'd like to further investigate other ways plants may adapt to climate change and the morphology of each genus.

For several of these genera, the number of specimens available to analyze was restricted. For example, within the genus *Helenium*, there were only 4 specimens available at Drew's Herbarium. This very small sample size could be the reason there was no trend. With additional specimens, a trend may begin to form. With other genera in this ‘category’, the sample size was not a restriction. For example, *Bidens* had a sample size of 12. If there was a trend here, it would have likely shown itself, or begun to show itself. Sample size could explain why these results were not statistically significant, but there may be other commonalities between genera that better explain this result.

Artemisia is a large, diverse genus of plants, with over 200 species. They are commonly known as mugwort, wormwood, or sagebrush. Species within this genus grow in temperate climates, usually in dry or semiarid climates (Flora of North America 2020). Most species have strong aromas and bitter tastes, which ultimately discourage herbivory (Flora of North America 2020). For this specific study, *Artemisia* showed some contradictory results, for the by-year analysis there was no statistical significance, but for the climate variables analysis, leaf area was statistically significant as a function of annual mean maximum temperature. More research needs to be done to investigate this genus and the link between mean maximum temperature and leaf area.

Bidens, more commonly known as beggarticks, are distributed throughout the tropical and warm temperature regions of the world (New South Wales Flora Online 2022). *Biden’s* specimens have a selective advantage against herbivory, their fruits are bristled and barbed (Flora of North America 2020). Most species within this genus are also zoochorous, meaning their seeds will stick to clothing, fur, or feathers. This strategy of increased transport of seeds is not present among the other genera analyzed in this study. Leaves for species within *Bidens* are usually simple and opposite (Flora of North America 2020). *Cirsium,* more commonly known as thistles, are mostly native to Eurasia and northern Africa, with only 60 species present in North America (Flora of North America 2020). Most species within this genus are considered weeds, but they support pollinators and are used as food for the larvae of several butterfly and moth species (Hicks et al. 2016). Their leaves are alternate, and some species' leaves may be slightly hairy (Flora of North America 2020).

The genus *Eurybia*, consisting of 23 species, is mostly native to North America, with only one species outside of the continent. The leaves are always alternate. The blades of the leaves are variable amongst different species, from cordate to elliptic (Flora of North America 2020). Species within *Eutrochium,* more commonly known as Joe-Pye weeds, are native to the United States and Canada. There are only 5 species present in this genus (Flora of North America 2020). Joe-Pye weeds have been historically used to treat a variety of ailments from fevers (Speck & Dodge 1945) to typhus (Audubon Society 1988*)* to kidney stones (Hemmerly 2000). Their leaves are usually alternative with serrate margins (Flora of North America 2020). Species within *Helenium,* more commonly known as sneezeweeds, are distributed in North America, Mexico, Cuba, Central America, and South America. They received their common name because their dried leaves were used in the making of snuff, herbal smokeless tobacco. It was inhaled to supposedly rid the body of evil spirits (Trull 2014). Their leaves are mostly alternate with margins entire or toothed, and with blades ranging from lanceolate to oblong (Flora of North America 2020).

Several genera, *Bidens, Cirsium, Eurybia, Eutrochium, and Helenium,* did not increase or decrease their leaf length or area as the climate changed over time. They may have instead increased their flowering time. A growing number of scientific studies have documented the relationship between phenological changes, like changes in flowering time, and changes in temperature (Primack et al. 2004). In addition, these genera may be changing their morphology in different ways, other than increasing or decreasing their leaf size. These specimens may also have faced different environmental pressures than the previous specimens, including less herbivory.

