UBC Social Ecological Economic Development Studies (SEEDS) Student Report

Environmental and developmental regulation of photosynthetic and transpiration rates, and leaf senescence in *Catalpa speciose*

Aman Chera

University of British Columbia
Directed Studies in Biology (BIOL 448)

December 5, 2016
(Revised: June 6, 2017)

Environmental and developmental regulation of photosynthetic and transpiration rates, and leaf senescence in *Catalpa speciose*

BIOL 448 – DIRECTED STUDIES IN BIOLOGY

Aman Chera

December 5, 2016
(Revised: June 6, 2017)

Research Supervisor: Dr. SANTOKH SINGH

Department of Botany, Faculty of Science, University of British Columbia

CONTENTS

Abstract:

An increase in the levels of greenhouse gases, such as carbon dioxide appears to induce changes in climate. Carbon can be sequestered by the leaves of terrestrial plants by a process known as photosynthesis when carbon dioxide enter the pores of leaves known as stomata. Similarly, stomatal opening allows water loss termed as transpiration, which is important for the circulation of the nutrients within the plant as well as the cooling effect on the leaf surface during the hot and dry season. The focus of this study was to examine the effects of environmental and developmental changes on photosynthesis and transpiration rates of Northern Catalpa (Catalpa speciose) leaves during late summer until fall season. As the season changed from summer to fall, the Northern Catalpa leaves started to age. To understand the leaf aging process, I investigated the role of plant hormones in regulating leaf senescence (leaf yellowing) in Northern Catalpa trees. Photosynthesis and transpiration rates were analyzed by CI-340 Handheld Photosynthesis System. The environmental factors such as precipitation levels, temperature, and photoperiod hours were also noted. Leaf senescence was measured by analyzing the chlorophyll and protein levels in the leaves. High temperatures and lower precipitation levels appear to decrease the photosynthesis and transpiration rates of Northern Catalpa leaves. In addition, the photosynthesis and transpiration rates of Northern Catalpa leaves progressively declined during the leaf ageing process, especially the onset of autumnal senescence, which could be due to low temperature and short photoperiod. Therefore, during this period, less carbon was sequestered by Northern catalpa. Plant hormone, auxin delayed senescence and increased the longevity of these leaves, whereas abscisic acid (ABA) enhanced leaf senescence. The optimal concentration for retarding senescence by natural auxin called Indole-3-acetic acid (IAA) is $2*10^{-4}$M and for synthetic auxin called Naphthaleneacetic acid (NAA), it is 10^{-4}.

Introduction:

The increase in the Earth's surface temperature, also known as global warming has become a major concern today. Environmental Protection Agency (EPA) states that the earth's temperature has increased by 0.8 degrees Celsius over the past hundred years. Intergovernmental Panel on Climate Change (IPCC) of the United Nations explain that the increased earth temperature is most likely due to the increase in greenhouse gas concentrations, mainly carbon dioxide (Florides and Christodoulides, 2009). Increased tree planting and decreased deforestation are some of the proposed ways to decrease the carbon dioxide concentration. The leaves of trees decrease carbon dioxide in the environment by converting carbon dioxide into carbohydrates through photosynthesis, so the leaves of many trees are being studied today (Palmer, 2012).

The physiological and biochemical aspect of leaves on one tree that hasn't been studied much before is *Catalpa speciose,* commonly known as Northern Catalpa. This deciduous tree is native to Illinois and Indiana in the United States of America. It has large simple leaves and two-lipped flowers. The fruits on this tree are long pods called capsules. This tree is the fastest growing and largest trees compared to the other Catalpa trees. This tree is known to be one of the last trees to grow leaves in spring. The leaves turn brown and shrivel when they come into contact with frost ("Catalpa speciose," 1928). It is used in pole ties and fence posts since there is very little sap to contaminate the wood and it is also used as an ornamental shade tree on the lawns and sides of streets ("The catalpa tree," 1904).

One of the aims of this research include studying the effects of environmental changes on photosynthesis and transpiration rates of different sized Northern Catalpa leaves during late summer until fall season. By analyzing the photosynthesis and transpiration rates of these leaves, we can understand the amount of carbon dioxide uptake by the Northern Catalpa throughout the different environmental changes. These environmental changes initiate and regulate the senescence process in deciduous trees, along with other plants (Gan and Amasino, 1997). Senescence is the process of aging of the plant (Templer, 2015). Another objective of this project is to investigate the hormones that effect catalpa leaf senescence and then vary the concentrations of these hormones to analyze the senescence response in catalpa leaves since the response to different hormone concentration is variable in different plant species (Qiu et al. 2015). By studying the hormones that effect leaf senescence, one can understand the hormonal factors that increase the longevity of these leaves.

Furthermore, one of environmental factors that changes throughout the seasons is temperature. Some of the past studies on some plant species have found that transitioning into or a constant high temperature causes a decrease in photosynthetic rates due to various changes in important photosynthetic enzymes including RuBP carboxylase/oxygenase. This enzyme is involved in the dark-independent reactions of photosynthesis, specifically in carbon fixation (Brooks & Farquhar, 1985). One study on *Spinacia oleracea L.*, commonly known as spinach, found that the specificity of the enzyme RuBP carboxylase/ oxygenase for oxygen and carbon dioxide decreases with increased temperature, resulting in decreased photosynthetic rates (Brooks & Fraquhar 1985).

Other studies have shown that transpiration rates increase with increased temperature. Transpiration is a process involving water evaporation from plants. Studies have showed that when temperatures increase from about 10 to 35 degrees, there is an increase in the transpiration in various plant species due to increased leaf stomata openings. The width of stomatal openings increase when temperature is increasing up until around 35 degrees Celsius in Solanum tuberosum, resulting in high transpiration rates. However, this trend can only be seen when the plants are not under water stress because when there is water stress, stomata close to conserve water in the plant (Hofstra & Hesketh, 1969).

Also, when the season changes, there are many environmental factors that change. These environmental factors initiate and regulate the senescence process in plants. Some of these environmental factors include temperature, water shortage, nutrient deficiency, pathogen infection, wounding, and photoperiods (Gan and Amasino, 1997). One type of environmental regulated senescence is called autumnal senescence which is a type of programed death that

results in the degradation of cellular constituents such as proteins, nucleic acids, lipids, and organelles of the leaf cell. This degradation results in photosynthetic decline. The specific cellular constituents of leaves involved in photosynthesis that decline include chlorophyll content, activities of Photosystem II (PSII) and photosystem I (PSI), and RuBP carboxylase/oxygenase levels. PSII and PSI are protein complexes involved in the light-dependent reactions of photosynthesis (He *et al.* 2002). Autumn senescence in most trees is mainly initiated by the decrease in the photoperiods. Previous studies on other deciduous trees have found that as autumnal senescence progresses, photosynthesis declines. Also, stomatal conductance also declines through senescence, resulting in lower transpiration rates. This decline is due to stomatal closures during senescence (Pessarakli, 2002). Stomatal conductance is the rate at which either water vapor exits or carbon dioxide enters through the stomata, which are the small pores of a plant (Bassow and Bazzaz, 1998).

Furthermore, studies have also shown the involvement of many plant hormones in this leaf senescence process of different plant species. Ethylene, abscisic acid (ABA), and Jasmonic acid (JA) are plant hormones that promote leaf senescence in various plant species. Cytokinin, gibberellic acid, and auxin have shown to delay senescence related processes in some plants including degradation of chlorophyll and enzymes involved in photosynthesis such as NADH-dependent hydroxypyruvate reductase and RuBP carboxylase/oxygenase (Liu et al. 2016).

One study on rice leaves shows that JA caused leaf senescence by causing chlorophyll degradation, cell membrane breakdown, and increasing the expression levels of senescence associated genes (SAGs). It promotes chlorophyll degradation through increasing the expression levels of chlorophyll degradation related genes (CDRG) known as *OsSGR* and *OsRCCR1* genes.

This hormone also promotes membrane breakdown by increasing lipid peroxidation and membrane permeability. Lipids are molecules that make up the membrane that are degraded by a process called lipid peroxidation. Increased membrane permeability will lead to leaf senescence because it allows ion leakage from the cells (Liu et al. 2016).

On the other hand, study on rice leaves have also shown that cytokinin slows chlorophyll degradation by down-regulating the expression levels of *CDRGs*. This hormone also delays senescence by delaying membrane deterioration by retarding the increases in lipid peroxidation and membrane permeability. And cytokinin also causes down regulates the expression of *SAGs* by inactivating the transcription factor that binds to the promoter of this gene called MYB, and therefore delay rice leaf senescence (Liu et al. 2016).

Studies have shown that ABA also promotes leaf senescence in *Arabidopsis* leaves through activating transcription factors known as ABF2 (AREB1), ABF3, and ABF4. These transcription factors bind to the promoter of *NYE1* gene. The products of NYE1 gene cause chlorophyll degradation, resulting in leaf senescence (Gao et al. 2016).

The exact molecular mechanism of auxin delaying leaf senescence is still unknown. However, studies have found that the delay of senescence occurs when auxin inactivates the auxin response factor 2 (ARF2), which is a transcription factor that represses auxin signaling. Studies found that plants with a ARF2 mutant can cause less repression of auxin signaling, resulting in delayed senescence by delaying degradation of chlorophyll content, maintaining photochemical efficiency of photosystem II, delaying membrane ion leakage, and the expression of senescence-associated genes. However, studies on genes or proteins that ARF2 targets still

need to be done in order to understand the senescence pathways that ARF2 regulates (Lim *et al.* 2010).

Other studies have shown that Ethylene causes leaf senescence in *Arabidopsis* species by triggering chlorophyll degradation through activating the transcription factor, ETHYLENE INSENSITIVE3 (EIN3). This transcription factor binds to 3 major chlorophyll catabolic genes: *NYE1*, *NYC1* and *PAO*, leading to chlorophyll degradation and then consequently leaf senescence (Qiu *et al.* 2015).

Based on prior studies on plant hormones, I predict that ethylene, ABA, and Jasmonic acid will cause senescence in leaves of *Catalpa speciose*. I also predict that auxin, cytokinin, and gibberellic acid will delay leaf senescence in leaves of northern catalpa. Furthermore, based on the past studies on the effect environmental changes on other deciduous trees, I predict that photosynthesis and transcription rates will decrease towards the fall season when leaf senescence is initiated in Northern Catalpa and respiration rates will stay constant.

Materials and Methods:

Plant: Leaves of *Catalpa speciose*

Chemicals: Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IAA), 6-Benzylaminopurine (BAP), Abscisic acid (ABA), 2 chloro-ethyl-phosphonic acid (CEPA), Jasmonic acid (JA), Gibberellic acid (GA_3), 5% bleach, ethanol, and Tween 20 (T-20).

Equipment: 95 petri-dishes (60 x 15 mm), filter paper, parafilm, CI-340 Handheld Photosynthesis System, chlorophyll meter, light intensity meter, beakers, autoclave machine, graduated cylinder, micropipettes.

In order to learn the effect of environmental changes on northern catalpa leaves, 3 different sized leaf samples were collected every week including a large, medium, and small leaf. The samples were then cut from the northern catalpa tree at the University of British Columbia (UBC) and then the petiole of the leaves were cut again under water in a beaker so there is continuous flow of water going through the xylem of the leaves. These samples were then taken to the lab and were put in the chamber of the CI-340 Handheld Photosynthesis system under a light intensity of 250 $\mu mol/m^2/s$ in order to measure photosynthesis and transpiration. After these measurements were collected, a picture of the leaves was taken. Then, the leaves were wrapped in aluminum foil, frozen in liquid nitrogen, and then put in the freezer. The leaves were frozen so that they can be further analyzed for protein analysis another day. Protein analysis was conducted using SDS page procedure which is stated in the laboratory manual (Singh, 2016). Also, from the picture of the leaf, the area of the leaf was measured using the program called "Image J."

In order to learn which hormones effect northern catalpa leaf senescence, a leaf disc experiment was set up. Northern Catalpa leaves were collected and then a rod was used to cut out small, circular disks from them. The disks were put in petri-dishes containing different hormone treatments (water, NAA, BAP, CEPA, ABA, JA, GA_3, and air). The hormone concentrations were 10^{-4}M. Six leaf disks were placed in each petri-dish and there was 5 replicate petri-dishes for each hormone treatment. The petri dishes were wrapped with aluminum foil and placed in a dark growth chamber at room temperature so the petri dishes aren't exposed to light and are under dark induced senescence conditions. Each week, all the treatments were analyzed for any morphological changes. Also, each week, leaf disks from one replicate dish of each treatment will be frozen with liquid nitrogen. A protein analysis will be conducted on these frozen samples

at the end of the month with SDS-PAGE and chlorophyll analysis will be conducted using spectrophotometer as explained in the procedure in the laboratory manual (Singh, 2016)

After the hormones that effect leaf senescence was known, another leaf disk experiment was conducted. The treatments of the leaf disks were varied concentrations of the hormones and mixture of hormones that effect leaf senescence. Also, leaf discs with no treatments were set up. Some leaf discs had airflow and some didn't. The ones that don't get airflow had their petri-dishes parafilmed and the ones that do get airflow did not have their petr-dishes parafilmed. The same procedure was used as the previous leaf disk experiment. However, the previous experiment had some contamination in the petri-dishes, so this experiment was conducted under sterile conditions. Everything was done under a laminar flow hood. Also, the equipment was autoclaved and the hormone solutions were sterilized using a syringe filter. Lastly, the leaves were sterilized by first being rinsed with 5% bleach and then rinsed 4 times with sterilized distill water.

Results:

Part 1: Effect of environmental changes on Photosynthesis and Transpiration Rates:

Morphological data of the leaves collected from late summer until fall season:

The Northern Catalpa tree has different sized leaves. In Figure 1.1, the leaves from each day are organized in three groups of sizes, including large, medium, and small sized leaves. For all the different sized leaves, the green color of the leaves start getting lighter from early October (Figure 1.1) and turn yellow by late October. In early October, half the Northern Catalpa tree had green leaves and half the tree had lighter green leaves. By mid-October, half the leaves were yellow but there was still half the tree that had lighter green leaves. By Late-October, all the leaves were yellow on the tree (Figure 1).

