UBC Social Ecological Economic Development Studies (SEEDS) Student Report

The Effects of Seasonal Changes on Photosynthesis Rates, Transpiration Rates and Protein Levels in the Leaves of Red Maple (*Acer rubrum*), Red Oak (*Quercus rubra*) and Western Red Cedar (*Thuja plicata*)

Amneet Dhillon

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

February 13, 2017
(Revised: June 6, 2017)

The Effects of Seasonal Changes on Photosynthesis Rates, Transpiration Rates and Protein Levels in the Leaves of Red Maple (*Acer rubrum*), Red Oak (*Quercus rubra*) and Western Red Cedar (*Thuja plicata*)

BIOL 448 – DIRECTED STUDIES IN BIOLOGY

AMNEET DHILLON

February 13, 2017
(Revised: June 6, 2017)

Research Supervisor: Dr. SANTOKH SINGH

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

CONTENTS

Abstract

This study focused on the physiological changes that occur throughout the year in *Acer rubrum, Quercus rubra* and *Thuja plicata*. The photosynthesis rates, transpiration rates and protein profiles were measured weekly using the CI-340 Handheld Photosynthesis Machine and SDS-PAGE from May to November of 2016. *T. plicata* maintained its needle-like leaves throughout the entire study while *A. rubrum* and *Q. rubra* began to show signs of senescence on October 19th, 2016. The chlorophyll levels were higher in *A. rubrum* than in *Q. rubra* but both species showed a dramatic decrease in early October. The *A. rubrum* and *Q. rubra* showed relatively low, but consistent photosynthesis and transpiration rates throughout the study while the *T. plicata* had relatively higher rates with a drastic peak in late July and early August. The protein profiles focused on RubisCO, the Light Harvesting Complex IIb (LHC IIb) and the D1 and D2 proteins of Photosystem II. *Q. rubra* showed drastically low protein profiles compared to *A. rubrum* and *T. plicata.* On the other hand, both *A. rubrum* and *T. plicata* showed very high amounts of both RubisCO and LHC IIb. *T. plicata* was the only species to show a seemingly random pattern of D1 and D2 protein abundance and the only species to maintain protein levels throughout the entire study. *A. rubrum* and *Q. rubra* showed minimum protein expression by the time their leaves changed colour. By tracking these physiological processes over the year, better insight is gained into how plants are responding to the changing climate.

Introduction

The earth is experiencing climate change as the levels of greenhouse gases in the atmosphere continue to rise (Solomon et al., 2008; Kammann et al., 2011). One of these greenhouse gases called carbon dioxide (CO_2) is directly released into the air by human activity, like the burning of fossil fuels. Because greenhouse gases trap heat, the increase of atmospheric CO_2 is related to the increasing global temperatures (Hansen et al., 2006). This can lead to hazardous consequences like higher sea levels, acidification of oceans and increased storm intensities (Solomon et al, 2008). However, plants may be a possible solution to this problem. They have the ability to capture CO_2 in the atmosphere and convert it into sugars to use as an energy source. If used correctly, plants can offset fossil-fuel emissions by 5 to 15% globally (Lal, 2004). However, the ability to sequester carbon depends not only on the type of plant, but also on its stage of development.

During the spring and summer, plants are focused on growing. They lengthen their roots and branches and create new leaves (Taiz et al, 2015). It is during these warmer months of the

year that environmental conditions, like increased sunlight and rainfall, favour high levels of the carbon-fixing process called photosynthesis.

Photosynthesis is the process by which plants convert sunlight, carbon dioxide and water into sugars for energy (Taiz et al., 2015). An important photosynthetic enzyme is ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This enzyme takes CO_2 in the air and uses it to carboxylate ribulose-1,5-bisphosphate into a form that the plant cell can use to create glucose (Ono et al., 2013). Another protein called the Light Harvesting Complex IIb (LHC IIb) also plays a critical role in photosynthesis. It serves to orient chlorophyll and associated carotenoid pigments in such a way that allows for the capture of energy from sunlight and the extraction of electrons from water (Dreyfuss and Thornber, 1994). Then, these electrons are passed to another set of important proteins called the D1 and D2 proteins, which make up the reaction core of Photosystem II (PSII). When the D1 and D2 proteins receive the electrons, they can indirectly create the power required to convert CO_2 into glucose (Kamiya and Shen, 2005; Kawamori et al., 2005). Due to the complexity of photosynthesis, there is no doubt that RubisCO, LHC IIb and the D1 and D2 proteins are essential for carbon fixation.