Three genera in this section, *Artemisia, Eutrochium,* and *Helenium*, have distinct smells, tastes, or medicinal properties, also known as secondary metabolites, or secondary compounds. Plant secondary compounds are substances manufactured by plants that make them more competitive in their environment, including in chemical and biological defense from herbivory (Teoh 2015, Holopainen et al. 2018). The most common and diverse secondary compounds in higher plants, or vascular plants, are terpenes, alkaloids, and phenolic compounds (Holopainen et al. 2018). Terpenes function as infochemicals, attractants, or repellants, and are responsible for the typical fragrance of many plants. High concentrations of terpenes can be toxic and are important weapons against pathogens and insects (Paduch et al. 2007). Alkaloids are bitter to taste and are believed to play a role in germination and protection from predation. Alkaloids are present in about 20% of higher plants (Teoh 2015). *Artemisia* contains alkaloids, characteristically the rupestine derivative, which possesses many important medical purposes, like antiparasitic properties, sleep-inducing agents, and opening blocked arteries (Rashid et al. 2019). There are three main important groups of phenols, flavonoids, the most studied and largest group of plant phenols, phenolic acids, and polyphenols also referred to as tannins. Plants need phenolic compounds for growth, pigmentation, reproduction, and resistance to pathogens (Oksana et al. 2012). Many species in the plant family Asteraceae are well known for their medicinal properties. Many of these species contain high amounts of phenolics, including within the genera *Achillea* and *Matricaria* (Oksana et al 2012)*,* which were not included in this study. The pressure of increasing temperatures, and/or increasing herbivory, may not have affected these three genera, *Artemisia, Eutrochium,* and *Helenium* due to the presence of these secondary compounds.

The other three genera, *Bidens, Cirsium,* and *Eurybia*, did not have any distinctive secondary compounds, but they still did not change in leaf size over time with a changing climate. Specimens within these genera could have reacted to changes in climate in a different way, possibly through phenological or molecular changes. Advancing phenology is one of the most sensitive plant responses to warming. Current estimates, from warming experiments and observations over time, suggest that changes in phenology are 1.9 to 3.3 days per degree Celsius for experiments and 2.5 to 5 days per degree Celsius for observations (Wolkovich et al. 2012). To sense change and adapt to the impacts of climate change, plants have evolved a wide spectrum of molecular programs (Ahuja et al. 2010). In response to increased CO2, *Arabidopsis,* a genus within the Brassicaceae family, showed downregulation of transcripts relating to photosynthesis and the Calvin cycle, and an upregulation of genes linked to carbon metabolism and cell wall proteins (Ahuja et al. 2010). More research about these phenological changes and molecular mechanisms is needed to make assumptions on why these three genera did not increase or decrease in leaf size.

Herbarium Specimen Bias & Challenges

Herbarium specimens can be amazing research subjects and can tell us a lot of information about the past, but there are some downsides. First, some herbarium specimens, specifically those before the 1960s, could have been treated with a toxic preservative, mercuric chloride (Webber et al. 2011). Mercuric chloride is an inorganic form of mercury that was used to treat herbarium specimens due to its lethality to all insects (Webber et al. 2011). Mercuric chloride vapor can contaminate untreated specimens within the same cabinet and can be a human health hazard (Oyarzun et al. 2007). With these specimens, handling them properly is essential. For the Drew Herbarium specifically, there was no evidence of whether preservatives were used or not. The oldest specimens here were from the 1930s, and this isn't a widely used herbarium, so this wasn't a huge concern for me, but I still handled them in a specific way. I wore rubber gloves, and a face mask, I kept the herbarium cabinet closed at all times, and I kept the room well ventilated.

Another challenge I faced while working with herbarium specimens was regarding the number of specimens available. For the Drew Herbarium, specimens were mainly collected when we had a Botany department, and staff dedicated to maintaining the herbarium. Most of the specimens in the Drew Herbarium are from the 1960s to the 1980s because of this. Specimens were mainly collected by two individuals, Robert and Florence Zuck. The Arboretum at Drew University is named after these two individuals for their commitment to education, conservation, and botany. In addition to this, although there were a lot of specimens in total, there were only a few of each species and/or genus. With this research project, some genera had over 30 specimens, but others had only 5 specimens. This caused some limitations on statistical analysis, specifically with identifying if there was a change over time as the climate changes, or if it was due to the chance that these 5 individuals were the same.