Molecular data of the leaves collected from late summer until fall season

The chlorophyll level of the Northern Catalpa leaves is higher in August and September (Figure 3) than in October. There is a great decline in chlorophyll levels from August to October. The chlorophyll levels in large sized leaves decreased by 79 percent from August 31st, 2016 until October 6, 2016 (Figure 3). The chlorophyll levels in medium sized leaves decreased by 33 percent from August 31st, 2016 until October 6, 2016 (Figure 3). The chlorophyll levels in small sized leaves decreased by 87 percent from August 31st, 2016 until October 6, 2016 (Figure 3).

All the samples run on the SDS-PAGE are only medium sized leaves because trend of decline and increase in photosynthesis and respiration rates are the same for the different sized leaves for the different days (Figure 4 and 5). The medium sized leaf of August 30 which had an area of 334.63cm has the brightest band for 56 KDa size and 25 KDa in the SDS-PAGE gel compared to the other medium leaf samples of other dates (Figure 2). This band represents the large subunit of RuBisCO protein and the high intensity of the band means that there are large quantities of this protein in this leaf sample. The 25 KDa band could be the light harvesting complex IIb because this protein is known to have a molecular weight between 25-28 KDa (Singh, 2016). This protein is responsible for orienting chlorophyll molecules in the right location so that they can absorb light energy efficiently (Dreyfuss and Philip, 1994). Medium sized green leaf samples from August 31, September 7, and September 29 also have bright bands with the size 56 KDa and 25 KDa but not as bright as the leaf sample from August 30. The light green leaf from October 6 also has this bright band. The green leaf samples from September 14 and October 6 and the yellow leaf sample from October 12 have extremely faded bands or no bands at all with 56 KDa size and 25 KDa size (Figure 2). These faded bands mean that there is

low levels of RubisCO and light harvesting complex IIb proteins and the absence of bands means that there are no proteins in these leaf samples.

Photosynthesis and Transpiration data:

In the net photosynthesis rate (NPR) graph in Figure 4, there is a general trend where the medium sized Northern Catalpa leaves have a higher net photosynthesis rate than the large and small sized leaves. The small and large leaves have similar net photosynthesis rates. In late August and early September, there are days when the NPR is lower by a factor of two compared to other days for all the different sized leaves. On September 7th, the NPR is higher by a factor of 2 compared to the NPR on August 30th. Also, on September 14, the NPR is lower by a factor of two compared to the NPR on September 7th. For all the different sized leaves, there is a decline in NPR in October. In mid-October, the NPR for these leaves are either very close to 0 or negative values. The negative values indicate that the respiration rate of these leaves are higher than the photosynthesis rates of these leaves.

The transpiration rates are fluctuating throughout the late summer to fall period (Figure 5). On all the days, either small or medium sized leaves have higher transpiration rates than large leaves. In late August and early September, there is generally higher transpiration rates of different sized leaves compared to rates in late October with a few exceptions. One exception is that on August 30, the small, medium, and large leaves have the lowest transpiration compared to other days of late summer and early fall including August 31 and September 7. The small leaves of August 31 have four times greater transpiration rates than small leaves on August 30. The large and medium leaves of August 31 have about two times higher transpiration rates than the large and medium leaves on August 30. Another exception is that the small leaves in late October have two times higher transpiration rates than early October including October 7th.

Environmental Data:

There is a decline in average temperature in Vancouver during from late summer until fall season (Figure 6). The precipitation levels were low in August and September but increase on some days in October as seen in Figure 7 ("climate.weather.gc"). The photoperiod also declines from late summer until fall season as seen in Figure 8 ("timeanddate").

Part 2: Effect of Different Hormones on Northern Catalpa Leaf Senescence:

Morphological data of leaf discs in different hormonal treatments:

Experiment 1:

In Table 2, all the leaf disc in the different hormonal treatments have the same color at day 7 as they did on day 0. Day 0 leaves are the leaf samples in the original state without any treatments. On day 14, ABA treated leaf disks all turn black and they remain this color until day 35. All the other different hormonal treatments still have the same green color on day 14 as day 0 but with black edges. NAA treated discs remains this color until day 35. On day 21, the treatments including control, BAP, CEPA, GA, AND JA have 2 or 3 leaf disks that turn black. By day 35, these samples all turned black

Experiment 2:

In Table 3 and 4, all the leaf discs in the different hormonal treatments have a lighter green color on day 7 compared to the color on day 0. The ABA treated leaf discs and the mixture of ABA and NAA treated leaf discs have thick band of black color on the edges on this day. The no treatment leaf discs are all dried up. As the days go by, all the treatments except NAA (10^{-4}M), IAA ($2*10^{-4}$M), and the no treatment with air flow discs start getting black spots and all the discs turn black by day 35.

Chlorophyll data of leaf discs in different hormonal treatments:

Experiment 1:

ABA treated leaf disks show a more rapid decline in chlorophyll a, chlorophyll b, and total chlorophyll levels over the time period of 35 days compared to the leaf disks in the control treatment which is the treatment with water (Figure 9). This rapid decline in chlorophyll levels suggest a role of ABA in accelerating senescence in Northern Catalpa leaves. The total chlorophyll amount in the ABA treated leaves decreased by 35 percent and by 24 percent in the control treated leaves by day 28. The chlorophyll levels measured at day 35 is the same as day 0 chlorophyll levels but this result is due to an experimental error.

BAP treated, JA treated, CEPA, and GA_3 leaf disks look very similar to the control leaf disks. Like the control, there isn't a decrease in chlorophyll a, chlorophyll b, and total chlorophyll levels for 14 days in the BAP treated and JA treated leaf disks and there is decline in these levels seen at day 21 (Figure 9). CEPA and GA_3 show decline in chlorophyll a levels by 11% and 21% during day 14 but show the same pattern of decline as the control treated leaf disks on the days after that. Since these treated disks look like the control, these hormones may have no role in senescence of Northern Catalpa leaves.

NAA treated leaf disks show a very small amount of decline in chlorophyll a, chlorphyll b, and total chlorophyll levels over the 35 days. This result indicates auxin's role in retarding the senescence of Northern Catalpa leaves. The chlorophyll levels during day 0, day 7, and day 14 are very close to being the same. Even at day 35, the chlorophyll levels only decreased by 6% (Figure 10).

Experiment 2:

The leaves for the next set of experiments were collected at the end of September when senescence of these leaves have been initiated so the chlorophyll levels are lower than the ones of the leaves in Figure 10 and 11.

Since auxin was shown to have a role in retarding the senescence of the Northern Catalpa, the concentration of natural and synthetic auxin was then varied to see what the optimal concentration for retarding senescence will be. Leaf disks in the NAA (10^{-4}M) and IAA ($2*10^{-4}$M) show little decline of chlorophyll a, chlorophyll b, and total chlorophyll levels over the 35 days compared to the control leaf disks (Figure 10). The IAA ($2*10^{-4}$M) had a 35% decrease by day 28 in content since day 0, NAA (10^{-4}M) had a 59% decrease and the control discs had 64% decrease. The IAA (10^{-5}M) treated and NAA (10^{-5}M) treated leaf disks show similar trend of decline like the control treated. The control and IAA (10^{-5}M) treated leaf disks on day 35 have the same amount of chlorophyll levels as day 0 but that may be due to experimental error. The IAA (10^{-4}M) and the NAA ($2*10^{-4}$M) show a great decline of chlorophyll a, chlorophyll b, and total chlorophyll levels over the 35 days. Also, there are days where the chlorophyll levels are shown to increase in Figure 10 and that will be explained further in the discussion.

Also, when auxin was found to delay senescence and ABA was shown to promote senescence, a mixture of these two hormones was then also used as a treatment to see the response in the northern catalpa leaf disks. The leaf disks exposed to the mixture of NAA (10^{-4}M) and ABA (10^{-4}M) show the rapid decline of chlorophyll levels like the ABA (10^{-4}M) treated leaf disks (Figure 11). This decline in this mixture treatment indicate ABA masking the effect of auxin on the northern catalpa leaves.

Some leaf disks were exposed to no treatment without airflow and some leaf disks were exposed to air flow because there was a leaf disk left on the bench for a week and the color of the

leaf didn't change. In the leaf disks with no treatment and had air flow show little decline of chlorophyll over the 35 days compared to the control leaf disks in water (Figure 11). No treatment leaf discs without airflow leaf discs show similar trend of decline like the control treated leaves. These no treatment leaf discs have days where the chlorophyll levels are shown to increase a lot but this is not possible since these leaf disks were placed in the dark and possible reasons why this may occur is explained in the discussion.

Protein levels data of leaf disks in different hormonal treatments:

In Figures 12, 13, and 14, most of the proteins in the different leaf samples have shown to be degraded since not a lot of bands are seen in the gel. Even though the leaves were kept frozen after the experiments so SDS-PAGE gels could be run later, most proteins may have degraded over this time. From the first experiment, a faded band of 56 KDa which is RuBisCO is seen in the leaf samples from day 0 which is the sample of the leaf in its original state without any treatment (Figure 12). This band is also seen in control samples in this Figure from day 7 and 14 in the first experiment. It is also seen in the NAA treated leaf sample from day 21 of this experiment (Figure 12). A huge smear is seen along the lane of the day 0 leaf sample in Figure (13) of the second experiment, indicating many cut up proteins. This day 0 sample is sample of the leaf in its original state without any treatment. The 56 KDa band which is indicative of the RuBisCO protein is also seen in NAA (10^{-4}M) treated samples from day 21 and 35 in Figure 13 of the second experiment. There are multiple bands seen in the leaf disk treatments only exposed to air flow in Figure 14.

Discussion:

One of the objectives of this study was to study effects of environmental changes on photosynthesis and transpiration rates of Northern Catalpa leaves. It was found through this study that these rates did fluctuate with environmental changes in the different sized leaves.

The general trend seen in Figure 4 where the medium sized Northern Catalpa leaves have a higher net photosynthetic rate than the large and small sized leaves even though chlorophyll levels of medium sized leaves are lower some days (Figure 3). For instance, on September 6, the small sized leaves have 1.5 times higher chlorophyll levels than medium sized leaves (Figure 3) and yet medium sized leaves have 1.7 times higher NPR than the small sized leaves that day (Figure 4). Also, on September 29, the large sized leaves have 1.3 times higher chlorophyll levels than medium sized leaves and yet medium sized leaves have 1.32 times higher NPR than the small large leaves that day. The reason that medium sized leaves have a higher NPR compared to the other sized leaves could be due to their ages. Age is a factor which could've also caused the transpiration rates of the small and medium leaves to be higher than large leaves seen in Figure 5. The small-sized leaves are the young leaves which haven't fully expanded yet and the medium sized leaves are the mature leaves which are more expanded causing the photosynthesis rates of these leaves to be higher (ConsTable and Rawson, 1980). Higher expansion allows the leaf to have more area to capture light. Higher amounts of light captured enables more photosynthesis to occur (Pantin *et al., 2011).* However, the large leaves on most days, exhibit lower photosynthesis rates than the medium sized leaves despite being mature like the medium sized leaves and more expanded. This difference could be due to the fact that the large leaves reached their full expansion. At full expansion, there are decreases in both photosynthesis and transpiration rates due to redistribution of resources, including nitrogen to

younger leaves (Kitajima *et al.*, 2002). Nitrogen is one main resource needed in photosynthesis machinery like chloroplasts (Liu *et al.*, 2013) and stomatal conductance (Schulze *et al.* 1994). Since nitrogen levels decrease in these fully expanded leaves, stomatal conductance will decrease which then consequently decreases transpiration rates (Schulze *et al.* 1994).

In late August and early September, there are days when the NPR is lower by a factor of about two compared to September 7th for all the different sized leaves. On these days, transpiration rates are low as well. The days when the NPR is lower is when the temperature is around 20 degrees Celsius (Figure 6) and when there is low or zero precipitation levels (Figure 7). The chlorophyll levels on these days vary for the different sized leaves. On a high temperature day like August 30, the small sized leaves had 27% lower levels, medium sized leaves had 37% higher levels and large leaves had about the same chlorophyll levels as leaves in September 7 when the temperature was lower (Figure 3). Since the temperature is high on these days, the photosynthesis rates are low. High temperatures result in low photosynthetic rates because there is less photosystem II function which is a protein complex involved in the light-dependent reactions of photosynthesis. The function of this complex is decreased because the electron acceptor side of photosystem II is sensitive to high temperatures (Yan *et al.*, 2011). Also, since on these days, the precipitation levels are very low or zero, there is water stress that causes stomata to close as ABA initiates a signaling pathway (Osakabe *et al.*, 2014). Stomata closures is indicated by the low transpiration rates on these days. Since stomata close, carbon dioxide was not being able to enter the leaves and less carbon was fixed, resulting in lower photosynthesis rates (Osakabe *et al.*, 2014). On the days when precipitation levels are high, the transpiration rates increase as seen in Figure 7 due to stomata remaining open (Osakabe *et al.*, 2014).

Also, on September 14, the photosynthesis rates of the different sized leaves are lower due to temperature explained above. However, for the medium sized leaf, it could also be due to some damage in the leaf which may have caused the proteins levels to decrease as seen in the SDS-PAGE gel in Figure (2). In this Figure, the proteins levels of this leaf is shown to be really low as the bands seen are really faded.

In mid-October, the NPR for these leaves are either very close to 0 or negative values and the transpiration rates are low as well because autumnal senescence in these leaves are triggered by the decrease in photoperiods during this time as seen in Figure 8 (Pessarakli, 2002). In this type of senescence, there is degradation of cell constituents including proteins like RuBP carboxylase/oxygenase levels and chlorophyll (He *et al.* 2002). The degradation of these constituents is seen Northern Catalpa leaves during this time. The decrease in chlorophyll levels in the Northern Catalpa leaves in October can be seen in Figure 3. The decrease in RuBP carboxylase/oxygenase levels during this time is indicated by the faded or absence of bands in the leaf samples on the SDS-PAGE gel in Figure 2. Also, stomatal conductance also declines through senescence, resulting in lower transpiration rates. This decline is due to stomatal closures during senescence (Pessarakli, 2002).

The second objective of this study was to learn the hormones that effect the senescence of northern catalpa leaves and it seems that auxin and ABA have the main effects on this process.