Transpiration, like photosynthesis, is another process that occurs in plants throughout the year. It is the upward movement of water within a plant that occurs when stomata, tiny pores on the underside of leaves, open to allow for carbon dioxide capture (Taiz et al., 2015). During this process, water is evaporated at the surface of leaves and thus, water loss is an inevitable consequence of photosynthesis.

In the fall, some plants shed their leaves. Leaf senescence is a highly regulated developmental process, involving a wide array of cellular mechanisms and morphological changes. It requires the production and sensation of hormones like ethylene and abscisic acid but

can also be affected by environmental conditions like darkness, drought and nutrient deficiency (Li et al., 2013; Kim et al., 2011; Buchanan-Wollaston et al., 2003). It occurs every year in deciduous species, leaving them leafless for two to seven months (Eamus, 1999). On the other hand, evergreen species maintain their needle-like leaves all year long and only old or damaged leaves senescence (Eamus, 1999). Nonetheless, when leaves are undergoing senescence, the ability to carry out cellular processes like photosynthesis and transpiration decreases.

The difference between photosynthesis and transpiration in deciduous and evergreen species is striking. Deciduous species can support higher photosynthetic rates because they invest large amounts of nitrogen into their flat, broad leaves (Eamus, 1999). In contrast, evergreen species have lower photosynthetic rates, which are partly explained by the decreased nitrogen investment in the leaves and use of RubisCO for nitrogen storage (Warren and Adams, 2004). The transpiration rate is typically higher in evergreen species than deciduous species due to their advanced root and vascular systems that can access deeper layers of soil and transport water more efficiently (Warren and Adams, 2004; Eamus, 1999; Goldstein et al., 1989). During the year, the leaves of evergreen species are able to support photosynthesis at a relatively constant rate (Eamus, 1999). As the leaves of deciduous species senescence, the ability to do photosynthesis will diminish as well (Sanchez et al., 2013). The developmental processes differ greatly between these two types of trees.

This study will investigate the physiological changes that occur throughout the year in the deciduous species, *Acer rubrum* (Red Maple) and *Quercus rubra* (Red Oak) and the evergreen species, *Thuja plicata* (Western Red Cedar). The Red Maple and the Red Oak are native to eastern North America. The Red Maple is used for landscaping and small-scale maple syrup production while the Red Oak is highly valued for lumber and veneer. The Western Red Cedar is

native to the west coast of North America and its wood is heavily relied upon in indigenous societies. By studying the carbon sequestration of these economically and culturally significant plants, it will allow for better insight of how plants will respond to future climate change and the impact that it will have on society.

The objectives of this study include the following: to determine net photosynthetic rate and transpiration rate differences within and between *A. rubrum, Q. rubra* and *T. plicata,* to track the morphological changes in the trees and their leaves and to examine the differences in leaf protein expression. I hypothesized that *T. plicata* will show consistent net photosynthetic and transpiration rates throughout the period of this experiment while *A. rubrum* and *Q. rubra* will show higher net photosynthetic and transpiration rates (relative to *T. plicata*) initially but both will dramatically decrease after the onset of senescence.

Materials and Methods

Collection of Samples

The trees used in this study are located near the Centre for Interactive Research on Sustainability. There is one *A. rubrum* and *Q. rubra* species each and two of the *T. plicata* species. The *T. plicata* closest to *A. rubrum* has been designated *T. plicata* (2) and the one furthest has been designated *T. plicata* (1).

The petioles of two leaves from each of the trees of *A. rubrum, Q. rubra* and *T. plicata* were cut, immediately placed under water and then cut again.

Experimental Setup

The CI-340 Handheld Photosynthesis Machine was set up on a clear, open bench space. A water-filled glass container and a light box were placed directly in front of the leaf chamber. The light was calibrated to 250 μmol/m^2/s with a LI-COR Photometer. The leaf sample was placed into the chamber and allowed to adjust to the light for two minutes before starting the analysis. Please note that a broad-leaf and a needle-leaf chamber were initially used for their respective samples but after having technical issues with the broad-leaf chamber, we switched to using only the needle-leaf chamber after August 9, 2016.

The experimental setup is shown in Figure 1. Please note that the light box and glass container are arranged at an angle to capture a better picture but do not depict the actual setup.

Analysis of Photosynthesis, Transpiration and Respiration

The CI-340 Handheld Photosynthesis Machine will automatically calculate photosynthesis and transpiration by measuring the levels of CO_2 and water vapor. Respiration is measured by turning the light off and covered the sample with a dark cloth. Each process was measured for five counts, which lasts about ten minutes.

Chlorophyll Measurements

The CL-01 Chlorophyll Content System was used to measure the relative chlorophyll of the leaves of *A. rubrum* and *Q. rubra*. Each leaf was sampled three times at various different locations.