With using herbarium specimens, there comes sampling bias. Ideally, specimens collected for morphology studies, like this current study, would be collected non-randomly to avoid sampling bias (Daru et al. 2017). However, a majority of the specimens in the Drew Herbarium were collected for their aesthetic value, for a specific lab course, or for systematic inquiries. There are several different types of sampling biases, taxonomic or phylogenetic bias, geographic bias, and temporal bias (Daru et al. 2017). Taxonomic or phylogenetic bias typically occurs from the scientific interests of the person collecting the plant or for the plant's attractiveness (Daru et al. 2017). Many specimens within the Drew Herbarium were collected by the Zucks. I assume that they collected plants based on their aesthetic value and interest. Geographic bias results from collecting specimens more frequently in one location over another (Daru et al. 2017). My study is refined to Morris County, but many specimens were collected in the Drew Forest or the Great Swamp. Temporal bias results from collecting specimens only during certain times throughout the year (Daru et al. 2017). Temporal bias in this study comes from collecting specimens only when plants are flowering, from August to October for genera within the Asteraceae family. The use of herbarium specimens comes with inherent biases, and there is still not a widespread, acceptable way to correct these biases. As more studies use herbarium specimens, a standardized correction of sampling bias will be needed. Although there are several challenges to using herbarium specimens, they are still a great research tool.

Other Challenges

Several challenges presented themselves throughout this study. To measure leaf size and area for this project, I used ImageJ, an open-source Java program that is used for many imaging applications, including in fields such as ecology, neuroscience, and microbiology (Abràmoff et al., 2004). I did not know how to use this program before starting this study. There was a large learning curve, and adapting a protocol that would work was challenging. ImageJ uses pixels to perform its measurements and relies heavily on digitized images. Drew's Herbarium was not digitized, and the process of taking pictures, uploading them, then taking leaf length and area measurements was tedious. After some time and with practice, ImageJ became easier to use, but the digitization effort was still a challenge. In the future, I hope that we can fully digitize Drew's Herbarium, and make the process and the collection accessible.

In addition to the technological challenges, this study was particularly difficult because I could not account for many factors. With herbarium specimens, there is an identification card that shows the basic information, but it does not necessarily provide microclimate or environmental information. For many of these herbarium specimens, I did not know if they grew in sun or shade, in wet or dry soil, or in nutrient-rich or nutrient-poor soils. There was a lot of noise in my data, which made analysis and discussion difficult, but I was still able to speculate reasons why there was an increase or decrease in leaf size over time. To address these other factors, or this noise, greenhouse experiments could be conducted to address one singular factor. Another way to address these other factors is for researchers to include as much information as they possibly can on the herbarium specimen identification card. With any ecology study, it is hard to account for all factors, but their study may help for future research and aid to identify new topics for research. Although there were some challenges while conducting this research, I thoroughly enjoyed the experience.

Future Climate Change & Plant Responses Projections

We are already seeing the impacts of climate change, and these impacts are only going to get worse. In regards to warming, between a 1 to 2 degrees Celsius increase above pre-industrial levels, vulnerable ecosystems will suffer greatly (Hare 2006). Above a 2 degrees Celsius increase, the risks increase, even more, involving large-scale extinctions, ecosystem collapses, food and water shortages, and socio-economic damage (Hare 2006). In regards to precipitation, there will be an increase in precipitation by at least 1%, with a possible increase of up to 12%, by 2100 (University Corporation for Atmospheric Research 2022). As the climate changes, we can also expect other impacts to worsen, including more snow and ice melt, rising sea levels, ocean acidification, more severe weather events, and risks to both aquatic and terrestrial ecosystems.

These changes in temperature and precipitation will alter the geographic ranges of many types of plants and animals (University Corporation for Atmospheric Research 2022). Many species will face extinction. Plant responses to climate change will vary greatly depending on the species and their environment. There may be changes in morphology, phenology, genetics, home range, etc. It is essential for studies to investigate the impacts of climate change on plant species. As climate change continues to worsen, this research may help us estimate how plants will respond in the future, and will help us to preserve plant biodiversity.

Conclusion

Although this project had some challenges, I was able to learn a lot. Through the protocol, I learned how to press herbarium specimens, how to write out identification cards, how to use ImageJ, and how to navigate through historical climate databases. Through the field excursions, I was able to learn more about field research, how to prepare for these trips, and how to be more aware of my surroundings. Through the literature research, I solidified my understanding of climate change. I learned more about the morphology of leaves, the relevance of secondary compounds to herbivory, and how climate change affects plant morphology, phenology, and molecular mechanisms.