In the natural senescence of these leaves, one can see that the leaves turn yellow (Figure 1.1) as the chlorophyll levels decrease (Figure 3) but in dark-induced senescence, when Northern Catalpa leaf discs are placed the different hormonal treatments, they initially get black spots and then they turn completely black as the days go by except the ones in the optimal concentrations of auxin and in the no treatment without any airflow (Table 2, 3, and 4). This delay in blackness

caused by auxin, indicates its role in slowing down senescence in these leaves. Also, the ones in the ABA treatment turn black fast really fast compared to the control which indicates its role in accelerating senescence. The reason they may turn black in the dark is that photosynthesis in these leaf discs are inhibited in the dark which leads to membrane damage of the leaf cells. Jones and Clayton-Greene (1992) lists two possible explanations for this damage including the increase in number of oxygen free radicals and increase in oxidations of phenols. Therefore, in this study it has been found that auxin may either decrease the level of oxygen free radicals or inhibit these oxidation reactions and ABA has the opposite action. Past studies have shown that auxin's ability to inhibit some oxidation reactions including oxidation of glutathione in horseradish (Stonier and Yang, 1973). Also, the no treatment discs without airflow may have no dark spots maybe due to the fact that the enzymes in these leaves are inactive to cause membrane damage. The inactivation of enzymes may be due to the fact these leaf disks had very little or no moisture left in their leaves because of airflow. This shortage of water may have inactivated the proteins and enzymes that cause the increase in oxygen free radicals or oxidation metabolism. There are many enzymes that are activated by the presence of moisture including the seed germination enzymes that require water in order to promote growth in the seeds (Vashisth and Nagarajan, 2010).

In this study, it has been found that ABA accelerates Northern Catalpa leaf senescence through its effect on chlorophyll levels, along with looking at the morphological features. It is found through the ABA treated leaf discs which show a more rapid decline in chlorophyll a, chlorophyll b, and total chlorophyll levels over the time period of 35 days compared to the leaf disks in the control treatment which is the treatment with water (Figure 9). More chlorophyll is being degraded in the ABA treated leaf disks each week compared to the leaf disks in the control

treatment which indicates a role of ABA of in promoting senescence in Northern Catalpa leaves. The chlorophyll levels measured at day 35 is the same as day 0 chlorophyll levels but this result is due to an experimental error. The experimental error is that when the mass of the leaf disk at day 35 was measured to calculate chlorophyll levels, it very damaged and degraded compared to the leaf disks of earlier days and it seems like a lot of the proteins and cellular constituents have oozed out compared to leaf disks of earlier days. Therefore, the mass was lower of the leaf disk measured was lower than it actually was if the leaf disk stayed intact so chlorophyll levels that were measured were higher than they actually were. Also, in Figure 12, there is no protein bands seen in the SDS-page gels of ABA treated leaf disks. However, one may argue that a lot of the proteins in the other hormonal treatments and control treatment also show no bands because the proteins have shown to deteriorate when the SDS-Page was conducted so the results in the gel may not be the result of the particular ABA treatment. It could be due to the fact that the leaves were frozen for a month after being in their particular treatments so the proteins degraded. However, studies on other plant species have shown that ABA promotes leaf senescence by decreasing chlorophyll levels and photosynthetic protein levels. Gao *et al.* (2016) explains that ABA promotes leaf senescence in an ethylene-independent way through activating transcription factors known as ABF2 (AREB1), ABF3, and ABF4. These transcription factors bind to the promoters of senescence associated genes (Gao *et al.* 2016). The products of genes cause chlorophyll degradation, less RuBisCO activity, and lower RuBisCO levels, resulting in leaf senescence (Gao et al. 2016).

While ABA is seen to accelerated leaf senescence, NAA is found to delay senescence in the northern catalpa leaves by delaying chlorophyll degradation and preventing some of the RuBisCO degradation. NAA treated leaf disks showed very small amount of decline in

chlorophyll a, chlorophyll b, and total chlorophyll levels over the 35 days. This result indicates an auxin's role in retarding the senescence of Northern Catalpa leaves. Also, all the proteins samples from treated leaf discs degraded before being run on the SDS-PAGE gel except for protein samples of the auxin treated leaf disk on day 21 (Figure 12). This data shows that auxin does delay senescence of the northern catalpa leaves by delaying the degradation of proteins, along with delaying the chlorophyll degradation. Past studies have confirmed this data on *Arabidopsis* plants. They found that a mutation in the ARF2 gene results in the delay of leaf senescence. This gene is a transcription factor that represses auxin-related signaling. The auxin signaling that delays degradation of chlorophyll content, maintains photochemical efficiency of photosystem II, delays membrane ion leakage, and delays the expression of senescence-associated genes is still unknown (Lim *et al.* 2010).

Varying concentrations of both NAA and IAA show different response in the leaves. The IAA (10^{-5}M) treated, IAA (10^{-4}M), and NAA (10^{-5}M) treated leaf disks show similar trend of decline like the control treated leaves where there is a decline of chlorophyll a, chlorophyll b, and total chlorophyll levels (Figure 10). This data shows that concentrations are not high enough to elicit the auxin response in leaves. The NAA ($2*10^{-4}$M) show a great decline of chlorophyll a, chlorophyll b, and total chlorophyll levels over the 35 days, indicating an inhibitory effect of high amounts of auxin. In other plant species, the molecular mechanism of the inhibitory effect high concentrations of auxin in leaf senescence is not known. High amounts of auxin is known to inhibit shoot growth by stimulating ethylene production which consequently increases the ABA levels in the shoots (Hanson, 2000). In leaves, high amounts of auxin may stimulate the product of a hormone that accelerates leaf senescence like ABA but further investigation needs to be done in order to make this conclusion. Leaf disks in the NAA (10^{-4}M) and IAA ($2*10^{-4}$M) show

little decline of chlorophyll a, chlorophyll b, and total chlorophyll levels over the 35 days, indicating that these are the optimal concentrations needed to delay senescence. Another indication that 10^{-4}M is an optimal concentration of NAA in leaf disks in delay senescence is that in the SDS-PAGE gel in Figure 13, some faded bands, representative of small levels proteins are still seen on leaf disks with 10^{-4}M treatment on day 21. Proteins in these samples means that the proteins didn't get degraded yet and senescence was delayed by this concentration of NAA.

The leaf disks exposed to the mixture of NAA (10^{-4}M) and ABA (10^{-4}M) show the rapid decline of chlorophyll levels like the ABA (10^{-4}M) treated leaf disks (Figure 11). This indicates that auxin doesn't inhibit the response of ABA. ABA is still able to activating transcription factors known as ABF2 (AREB1), ABF3, and ABF4. These transcription factors bind to the promoters of senescence associated genes (Gao *et al.* 2016).

Also, the leaf disks with no treatment but experienced air flow also show little decline of chlorophyll levels and proteins over the 35 days. In the SDS-PAGE gel, there are multiple bands and the brightness of these bands stay the same for the samples from day 7, 14, 21, and 35, indicating that the proteins levels stay constant (Figure 14). Whereas, the no treatment leaf disks with no airflow show similar trend of chlorophyll decline like the control treated leaves. This may be due to the fact that the leaf disks with no treatment that experienced air flow had very little or no moisture left in their leaves. This may have inactivated the proteins and enzymes that degrade chlorophylls and other proteins during senescence induced by the dark and wounding of the leaves after being cut (Vashisth and Nagarajan, 2010). However, in the no treatment samples that experienced no airflow had the moisture to keep their enzymes active so the chlorophyll levels degraded similar to the control treatment discs (Figure 11).

In Figures 10 and 11, there are days in some of the treatments where chlorophyll levels are shown to increase but this is not possible because chlorophyll formation is a light-regulated process (Biswal *et al.,* 2012). As mentioned before, leaves for this experiment were taken at the end of September when senescence was initiated in some these leaves. This can also be seen in Figure 13, in the day 0 lane, where the leaves from this time showed that enzymes had already cut proteins in this leaf sample. Multiple proteins are degraded into smaller sizes which is represented by the smear in this lane. For some of the hormonal treatments, different leaves were used to test the hormone effects for a different amount of days. Some of these leaves may be in different stages in senescence so there may have a different initial chlorophyll levels and there may be more degradation of this pigment in some leaves over others in some leaves so a higher chlorophyll level may be seen later days compared to earlier days. For instance, on day 14, there may be higher chlorophyll levels measured compared to day 7 because two different leaves were being tested for these amount of days. Next time, to see a clearer effect of hormones on chlorophyll levels, the experiment should be done when the leaves aren't going senescence and the same leaf should be used to test the effect of the hormone for different amount of days.

All in all, through this study, it has been found that high temperatures and lower precipitation levels lower the photosynthesis and transpiration rates of northern catalpa leaves, resulting in less carbon sequestration. Also when the season changes to fall season, autumnal senescence is triggered in these leaves which also causes the lowering of these rates, thereby, reducing the carbon intake from the atmosphere. Also, it has been found through this study that auxin and ABA play major roles in the senescence process of these leaves by looking at the morphology, chlorophyll levels, and protein levels of these leaves. Optimal concentrations of

synthetic and natural auxin delay senescence and ABA accelerates the senescence process in these leaves.

Acknowledgments:

I would like to sincerely thank Dr. Singh and Mr. Mehroke for their continuous help and guidance throughout this whole study. I would also like to thank the volunteer and work learn student in Dr. Singh's lab who helped my complete various experiments in this study. Their names are Karthik Arora and Amy Dhillon.

References

Bassow, S. L., & Bazzaz, F. A. (1998). How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species. *Ecology, 79*(8), 2660-2675.

Biswal, A. K., Pattanayak, G. K., Pandey, S. S., Leelavathi, S., Reddy, V. S., Govindjee, & Tripathy, B. C. (2012). Light intensity-dependent modulation of chlorophyll b biosynthesis and photosynthesis by overexpression of chlorophyllide a oxygenase in tobacco. *Plant Physiology, 159*(1), 433-449. doi:10.1104/pp.112.195859

Brooks, A., & Farquhar, G. D. (1985). Effect of temperature on the CO2/O 2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light : Estimates from gas-exchange measurements on spinach. *Planta, 165*(3), 397.

Catalpa speciosa. (1928). *Bulletin of Popular Information (Arnold Arboretum, Harvard University), 2*(14), 54-54.

The catalpa tree. (1904). *Scientific American (1845-1908), XC.*(No. 21.), 398.

Constable, G., & Rawson, H. (1980). Effect of Leaf Position, Expansion and Age on Photosynthesis, Transpiration and Water Use Efficiency of Cotton. Australian Journal of Plant Physiology, 7(1), 89. doi:10.1071/pp9800089

Dreyfuss, B. W., & Thornber, J. P. (1994). Assembly of the light-harvesting complexes (LHCs) of photosystem II: Monomeric LHC IIb complexes are intermediates in the formation of oligomeric LHC IIb complexes. *Plant Physiology, 106*(3), 829-839.

Gan, S., & Amasino, R. M. (1997). Making sense of senescence: Molecular genetic regulation and manipulation of leaf senescence. *Plant Physiology, 113*(2), 313-319.

Gao, S., Gao, J., Zhu, X., Song, Y., Li, Z., Ren, G., . . . Kuai, B. (2016). ABF2, ABF3, and ABF4 promote ABA-mediated chlorophyll degradation and leaf senescence by transcriptional activation of chlorophyll catabolic genes and senescence-associated genes in arabidopsis. *Molecular Plant, 9*(9), 1272-1285. doi:10.1016/j.molp.2016.06.006

Florides, G. A., & Christodoulides, P. (2009). Global warming and carbon dioxide through sciences. *Environment International, 35*(2), 390-401. doi:10.1016/j.envint.2008.07.007

Hansen, H., & Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth Inhibition1. *Plant Physiology, 124*(3), 1437-1448.

He, P., Osaki, M., Takebe, M., & Shinano, T. (2002). Changes of photosynthetic characteristics in relation to leaf senescence in two maize hybrids with different senescent appearance. *Photosynthetica, 40*(4), 547-552. doi:10.1023/A:1024347918199

Hofstra, G., & Hesketh, J. D. (1969). The effect of temperature on stomatal aperture in different species. *Canadian Journal of Botany, 47*(8), 1307-1310. doi:10.1139/b69-184

Jones, R. B., & Clayton-Greene, K. A. (1992). The role of photosynthesis and oxidative reactions in leaf blackening of protea neriifolia R. br. leaves. *Scientia Horticulturae, 50*(1), 137-145. doi:10.1016/S0304-4238(05)80016-0

Keskitalo, J., Bergquist, G., Gardeström, P., & Jansson, S. (2005). A cellular timetable of autumn Senescence1. *Plant Physiology, 139*(4), 1635-1648. doi:10.1104/pp.105.066845

Kitajima, K., Mulkey, S. S., Samaniego, M., & Joseph Wright, S. (2002). Decline of photosynthetic capacity with leaf age and position in two tropical pioneer tree species. *American Journal of Botany, 89*(12), 1925-1932. doi:10.3732/ajb.89.12.1925

Liu, X., Fan, Y., Long, J., Wei, R., Kjelgren, R., Gong, C., & Zhao, J. (2013). Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of robinia pseudoacacia seedlings. *Journal of Environmental Sciences (China), 25*(3), 585-595. doi:10.1016/S1001-0742(12)60081-3

Liu, L., Li, H., Zeng, H., Cai, Q., Zhou, X., & Yin, C. (2016;2015;). Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. *Journal of Plant Growth Regulation, 35*(2), 366-376. doi:10.1007/s00344-015-9539-0

Lim, P. O., Lee, I. C., Kim, J., Kim, H. J., Ryu, J. S., Woo, H. R., & Nam, H. G. (2010). Auxin response factor 2 (ARF2) plays a major role in regulating auxin-mediated leaf longevity. *Journal of Experimental Botany, 61*(5), 1419-1430. doi:10.1093/jxb/erq010

Osakabe, Y., Osakabe, K., Shinozaki, K., & Tran, L. P. (2014). Response of plants to water stress. *Frontiers in Plant Science, 5* doi:10.3389/fpls.2014.00086

Palmer, C. (2012). To what extent could planting trees help solve climate change? Retrieved December 05, 2016, from https://www.theguardian.com/environment/2012/nov/29/planting-trees-climate-change

Pantin, F., Simonneau, T., Rolland, G., Dauzat, M., & Muller, B. (2011). Control of leaf expansion: A developmental switch from metabolics to Hydraulics1[W][OA]. *Plant Physiology, 156*(2), 803-815. doi:10.1104/pp.111.176289

Pessarakli, M. (2002). *Handbook of plant and crop physiology* (2nd, rev. and expand ed.). New York: M. Dekker.