To summarize the takeaways of this thesis, there were several interesting results and several instances that need additional investigation. *Euthamia* showed a statistically significant increase in leaf area by year, but not leaf length, and this was consistent across all three climate variables. My hypothesis was not supported by these findings, but it does support the idea that different organisms are responding to climate change in different ways. From 1950 to 1980, *Ambrosia* and *Symphyotrichum* showed a statistically significant decrease in leaf length and area as a function of the year, with *Symphyotrichum* showing a statistically significant decrease for both dependent variables as a function of mean maximum temperature. This finding did support my hypothesis of a decrease in leaf size over time as climate changes, but there are several other unknown factors over time that weren't accounted for. Additional research is needed to further investigate some genera, specifically those that had a small representative sample in this study, and those that showed a significant change in leaf size by climate or by year, but not both. Additional research is also needed to further examine all the ways organisms, specifically, plants, are being affected by anthropogenic climate change, and their responses.

Climate change has been an important topic to research over the past few decades, and we will continue to need dedicated scientists who research its impacts as temperatures increase, precipitation increases, sea levels rise, and ice sheets melt. In conclusion, I am thankful I was able to complete this research project and contribute to the vast amount of research to further understand how climate change is influencing organisms and ecosystems.

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Appendix A

Standard Operating Procedure

Herbarium Specimen Leaf Length and Area Quantification

General Purpose: To determine average leaf length and area of herbarium specimens using ImageJ

Theory: Herbarium specimens can be utilized in various ways in scientific research, including determining how plants may respond to climate change. Understanding how plants respond to changes in climate is essential because humans and natural systems rely on these organisms for many resources and services. Climate change impacts plants in a multitude of ways, including affecting their growth and reproduction. By measuring leaf length and leaf area of herbarium specimens, we can determine how leaf size may be related to climate change. Through this, we can also begin to conclude why exactly plants could be responding to climate change in this way, e.g. through an effort to reduce water loss, to increase leaf water use efficiency, to maintain sufficient leaf water supply, or a combination of these and other factors.

Equipment:

Disposable gloves
Herbarium specimens
Kodak PixPro AZ421 Digital Camera
SD card
SD card reader
67” Camera Tripod Stand
15 cm Ruler
Computer
Notebook and pen
ImageJ Software (ImageJ bundled with Java 1.8.0_172)
Microsoft Excel

Procedure:

#1. To select an herbarium specimen (Figure 1)*, look at your datasheet or through your herbarium’s database to find its catalog number. Open the herbarium cabinet and look for the family name of the specimen. The herbarium is organized numerically and alphabetically by family name. Take out a folder with the specific family name and close the herbarium door. Look through this folder and see if you can find the specimen with the correct catalog number. If it is here, proceed with the following steps. For each family, there are several folders. If you cannot find the specimen in the first folder, take out another folder from the cabinet and look for the specimen. Be sure to close the cabinet door after you remove and put back the folders. Make sure to keep all specimens in numerical order.

**Note:* Some old university herbarium specimens, specifically those from the 1800s to early 1900s, were treated with mercuric chloride as a preservative. Mercuric chloride-treated samples may thus be a source of mercury exposure and contamination, which could be very hazardous to one's health. While it is highly unlikely that any of Drew's samples were treated in this way, be careful in handling these possibly treated specimens by wearing disposable gloves whenever handling pressings, closing the herbarium specimen cabinet immediately after use, not breathing the air in the cabinet directly, and keeping the workspace well-ventilated.

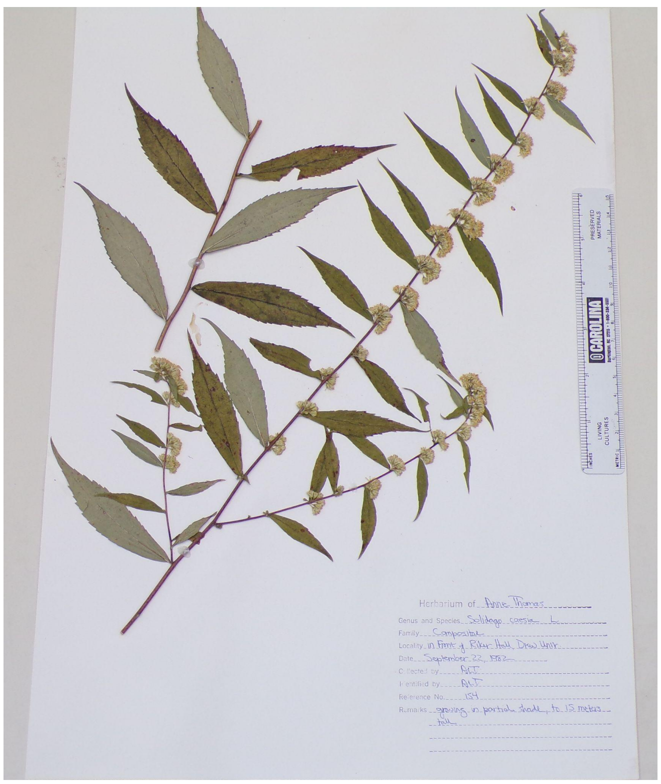


Figure 1. An herbarium specimen from Drew University's Herbarium, with an identification card and ruler present.

#2. To prepare images to measure in ImageJ, place an herbarium specimen on a table against a white background. Put your tripod and camera, with an SD card in it, directly above the herbarium specimen (the pictures taken should be as straight up-and-down as possible). Turn on macro focus and flash in the camera's settings. Place a ruler on or next to the herbarium specimen to provide an indicator of scale. Take a picture. Once you have a clear picture, transfer the picture from the SD card to your computer using an SD card reader. You can put the images into your spreadsheet under "Image" or you can keep them readily accessible in a folder named

"Herbarium Specimens". If you are storing them in a folder, you will need to change the names of the files. Change the name of each file to its appropriate catalog number.

#3. Download and/or open the ImageJ software. There are options for Mac, Linux, and Windows.

#4. Prepare and/or locate your master datasheet. This should contain the following columns: Species name, Catalog #, Year Collected, Leaf Length Average, and Leaf Area Average. This can also contain an "Image" column for file names of specimen photographs for reference.

#5. In the ImageJ app, drag an image of interest into the ImageJ bar (Figure 2).

Figure 2. Drag and Drop bar feature.

#6. Select three leaves on your chosen specimen image to measure. Do not choose the most basal or terminal leaves as these leaves may be at abnormal stages in their maturity, especially near the tip. Select one leaf towards the bottom. To do this, move two leaves up from the bottom of the specimen. Be sure that there is nothing in the way of measuring the length and area for this leaf. If there is something, for example, another leaf, in its way, move one or more leaves up and follow the same steps. Select one leaf near the top. To do this, move two leaves down from the top of the specimen. Be sure that there is nothing in the way of measuring the length and area for this leaf. If there is something in its way, move one or more leaves down and follow the same steps. Select another leaf from the middle. To determine the exact middle of the specimen for leaf selection, use the ImageJ measurement tool (described below) to measure the whole specimen, then divide this number in half. Select the leaf nearest the middle, and make sure there is no obstructed view of this specific leaf. If there is an obstructed view, move either one leaf to the top or the bottom.

Using the multi-point tool, click on the three leaves you selected. This will give each leaf a number. Take a screenshot of this and rename the picture with the catalog number followed by an L. This will indicate that this is the image with the leaf selections for this specific specimen. Put this image in your Herbarium folder.

#7. To begin the first leaf length measurement, change the image from RBG to 8-bit*. To do this, select Image in the Menu Bar (Figure 3), then Image Type, then 8-bit. Then, set the scale of your image. To do this, select the magnifying glass tool (Figure 4) and zoom in to the ruler on your image. Select the straight line tool (Figure 5) and draw a straight line over a 2cm range of the ruler. Select the magnifying glass again and right-click to zoom back out. Next, click Analyze in the Menu Bar, followed by Set Scale (Figure 6). Put in 2 for the known length, and centimeters for the units.

**Note:* Each pixel in an 8-bit image is represented by 8-bits or 1 byte, the basic unit of computer storage and processing information. This conversion from RGB to 8-bit makes it easier for the software to analyze the pixels, which helps in calculating length and area using this program.

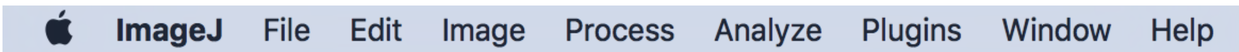


Figure 3. ImageJ menu bar.

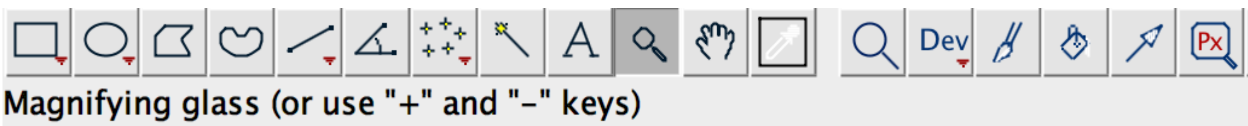


Figure 4. Magnifying glass tool. Use to zoom in and out on the specimen of interest.