Qiu, K., Li, Z., Yang, Z., Chen, J., Wu, S., Zhu, X., . . . Zhou, X. (2015). EIN3 and ORE1 accelerate degreening during ethylene-mediated leaf senescence by directly activating chlorophyll catabolic genes in arabidopsis. *PLoS Genetics, 11*(7), e1005399. doi:10.1371/journal.pgen.1005399

Stonier, T., & Yang, H. (1973). Studies on auxin protectors: XI. inhibition of peroxidase-catalyzed oxidation of glutathione by auxin protectors and. *Plant Physiology, 51*(2), 391-395.

Schulze, E., Kelliher, F. M., Korner, C., Lloyd, J., & Leuning, R. (1994). Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: A global ecology scaling exercise. *Annual Review of Ecology and Systematics, 25*(1), 629-662. doi:10.1146/annurev.es.25.110194.003213

Yan, K., Chen, P., Shao, H., Zhang, L., & Xu, G. (2011). Effects of Short-Term high temperature on photosynthesis and photosystem II performance in sorghum. *Journal of Agronomy and Crop Science, 197*(5), 400-408. doi:10.1111/j.1439-037X.2011.00469.x

Retrieved November 05, 2016, from http://climate.weather.gc.ca/climate_data/hourly_data_e.html?hlyRange=1976-01-20|2016-11-29&dlyRange=1925-11-01|2016-11-29&mlyRange=1925-01-01|2007-02-01&StationID=888&Prov=BC&urlExtension=_e.html&searchType=stnName&optLimit=yearRange&StartYear=1840&EndYear=2016&selRowPerPage=25&Line=35&searchMethod=contains&Month=11&Day=29&txtStationName=vancouver&timeframe=1&Year=2016

Singh, S. 2016. Lab Unit 3: Senescence and regrening of cumcumber cotyledons: light and hormonal regulation of proteins and chlorophyll. Biology 352 Plant Physiology II: Plant Development. Plant Physiology Teaching Laboratory, Department of Botany, University of British Columbia. 38-55. PDF downloaded Jan 9, 2016 from http://elearning.ubc.ca/connect/

Templer, P. H. (2015). Changes in autumn senescence in northern hemisphere deciduous trees: A meta-analysis of autumn phenology studies. *Annals of Botany, 116*(6), 875. doi:10.1093/aob/mcv055

Vancouver, British Columbia, Canada - Sunrise, Sunset, and Daylength, Retrieved November 05, 2016, from https://www.timeanddate.com/sun/canada/vancouver

Vashisth, A., & Nagarajan, S. (2010). Characterization of water distribution and activities of enzymes during germination in magnetically-exposed maize (zea mays L) seeds. *Indian Journal of Biochemistry & Biophysics, 47*(5), 311.

Appendix:

Part 1: Figures and Tables

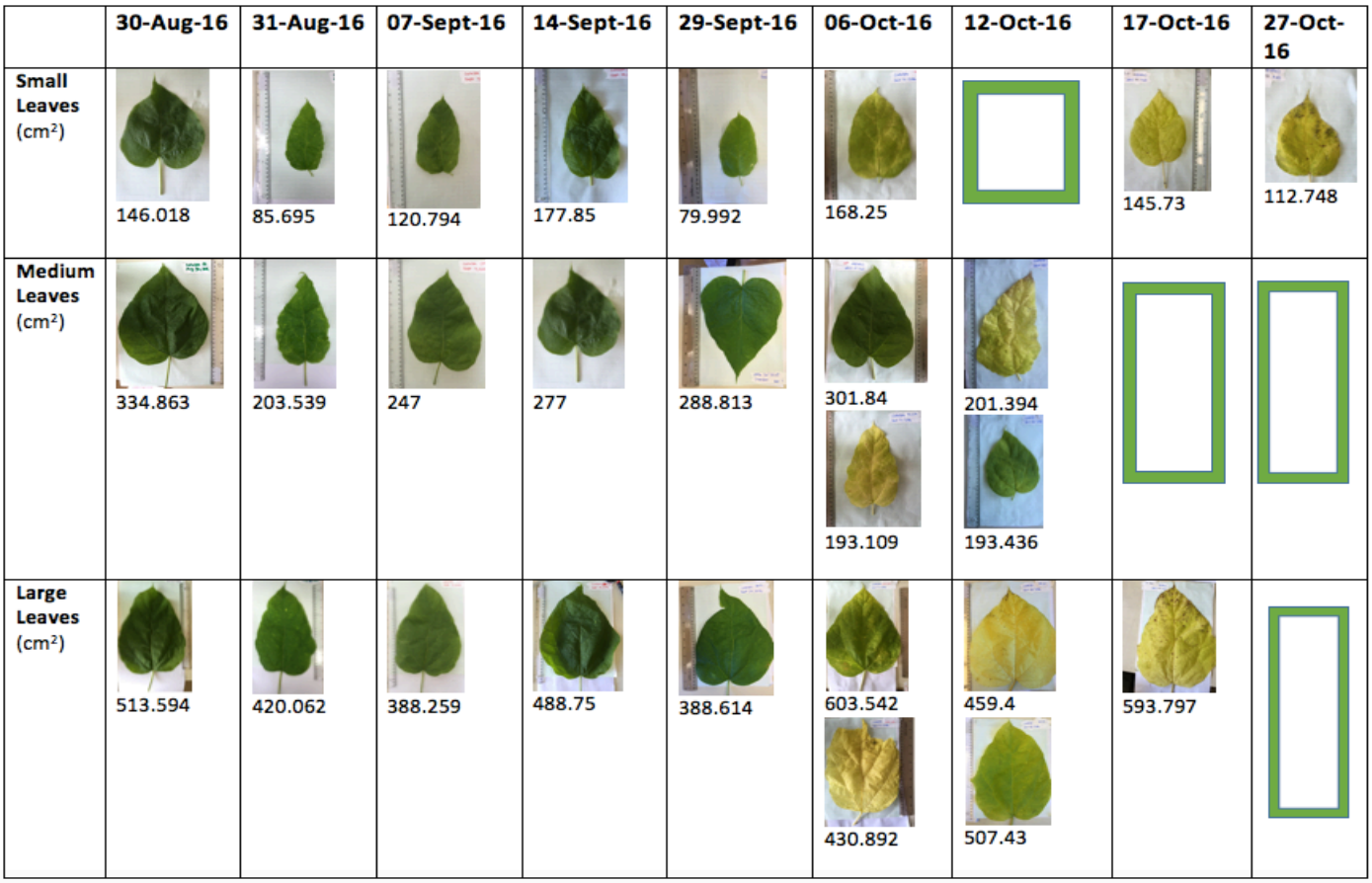


Figure 1.1. Morphological changes in small, medium, and large sized Northern Catalpa leaves from August to October 2016. The area of each leaf is also provided below each picture of the leaves in cm^2. The green boxes indicate no data for that category.

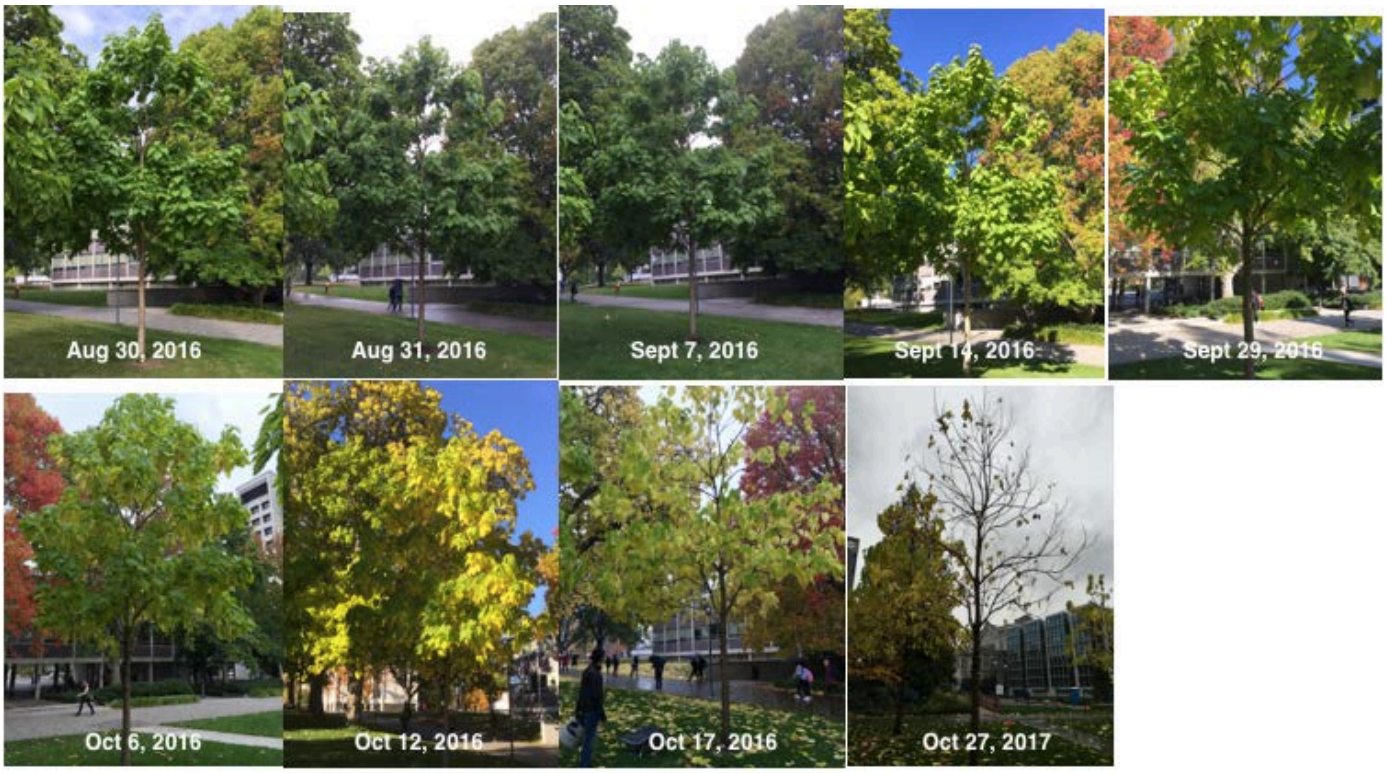


Figure 1. The images of the Northern Catalpa tree from August 30, 2016 until October 27, 2017.

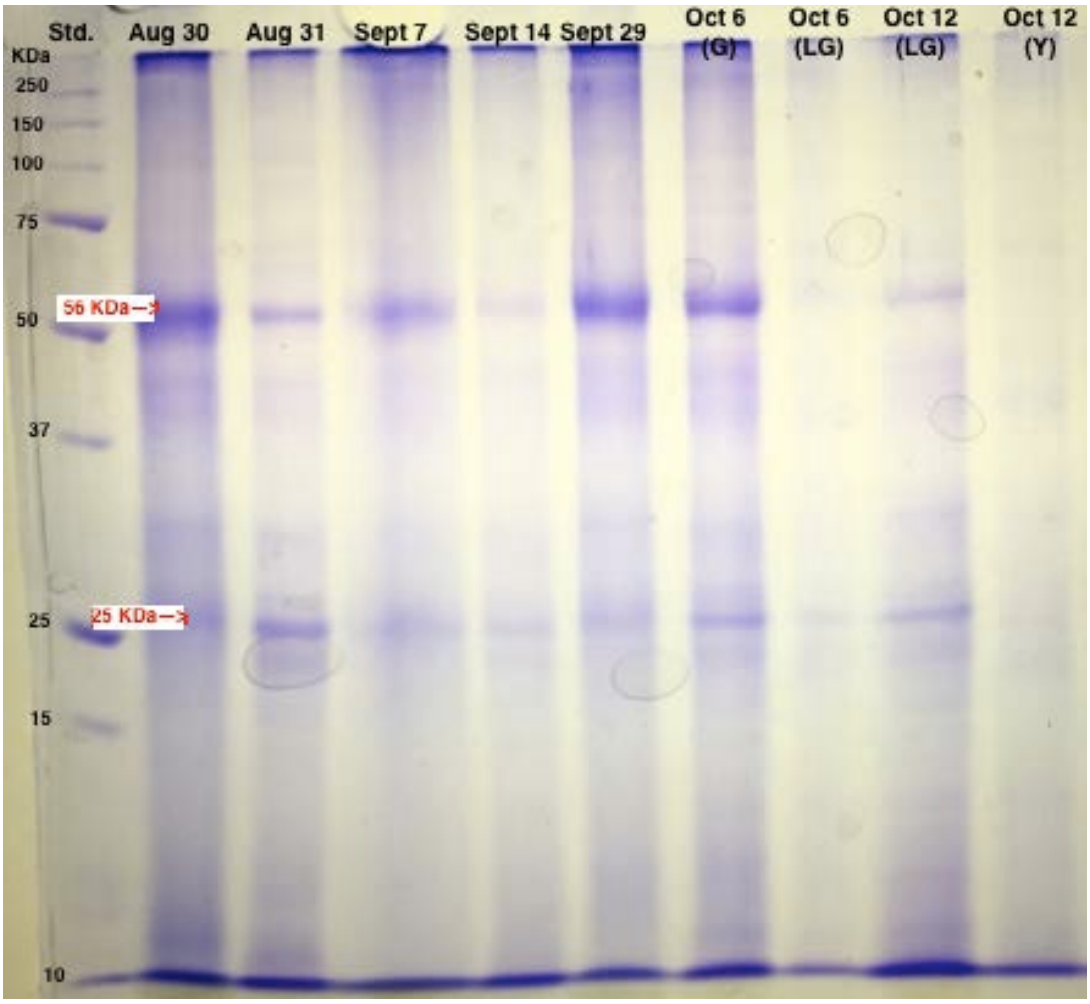


Figure 2. SDS-PAGE gel with Coomassie blue staining. The first lane contains protein standards (Std.). The lanes dated from August 30, 2016 until September 29, 2016 have proteins from green leaves dated from August 30, 2016 until September 29, 2016. There are proteins from a green leaf in the lane "October 6 (G)" and proteins from a lighter green leaf in lane "Oct 6 (LG)." There are proteins from a lighter green leaf in the lane "October 12 (LG)" and proteins from a yellow leaf in "October 12 (Y)." All the leaves used in the gel are medium sized leaves.