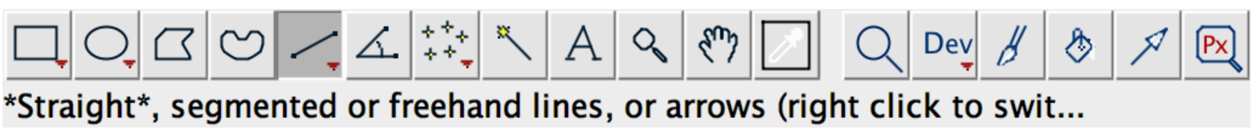


Figure 5. Straight-line tool & Multi-point tool. Use the straight-line tool to set the scale and to measure the leaf of interest from petiole to leaf tip. Two options to the right is the multi-point tool (shown by 5 "crosshairs"). Use this tool to select your leaves.

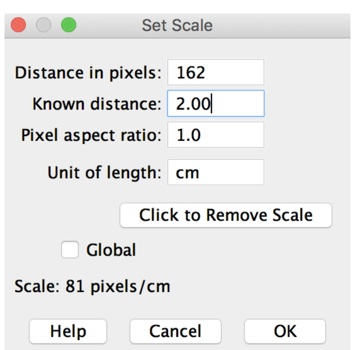


Figure 6. The set scale pop-up box. Use this to put in the known distance and the unit of length.

#8. To measure the first leaf of interest on the specimen, select the straight line tool (Figure 4). Then put a line from the petiole to the tip of the leaf. For lobed leaves, select the longest available lobe, and drag the straight line from the petiole to the tip of the leaf. Click Analyze in the Menu Bar (Figure 2), then Measure. Click Edit in the Result Menu Bar (Figure 7), then select Fill (Figure 8). For the next two length measurements, select the straight line tool and put a line from the beginning of the leaf until the end, as above. Then, use hotkeys as a shortcut: Control M to measure and Control F to fill. To obtain your results, click Results in the Result Menu Bar, then Summarize. In the new Results window (Figure 9), the three-leaf measurements and the mean, standard deviation, minimum and maximum, will be visible. When you are finished with this specimen's measurements, you can close the window. Before the window closes, the program will ask if you want to save these results as an Excel file. I suggest naming the file with the specimens catalog number, followed by "Leaf Length". Copy and paste these results into a single file. Calculate the average of these three values, and copy and paste this value into the master data sheet under Leaf Length Average.

Figure 7. The results menu bar. This menu bar is different from the ImageJ menu bar (Figure 2).

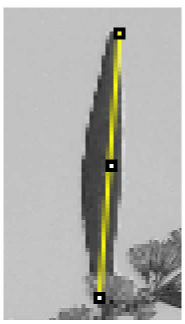
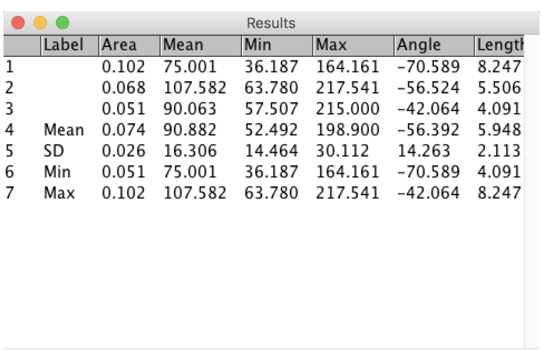


Figure 8. A straight line is drawn from the petiole to tip with the line filled.



Results

	Label	Area	Mean	Min	Max	Angle	Length
1		0.102	75.001	36.187	164.161	-70.589	8.247
2		0.068	107.582	63.780	217.541	-56.524	5.506
3		0.051	90.063	57.507	215.000	-42.064	4.091
4	Mean	0.074	90.882	52.492	198.900	-56.392	5.948
5	SD	0.026	16.306	14.464	30.112	14.263	2.113
6	Min	0.051	75.001	36.187	164.161	-70.589	4.091
7	Max	0.102	107.582	63.780	217.541	-42.064	8.247

Figure 9. The new results window with the three leaf measurements and the mean, SD, min, and max.