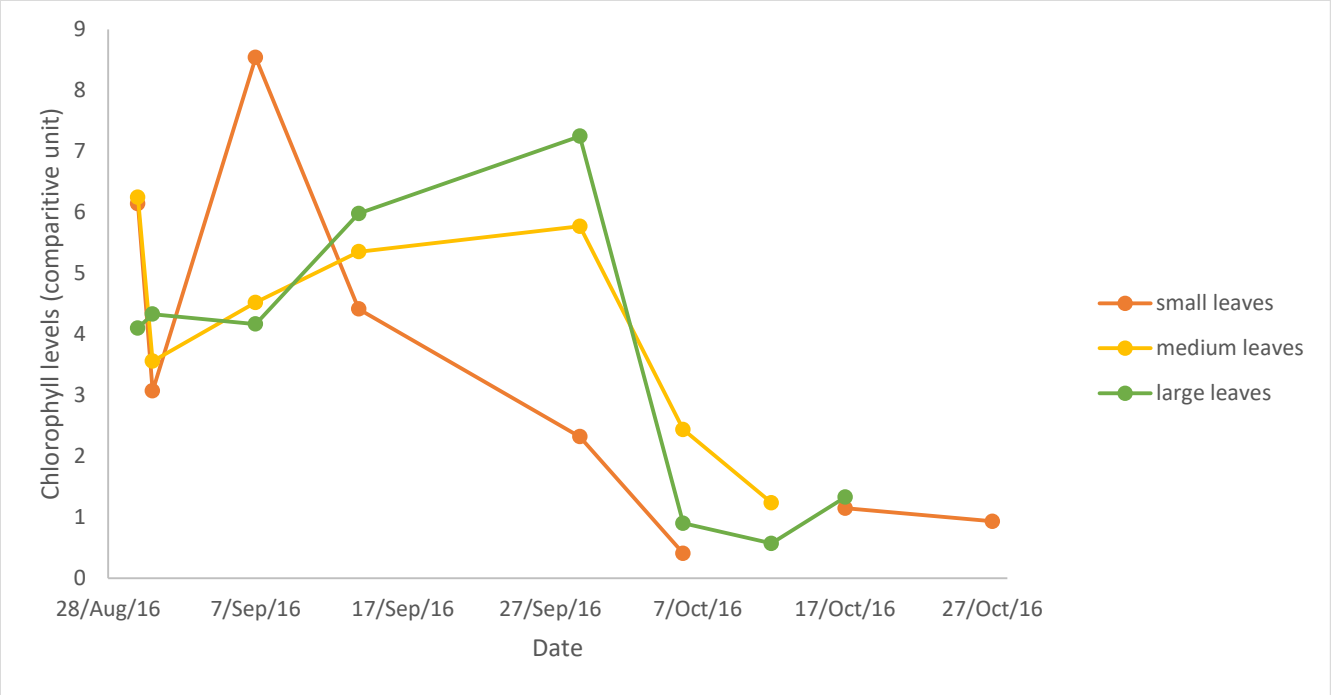


Figure 3. The chlorophyll levels of small, medium, and large sized Northern Catalpa leaves from August to October 2016.

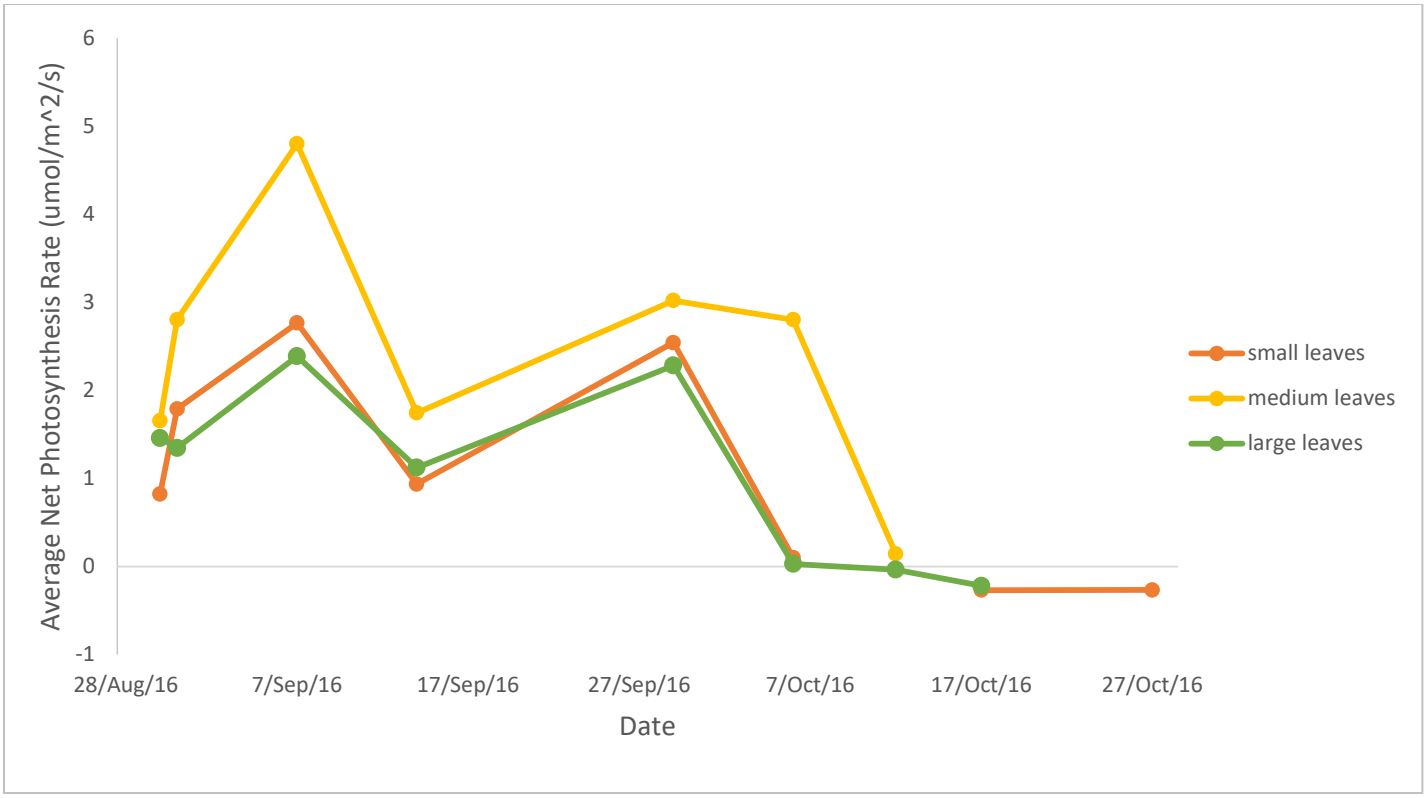


Figure 4. Average net photosynthetic rates for small, medium, large Catalpa leaves during late summer and early fall season.

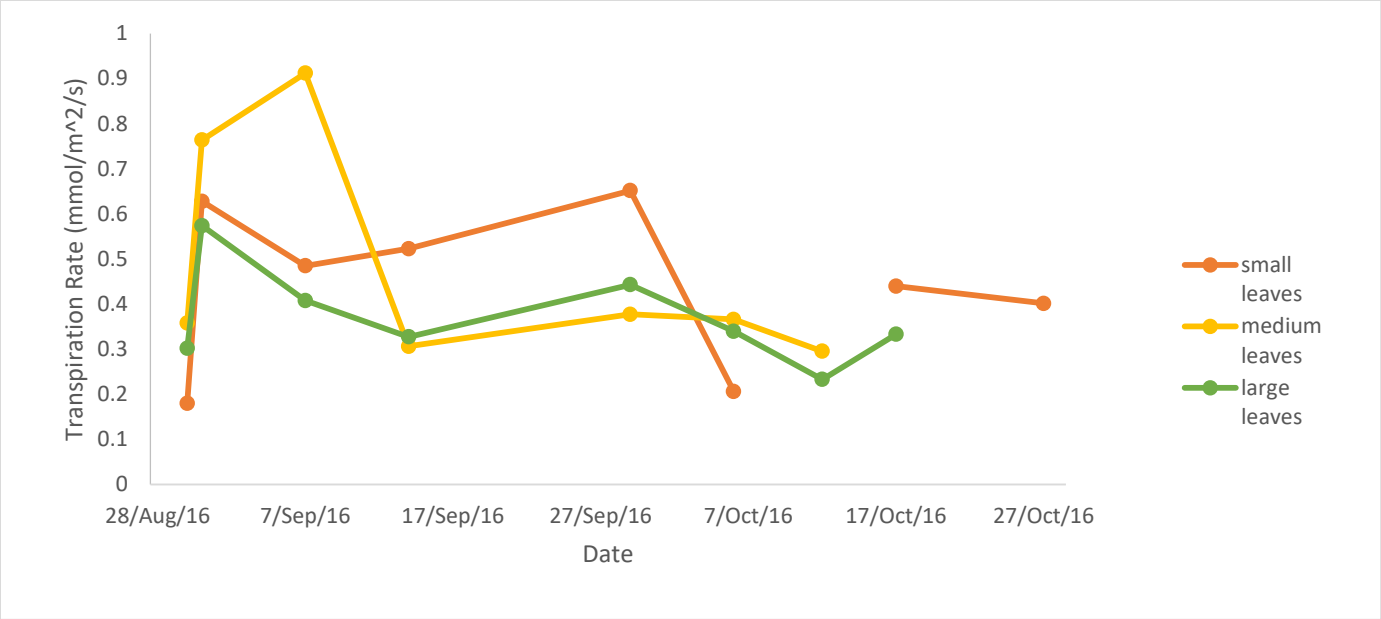


Figure 5. Average transpiration rates for small, medium, large Catalpa leaves during late summer and early fall season.

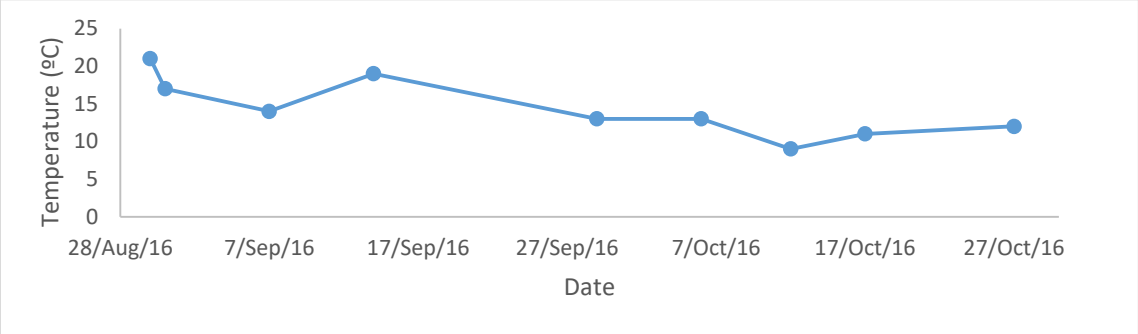


Figure 6. The average temperatures in Vancouver during late summer until fall season.

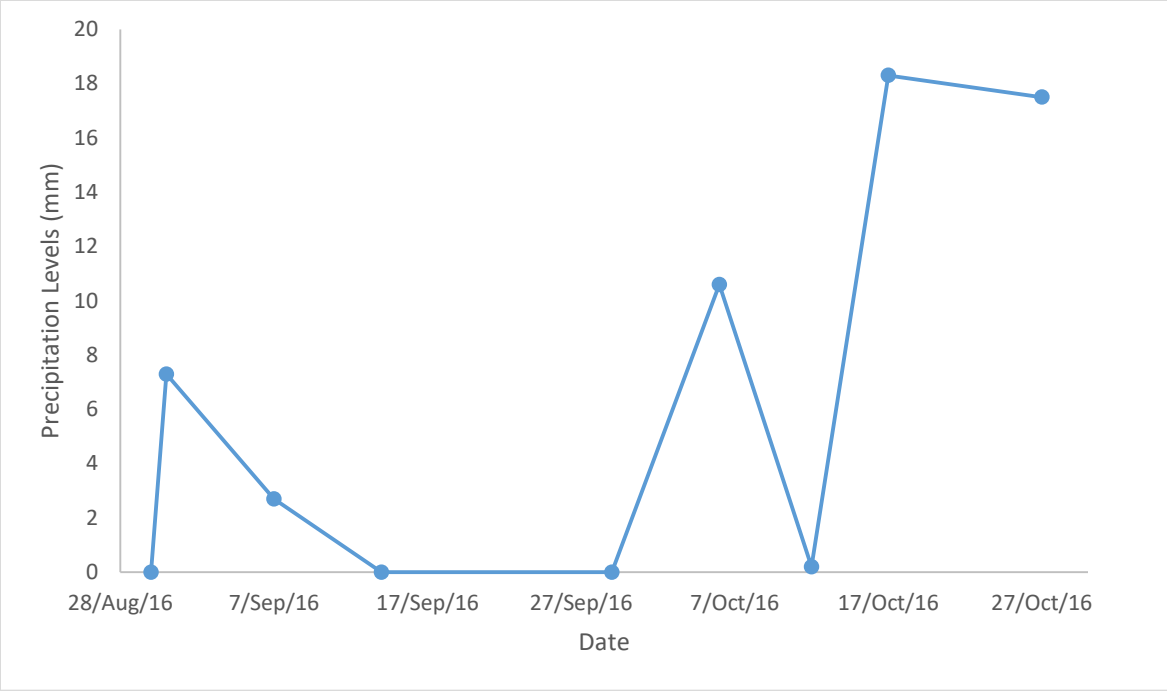


Figure 7. The average precipitation levels in Vancouver during late summer until fall season.

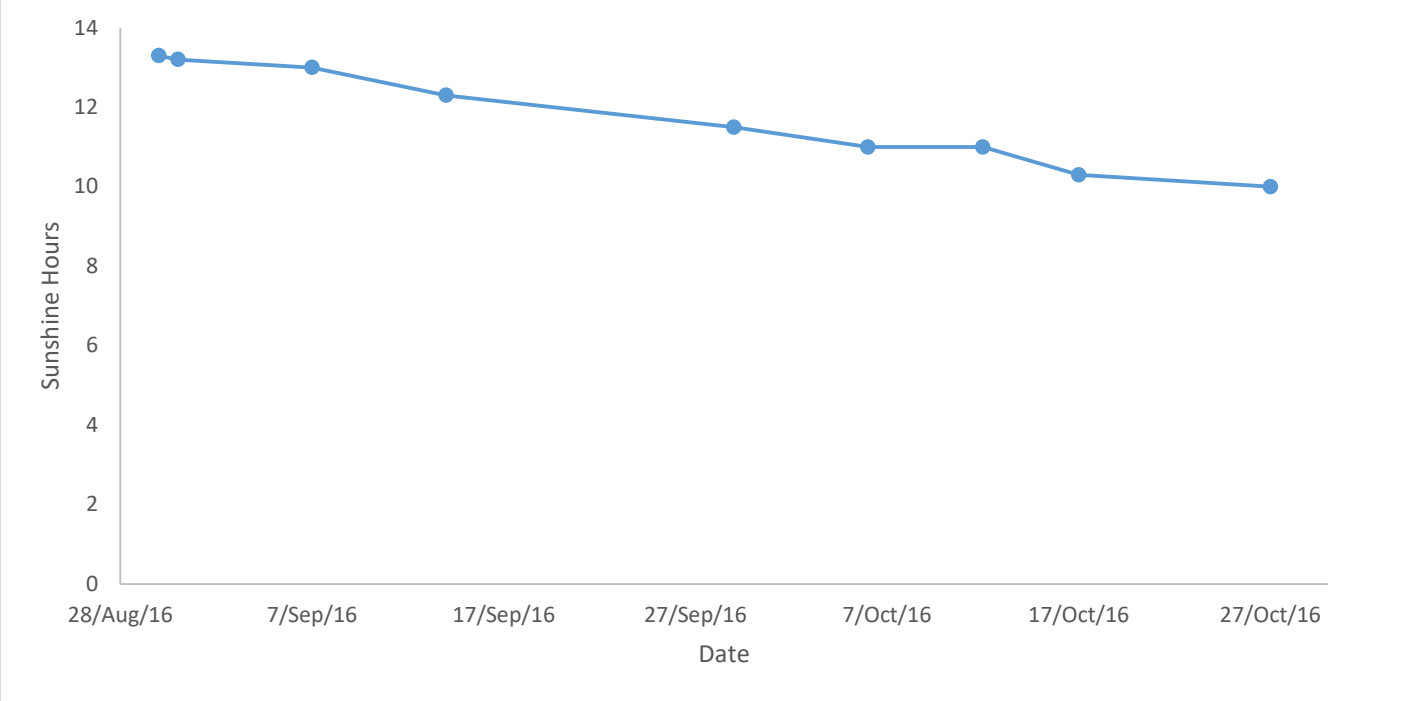


Figure 8. The average photoperiod (sunshine hours) in Vancouver during late summer until fall season.

Table 2. Morphological changes of Northern Catalpa leaf disc in different hormonal treatments including the control, BAP, CEPA, GA3, NAA, JA, and ABA.

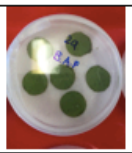
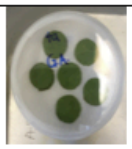
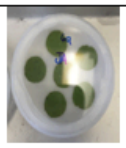
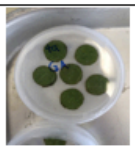
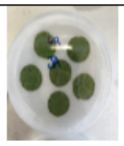
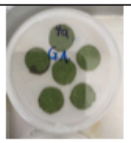
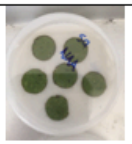
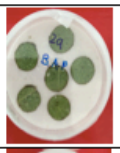
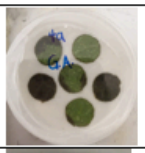
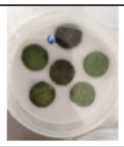
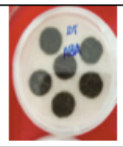
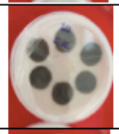
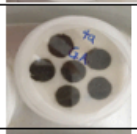
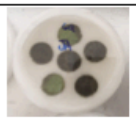
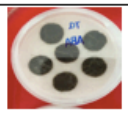
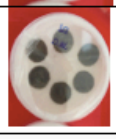
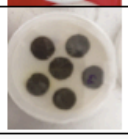
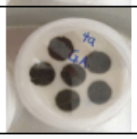
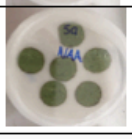
	Control (Water)	BAP (10^{-4}M)	CEPA (10^{-4}M)	GA_3 (10^{-4}M)	NAA (10^{-4}M)	JA (10^{-4}M)	ABA (10^{-4}M)
Day 0							
Day 7							
Day 14							
Day 21							
Day 28							
Day 35							

Table 3. Morphological changes of Northern Catalpa leaf disc in treatments containing varied concentrations of IAA and NAA.

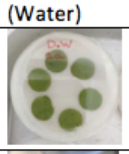
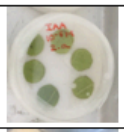
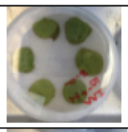
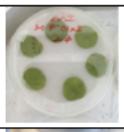
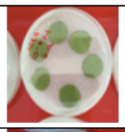
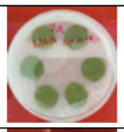
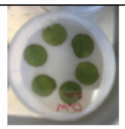
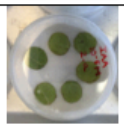
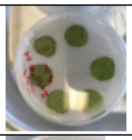
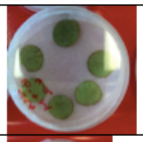
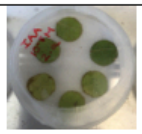
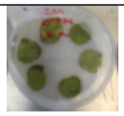
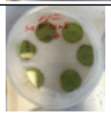
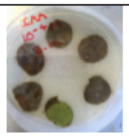
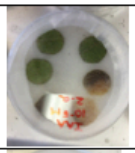
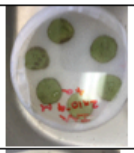
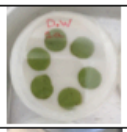
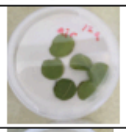
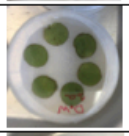
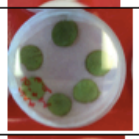
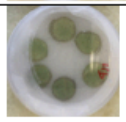
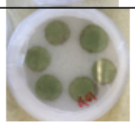
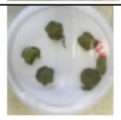
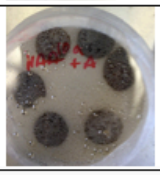
	Control (Water)	IAA (10^{-5}M)	IAA (10^{-4}M)	IAA ($2^{\wedge}10^{-4}$M)	NAA (10^{-5}M)	NAA (10^{-4})M	NAA ($2*10^{-4}$)M
Day 0							
Day 7							
Day 14							
Day 21							
Day 28							
Day 35							

Table 4. Morphological changes of Northern Catalpa leaf discs in treatments including, control, NAA, ABA, mixture of NAA and ABA, no treatment without airflow, and no treatment with airflow.

	Control (Water)	NAA (10^{-4}M)	ABA (10^{-4}M)	NAA (10^{-4}M)and ABA(10^{-4}M)	No treatment without airflow	No treatment with airflow
Day 0						
Day 7						
Day 14						
Day 21						
Day 28						
Day 35						

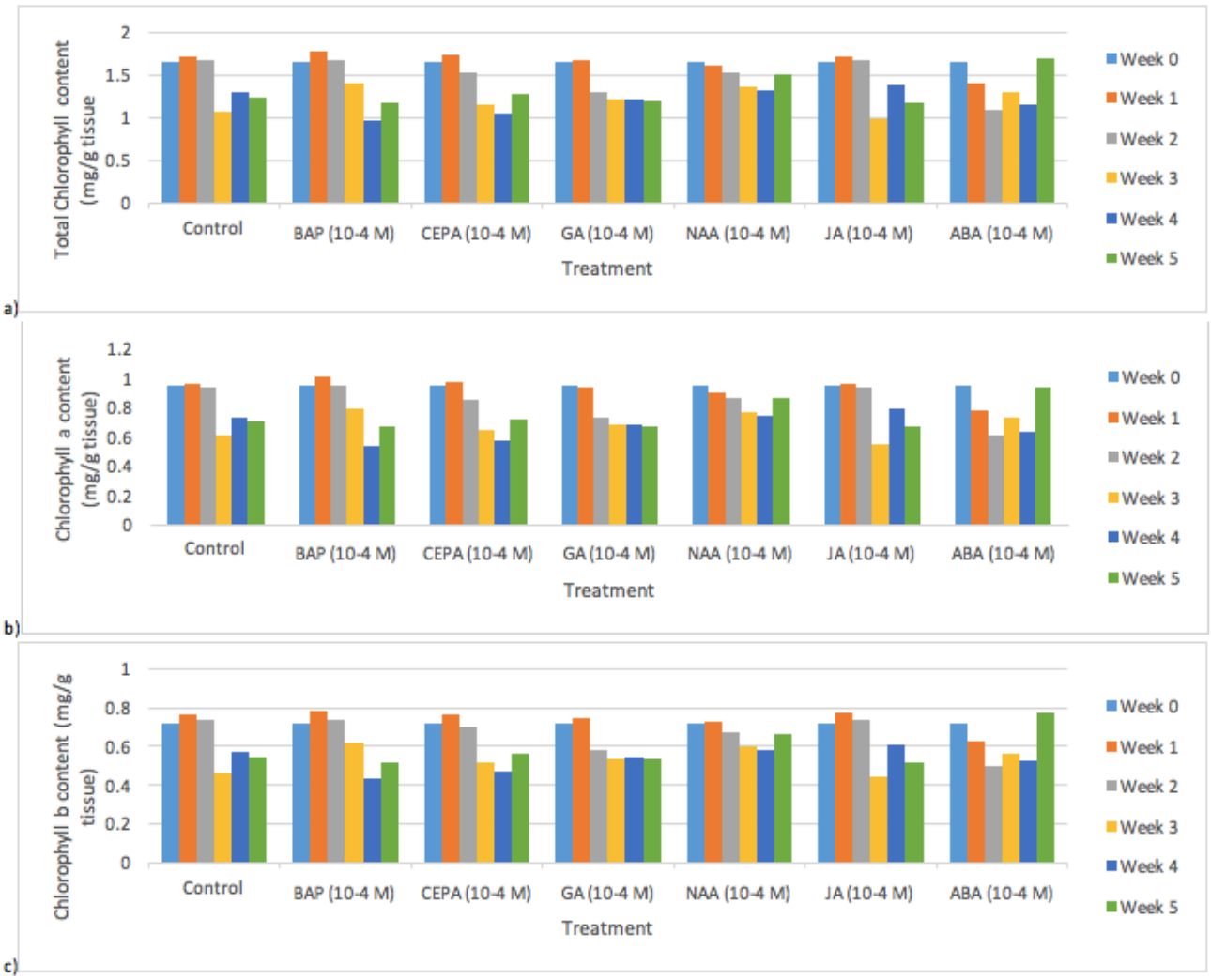


Figure 9. The chlorophyll levels of the leaf disks exogenously treated with different hormones. a) The total chlorophyll levels of leaf disks exogenously treated with different hormones. b) The chlorophyll a levels leaf disks exogenously treated with different hormones. c) The chlorophyll b levels of leaf disks exogenously treated with different hormones.

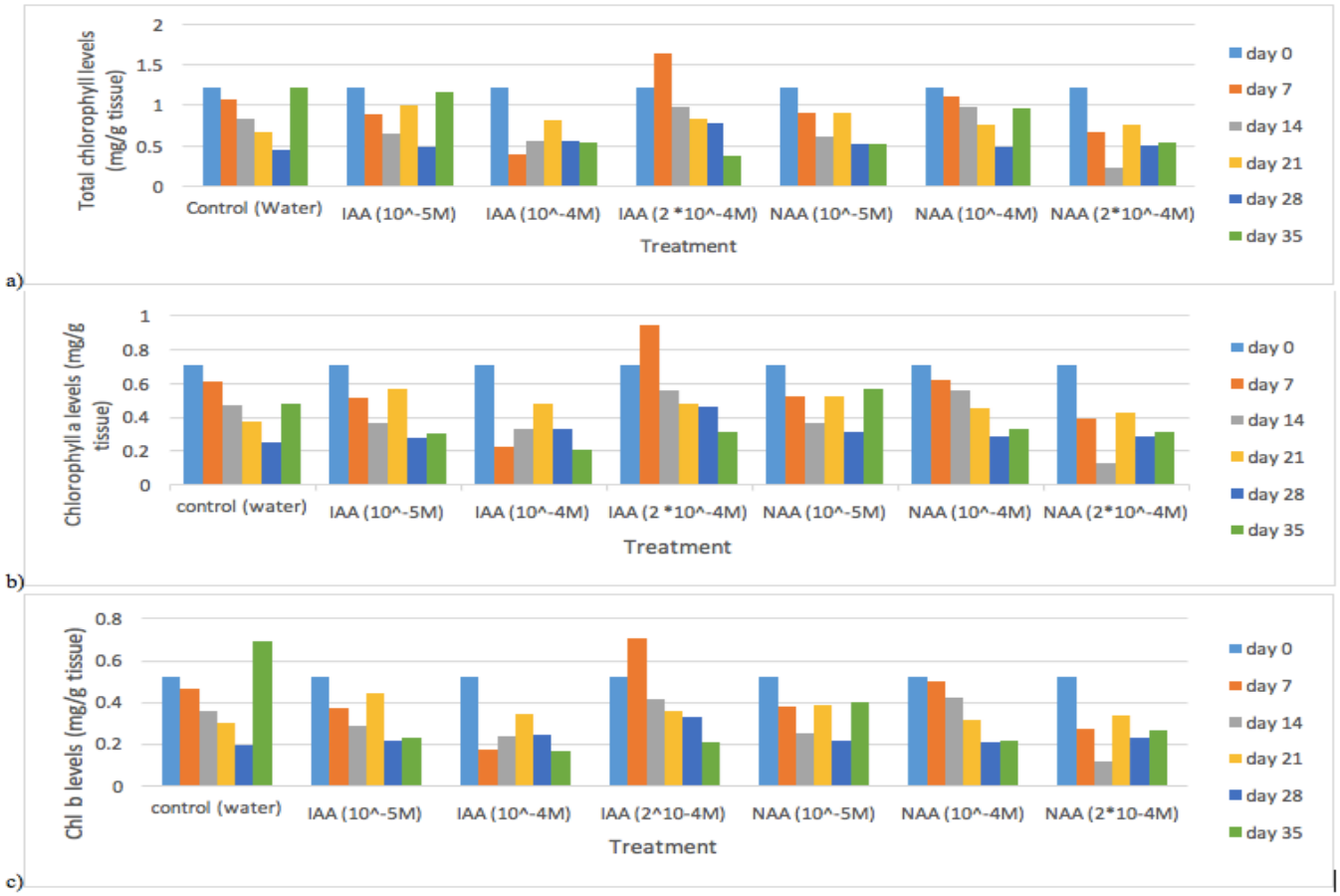


Figure 10. The chlorophyll levels of the leaf disks exogenously treated with different concentrations of natural and synthetic auxin which is IAA and NAA respectively. a) The total chlorophyll levels of leaf disks exogenously treated with different concentrations of IAA and NAA. b) The chlorophyll a levels leaf disks exogenously treated with different concentrations of IAA and NAA. c) The chlorophyll b levels of leaf disks exogenously treated with different concentrations of IAA and NAA.