Note: If you are completing leaf area measurements directly after leaf length, you do not need to reset the scale. If you re-upload another image, or exit out and come back into the program, you will need to re-convert the image from RBG to 8-bit and set the scale again, described in Step 4.

#9. To begin leaf area measurements, click on the rectangle tool (Figure 10) in the toolbar and place a rectangle around your leaf of interest. Then, select the Image tab in the Menu Bar, then select Duplicate. The specific area will come into a separate window, making an enlarged inset of the area of interest (Figure 11). Select the Image tab in the Menu bar, then click Adjust, then Threshold*. A new window will come up (Figure 12). The automatic color associated with thresholding is red. Slide the thresholding bar until the color red completely covers the background (Figure 13). Once the background is completely red, and the leaf is completely gray, select Apply.

**Note:* Thresholding is an image processing technique for dividing an image into two, or more, classes of pixels typically called foreground (black) and background (white). By using the rectangle tool, you are highlighting pixels in the image of interest (Figure 11). By designating all pixels as either part of the area of interest (foreground) or not (background), the program can calculate several metrics of interest, such as the area of the foreground.

Figure 10. Rectangle tool. Use to place a rectangle around the leaf of interest.

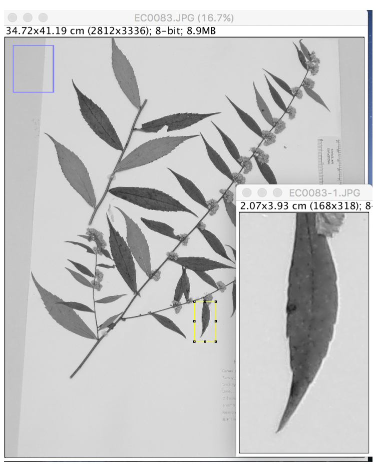


Figure 11. The specimen plus the enlarged inset of the leaf of interest.

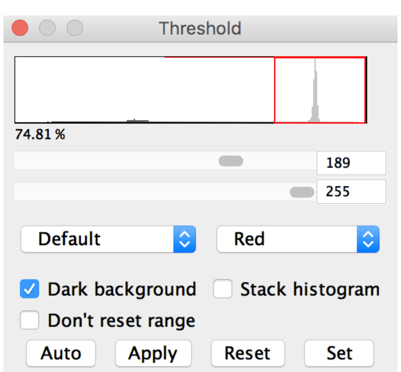


Figure 12. The threshold window. Slide the top bar until the background is completely covered with red.

Figure 13. The end of the thresholding process. Notice that the background is completely covered with red, and the leaf is completely gray.

#10. To obtain your calculated leaf area, click Analyze in the Menu bar. Select Analyze Particles, then Display Results. In the Display Results box (Figure 14), select “Display Results,” and “Show Outlines.” Make sure the size range is from 0 to Infinity. Click Ok. Another window will pop up with the area calculation for one leaf (Figure 15). Unlike the leaf length measurements, not all three measurements come up at once in the Results table. You have to do the leaf area process separately for each leaf to get each measurement. You can then save these in the same file as the leaf length calculations, labeled with their catalog number, “Leaf Area”, and which leaf it is for that specimen (1, 2, or 3). Calculate the average of these values, and copy and paste this value into the master data sheet under Leaf Area Average.

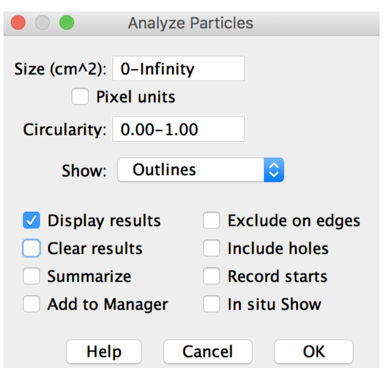


Figure 14. The Display Results pop-up box.

	A	B
1		Area
2	1	5.298
3		

Figure 15. The leaf area calculation for one leaf in an Excel spreadsheet.

#11. To place your herbarium specimen back into the cabinet, first pull out the folder where you found this specimen. Look at the catalog numbers and place this specimen in its appropriate, sequential spot. Place this folder back into the cabinet in the same spot where you removed it.

#12. Repeat this procedure for all of the herbarium specimens in your datasheet