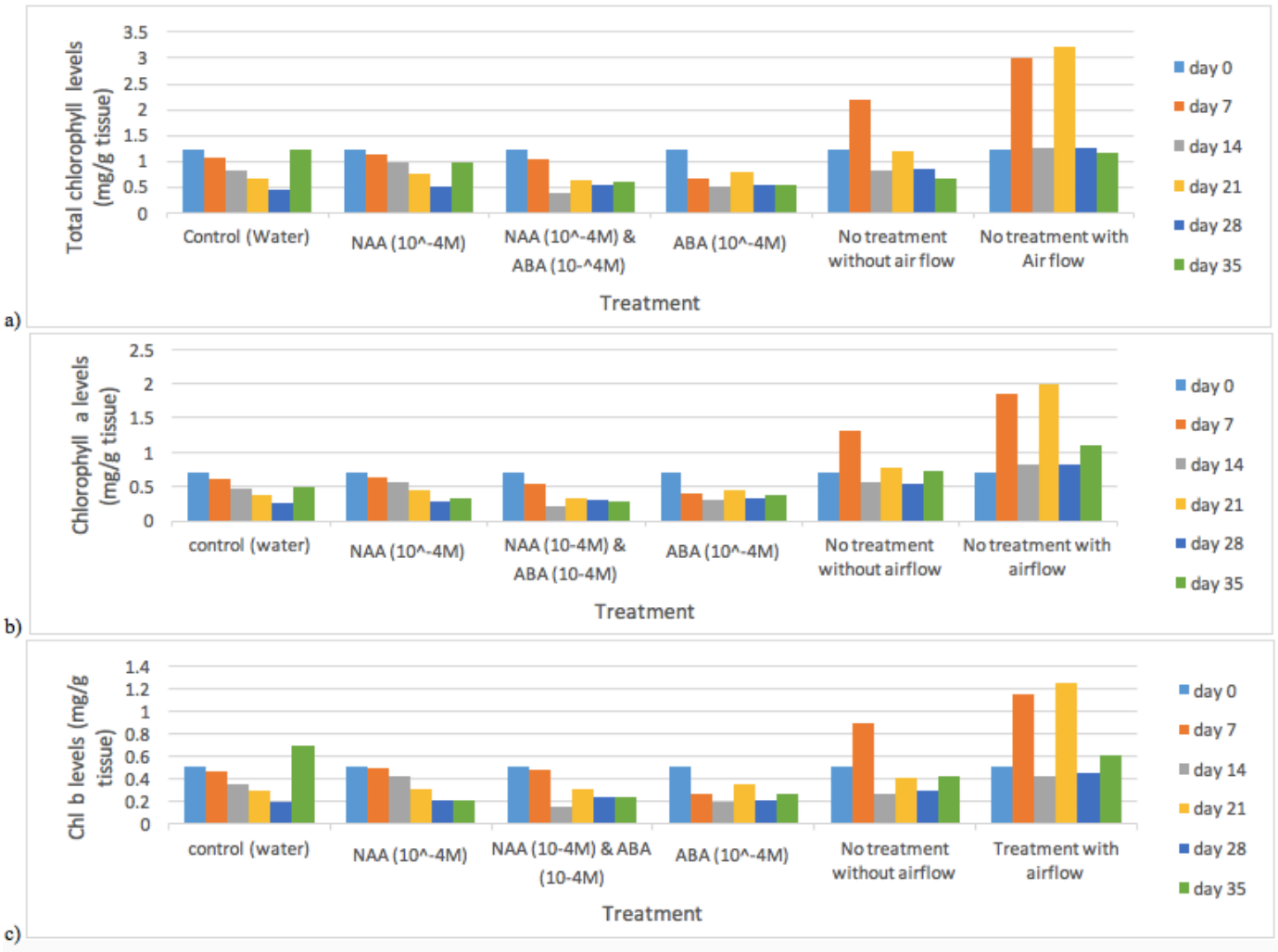


Figure 11. The chlorophyll levels of the leaf disks treated with NAA (10^{-4}M), ABA 10^{-4}M), mixture of NAA 10^{-4}M) and ABA 10^{-4}M), and with no treatments. a) The total chlorophyll levels of leaf disks exogenously treated with with NAA (10^{-4}M), ABA 10^{-4}M), mixture of NAA 10^{-4}M) and ABA 10^{-4}M), and with no treatments. b) The chlorophyll a levels leaf disks exogenously treated with with NAA (10^{-4}M), ABA 10^{-4}M), mixture of NAA 10^{-4}M) and ABA 10^{-4}M), and with no treatment. c) The chlorophyll b levels of leaf disks exogenously treated with with NAA (10^{-4}M), ABA 10^{-4}M), mixture of NAA 10^{-4}M) and ABA 10^{-4}M), and with no treatment.

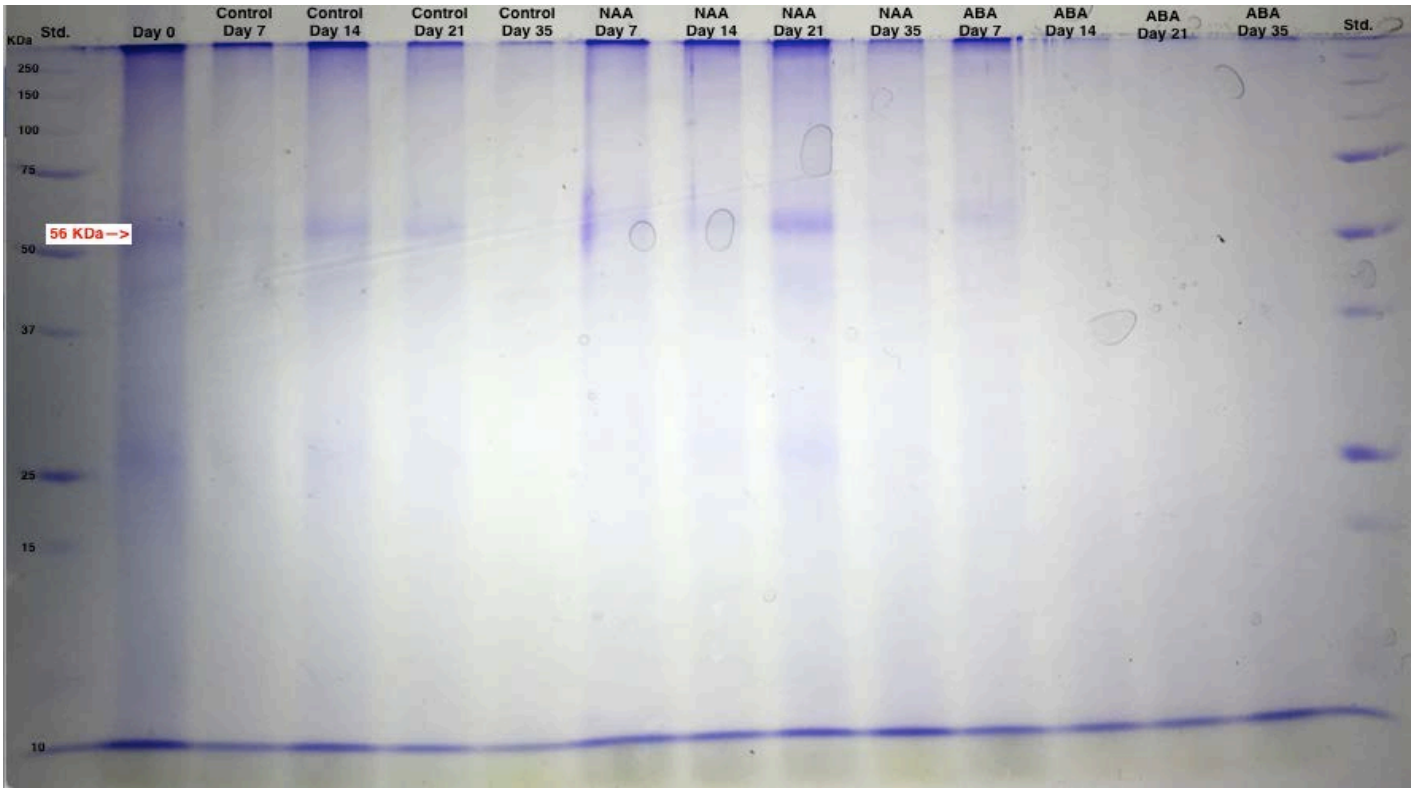


Figure 12. SDS-PAGE gel with Coomassie blue staining. The first and last lane contains protein standards (Std.). The second lane contains proteins from leaves in their original state which didn't undergo any treatment. Rest of the lanes contain proteins from leaf disks in the control, NAA (10^{-4}M), and ABA (10^{-4}M) treatments from day 7 until day 35.

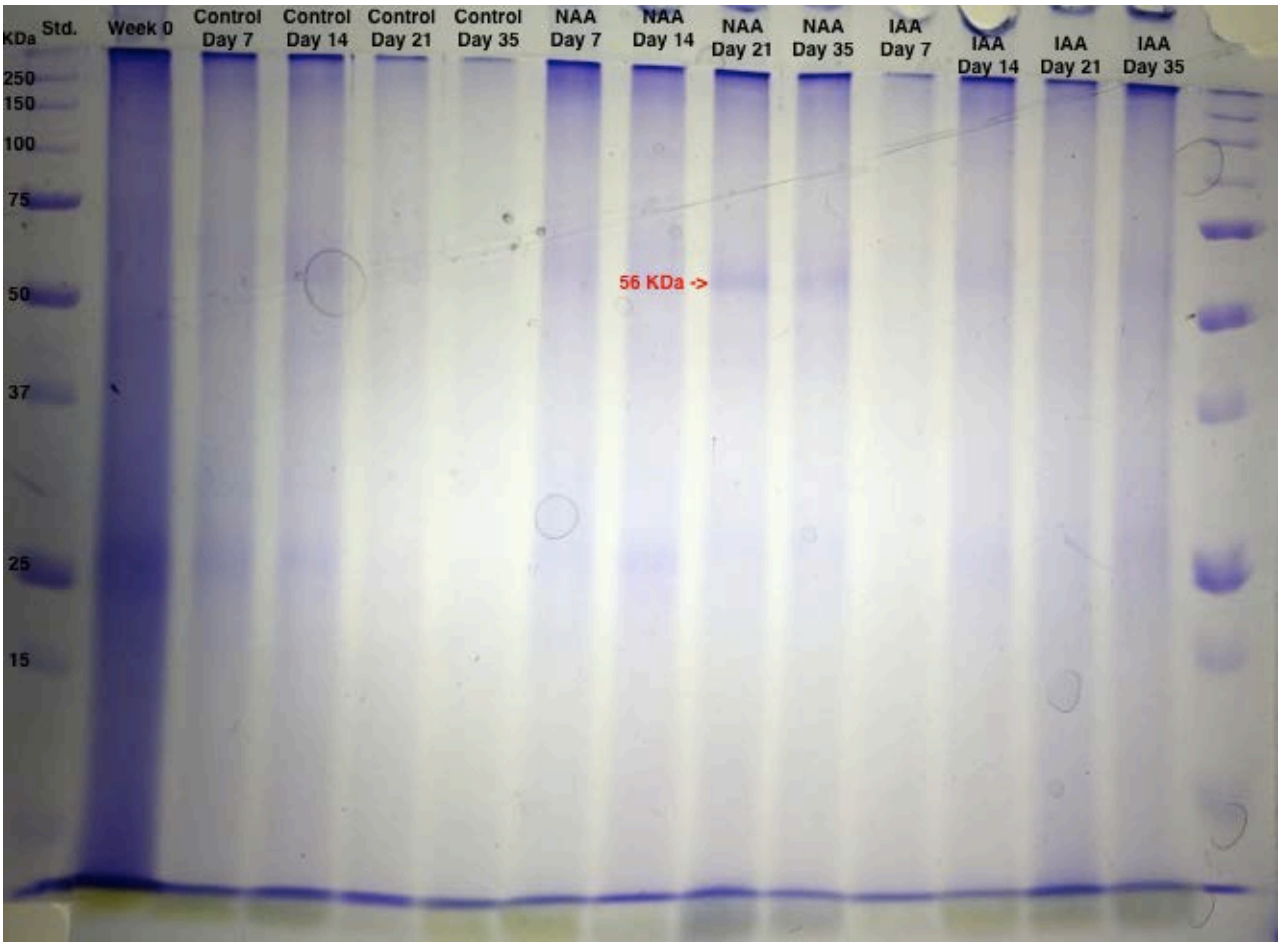


Figure 13. SDS-PAGE gel with Coomassie blue staining. The first and last lane contains protein standards (Std.). The second lane contains proteins from leaves in their original state which didn't undergo any treatment. Rest of the lanes contain proteins from leaf disks in the control, NAA (10^{-4}M), and IAA ($2*10^{-4}$M) treatments from day 7 until day 35.

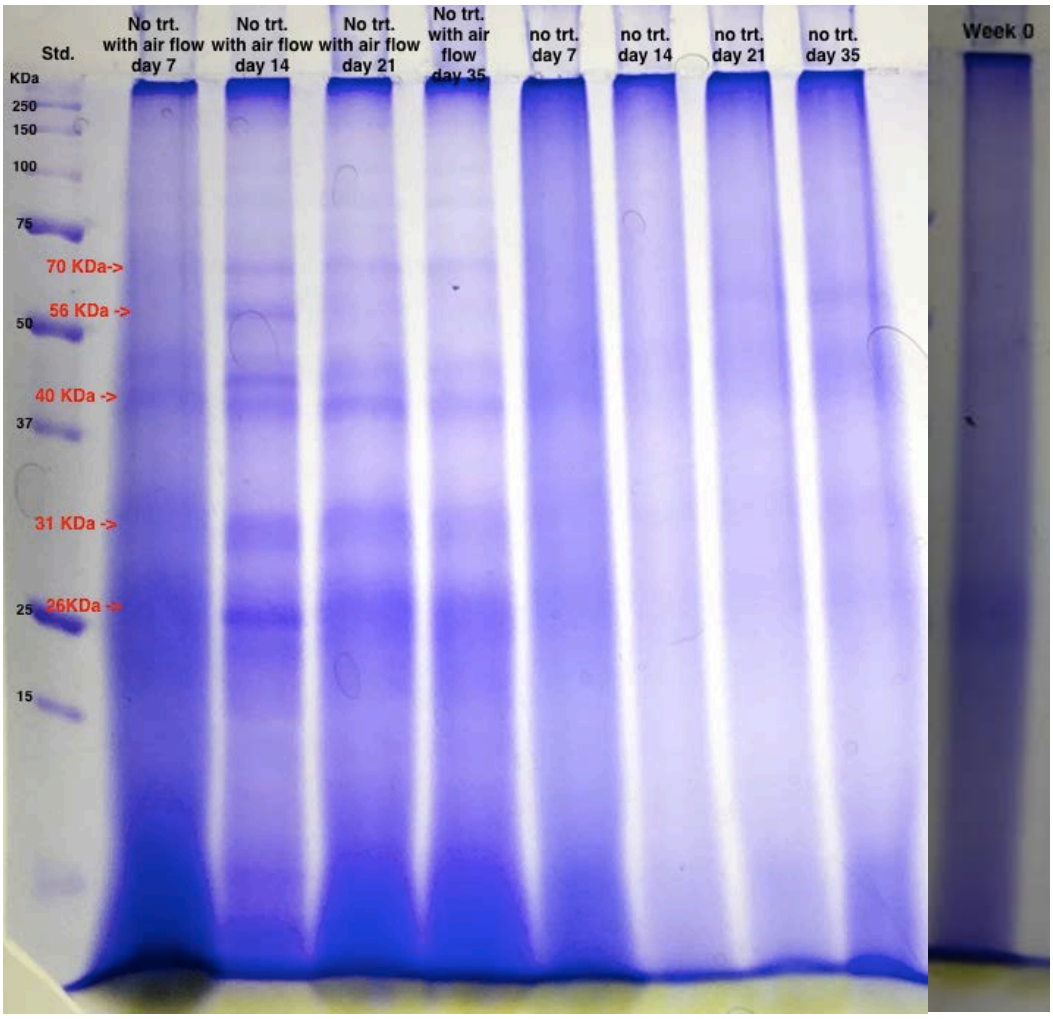


Figure 14. SDS-PAGE gel with Coomassie blue staining. The first contains protein standards (Std.). The last lane contains proteins from leaves in their original state which didn't undergo any treatment. Rest of the lanes contain proteins from leaf disks with no treatment without airflow and leaf disks with no treatment with airflow from day 7 until day 35.

Part 2: Raw Data

Table 5. Average NPR, Transpiration Rate, and Chlorophyll leaves of one small sized Northern Catalpa leaf. The averages of all the different sized leaves were used by making this type of table and the averages were used to make figures 3, 4, and 5.

Date	Aug 30		
Sample:	Small		
Area:	146.018 cm^2		
Number of counts:	**NPR (umol/m^2/s)**	**Transpiration rate mmol/m^2/s**	**Chlorophyll levels (Comparative unit)**
1	0.66	0.17	6.05
2	0.62	0.18	6.07
3	0.88	0.19	6.31
4	1.13	0.21	
Average:	0.8225	0.18	6.14

*Sample calculation:

Average NPR= (0.66+0.62+0.88+1.13)/4= 0.8225

Experiment 1 chlorophyll data tables:

Table 6. Chlorophyll (Chl) content for control treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0401	0.860	0.512	**0.959812469**	**0.7680798**	**1.719660848**
Week 2	0.0409	0.860	0.504	0.943143276	0.735139364	1.670220049

Week 3	0.0502	0.676	0.392	0.604905179	0.463196813	1.062941833
Week 4	0.0453	0.736	0.432	0.728596909	0.569388079	1.291754525
Week 5	0.0441	0.692	0.404	0.7042104308	0.5454004535	1.243595465

Table 7. Chlorophyll (Chl) content for BAP (10^{-4} M) treated leaf disks

Duration of BAP Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0362	0.782	0.460	1.009727072	0.788066298	1.789160221
Week 2	0.0400	0.825	0.500	0.947869	0.7358	1.67557
Week 3	0.0409	0.742	0.440	0.796575061	0.617302689	1.407072861
Week 4	0.0538	0.640	0.384	0.542805948	0.430337546	0.96849368
Week 5	0.0499	0.756	0.446	0.6671519038	0.5183198397	1.179770741

Table 8. Chlorophyll (Chl) content for CEPA (10^{-4} M) treated leaf disks

Duration of CEPA Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0360	0.825	0.496	0.97382	0.763804444	1.729293333
Week 2	0.0434	0.654	0.390	0.848548387	0.699447005	1.540691244
Week 3	0.0513	0.578	0.341	0.64768655	0.514888109	1.157024561
Week 4	0.0494	0.680	0.402	0.579161134	0.469506073	1.043692308
Week 5	0.0472	0.694	0.414	0.7177542373	0.5657050847	1.277315254

Table 9. Chlorophyll (Chl) content for GA (10^{-4} M) treated leaf disks

Duration of GA Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0397	0.825	0.496	0.941603023	0.750690176	1.684221662
Week 2	0.0403	0.654	0.390	0.726110174	0.582658065	1.302538958
Week 3	0.0381	0.578	0.341	0.679824672	0.53583832	1.20984357
Week 4	0.0444	0.680	0.402	0.68610991	0.542648649	1.222882883
Week 5	0.0462	0.694	0.414	0.6720865801	0.539625974	1.20594632

Table 10. Chlorophyll (Chl) content for NAA (10^{-4} M) treated leaf disks

Duration of NAA Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0403	0.815	0.486	0.904864516	0.726074442	1.623176179
Week 2	0.0388	0.744	0.436	0.860094845	0.670358763	1.523101031
Week 3	0.0455	0.784	0.458	0.773216703	0.59947956	1.366090549
Week 4	0.0395	0.658	0.384	0.747631392	0.578649114	1.319894684
Week 5	0.0364	0.698	0.406	0.8610197802	0.6627208791	1.516391209

Table 11. Chlorophyll (Chl) content for JA (10^{-4} M) treated leaf disks

Duration of JA Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)

Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0365	0.788	0.468	0.966531507	0.770340822	1.728587397
Week 2	0.0385	0.806	0.476	0.938005195	0.740604675	1.670578701
Week 3	0.0549	0.676	0.406	0.550375228	0.446901275	0.992547905
Week 4	0.0407	0.714	0.418	0.786991646	0.612351843	1.392617199
Week 5	0.0431	0.640	0.374	0.6663146172	0.5168816705	1.17750348

Table 12. Chlorophyll (Chl) content for ABA (10^{-4} M) treated leaf disks

Duration of ABA Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0353	0.746	0.430	0.9501008499	0.7201949008	1.662200567
Week 1	0.0430	0.666	0.396	0.779954651	0.622833488	1.39610093
Week 2	0.0550	0.750	0.450	0.6096	0.494181818	1.098545455
Week 3	0.0438	0.714	0.416	0.731782648	0.564829224	1.29036347
Week 4	0.0466	0.658	0.400	0.630027468	0.521936481	1.146537339
Week 5	0.0350	0.740	0.446	0.9445554286	0.7714514286	1.707885714

Experiment 2 chlorophyll data tables:

Table 13. Chlorophyll (Chl) content for control treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0158	0.214	0.124	0.608481013	0.465336709	1.068627848

Week 2	0.0201	0.210	0.122	0.46919801	0.36039801	0.82559204
Week 3	0.0167	0.139	0.083	0.37234491	0.299444311	0.668594012
Week 4	0.0262	0.148	0.085	0.254087023	0.191429008	0.443352672
Week 5	0.0146	0.168	0.145	0.481827397	0.694317808	1.171605479

Table 14. Chlorophyll (Chl) content for IAA (10^-5M) treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0210	0.241	0.135	0.517950476	0.374022857	0.887584762
Week 2	0.0176	0.145	0.085	0.369522727	0.288159091	0.654522727
Week 3	0.0202	0.257	0.150	0.57099604	0.442027723	1.008146535
Week 4	0.0163	0.102	0.059	0.281195092	0.214414724	0.49321227
Week 5	0.0242	0.165	0.095	0.306578512	0.231950413	0.535917355

Table 15. Chlorophyll (Chl) content for IAA (10^-4 M) treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0265	0.132	0.145	0.223975849	0.169455094	0.391523019
Week 2	0.0198	0.145	0.081	0.330638384	0.237636364	0.565474747
Week 3	0.0178	0.189	0.105	0.47974382	0.341568539	0.817253933
Week 4	0.0160	0.117	0.066	0.3297225	0.24096	0.567885

Week 5	0.0154	0.071	0.042	0.206522078	0.163511688	0.368264935

Table 16. Chlorophyll (Chl) content for IAA (2*10^-4M) treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0193	0.406	0.232	0.946872539	0.707299482	1.646118135
Week 2	0.0200	0.249	0.142	0.560546	0.417296	0.973076
Week 3	0.0150	0.161	0.092	0.483122667	0.360885333	0.839898667
Week 4	0.0177	0.180	0.100	0.459480226	0.327141243	0.782734463
Week 5	0.0241	0.165	0.089	0.310529461	0.210107884	0.518024896

Table 17. Chlorophyll (Chl) content for NAA (10^-5 M) treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0188	0.218	0.123	0.522840426	0.382225532	0.900629787
Week 2	0.0182	0.146	0.080	0.363107692	0.252465934	0.61250989
Week 3	0.0186	0.216	0.122	0.523539785	0.383423656	0.90252043
Week 4	0.0188	0.129	0.071	0.310408511	0.217485106	0.52527234
Week 5	0.0202	0.253	0.140	0.566192079	0.400388119	0.961794059

Table 18. Chlorophyll (Chl) content for for NAA (10^-4 M) treated leaf disks

Duration of Control Treatment	Weight of Tissue	Abs @ 663	Abs @ 645	Chl A content (mg/g.	Chl B content (mg/g.	Total Chl content (mg/g.

	(g)	nm	nm	tissue)	tissue)	tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0141	0.196	0.117	0.621875177	0.49986383	1.116402837
Week 2	0.0183	0.226	0.131	0.554786885	0.424528962	0.974583607
Week 3	0.0223	0.224	0.122	0.455027803	0.313090583	0.764283408
Week 4	0.0378	0.237	0.135	0.282336508	0.209771429	0.489707937
Week 5	0.0294	0.216	0.114	0.334146939	0.21764898	0.548995918

Table 19. Chlorophyll (Chl) content for for NAA (2 *10^-4 M) treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0281	0.243	0.134	0.391104626	0.27492669	0.662727402
Week 2	0.0254	0.071	0.046	0.123519685	0.113562205	0.23600315
Week 3	0.0222	0.218	0.125	0.425664865	0.337841441	0.759859459
Week 4	0.0185	0.118	0.070	0.285604324	0.227191351	0.510348108
Week 5	0.173	0.120	0.075	0.030821965	0.026726012	0.057280925

Table 20. Chlorophyll (Chl) content for NAA (10^-4 M) and ABA (10^-4 M)treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0141	0.175	0.110	0.551021277	0.482269504	1.028510638
Week 2	0.0154	0.072	0.041	0.210542857	0.156348052	0.365101299
Week 3	0.0206	0.169	0.100	0.317807767	0.309258252	0.624267961

Week 4	0.0212	0.144	0.085	0.304360377	0.240109434	0.541864151
Week 5	0.0209	0.134	0.082	0.285795215	0.239364593	0.522694737

Table 21. Chlorophyll (Chl) content for ABA treated leaf disks

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0150	0.133	0.072	0.401970667	0.273696	0.672282667
Week 2	0.0185	0.122	0.065	0.299574054	0.198387027	0.495446486
Week 3	0.0248	0.248	0.146	0.448254839	0.352058065	0.796477419
Week 4	0.0240	0.173	0.090	0.328428333	0.20856	0.534243333
Week 5	0.0198	0.169	0.093	0.386129293	0.270460606	0.653329293

Table 22. Chlorophyll (Chl) content for no treatment leaf disks without airflow

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0071	0.204	0.111	1.301729577	0.894185915	2.184946479
Week 2	0.0050	0.060	0.027	0.555816	0.27	0.82128
Week 3	0.0054	0.090	0.043	0.766985185	0.417407407	1.178074074
Week 4	0.0062	0.074	0.035	0.549877419	0.293664516	0.839019355
Week 5	0.0058	0.092	0.046	0.726165517	0.429544828	1.149682759

Table 23. Chlorophyll (Chl) content for no treatment leaf disks with airflow

Duration of Control Treatment	Weight of Tissue (g)	Abs @ 663 nm	Abs @ 645 nm	Chl A content (mg/g. tissue)	Chl B content (mg/g. tissue)	Total Chl content (mg/g. tissue)
Day 0	0.0117	0.185	0.104	0.713295726	0.518222222	1.225470085
Week 1	0.0032	0.130	0.067	1.8530875	1.157375	2.995
Week 2	0.0051	0.091	0.042	0.824243137	0.420329412	1.237819608
Week 3	0.0058	0.252	0.131	1.979786207	1.255544828	3.218786207
Week 4	0.0056	0.100	0.048	0.821342857	0.450857143	1.265428571
Week 5	0.0050	0.120	0.058	1.103024	0.61328	1.7072