Protein Analysis

Each of the samples were frozen in liquid nitrogen and stored in a freezer. On the final days of the study, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was run on the samples from the following days: May 4, May 31, June 22, July 13, July 27, August 9, September 14, October 19 and October 26. Please refer to Lab Unit 3: Senescence and Regrening of Cucumber Cotyledons: Light and Hormonal Regulation of Proteins and Chlorophyll in the BIOLOGY 352 Plant Physiology II: Plant Development Laboratory Manual for a detailed methodology (Singh, 2016).

Results

The leaf morphology of *Acer rubrum*, *Quercus rubra* and *Thuja plicata* from May to November 2016 is shown in Table 1. In both *A. rubrum* and *Q. rubra* trees, the leaves maintained their green colour until the week of October 3rd. The leaves of *A. rubrum* began to show colour change on October 19th. The edges had begun to turn yellow. In the next week, the leaf continued to yellow toward the center and the edges turned brown. By the week of November 9th, the leaf was entirely yellow with brown edges. This was the final week before all the leaves of *A. rubrum* fell off. Similarly, the leaves of *Q. rubra* turned to a light green in the center and yellow around the edges on October 19th. By the next week, *Q. rubra* had only brown leaves. This was the final week before *Q. rubra* lost all its leaves. *T. plicata* (1) and (2) had dark green, needle-like leaves from the beginning of the experiment on May 4th and maintained this morphology until the end of the experiment on November 15th.

The tree morphology for *A. rubrum*, *Q. rubra* and *T. plicata* is shown in Table 2. The *A. rubrum* maintained a leafy, green foliage until August 9th. Then, during the week of September 9th, a small section of the foliage turned red and was preserved until the week of October 19th. During this week, the upper half of the foliage turned different shades of yellow, orange and red. By November 2nd, there were no more green leaves and much of the foliage had already dropped. The tree fully senesced on November 15th. The *Q. rubra* initially had a full, leafy green foliage

as well. This tree maintained this morphology perfectly until the week of October 19th, where the entire foliage turned orange and light green. By the following week, the leaves had all turned orange or brown. On November 2nd, the leaves had fully senescenced and left its branches bare. *T. plicata* (1) and (2) had a bushy and full foliage during the early weeks of this experiment and maintained this exact morphology for the duration of the study.

The average chlorophyll levels of *A. rubrum* and *Q. rubra* are presented in Figure 5. In *A. rubrum*, the initial reading was +5.91 relative chlorophyll units (rcu) and the values increased slowly over the weeks to reach a peak of +18.53 rcu on July 13th. The following eight weeks had chlorophyll levels that ranged between +12.53 and +17.66 rcu. On October 5th, the value dropped to +11.67 rcu. From the onset of senescence (on October 19th as shown in Table 1), the chlorophyll reading was +3.57 rcu. On November 2nd, the reading was a lowly +0.33 rcu. *Q. rubra* followed a similar pattern but peaked at only +13.90 rcu on July 27th. Following this peak, the chlorophyll levels dropped dramatically. By September 28th, the reading had dropped to +5.68 rcu, a value less than half the maximum. After *Q. rubra* began to show signs of senescence, the chlorophyll readings were +2.01 and +1.19 rcu on October 19th and 26th respectively.

For *A. rubrum*, the average photosynthetic rates are shown in Figure 6 and are represented by the red line. In the first week of May, *A. rubrum* showed its highest photosynthetic rate at +6.068 μmol/m^2/s. The rest of May showed heavy fluctuations, dropping to +0.308 μmol/m^2/s before rising to +2.338 μmol/m^2/s. From early July to early August, the photosynthetic rates were fairly constant, averaging around +0.523 μmol/m^2/s. Then, *A. rubrum* showed a slight increase to +1.925 μmol/m^2/s on September 19th. For the next two weeks, the photosynthetic rate dropped to +0.158 μmol/m^2/s. On October 19th, the first signs of senescence

in *A. rubrum* occurred as shown in Table 1. Interestingly, this week's rates were slightly higher at +0.845 μmol/m^2/s but after a more complete yellowing of the leaves occurred, photosynthetic rates dropped completely. The rates for October 26th and November 2nd were -0.234 and -0.045 μmol/m^2/s.

The average transpiration rates for *A. rubrum* are shown in Figure 7 and are also represented by the red line. The highest transpiration rate was on May 4th with a value of +1.182 mmol/m^2/s. Then, the rates dropped off until early June, averaging at around +0.233 mmol/m^2/s before showing a dramatic increase to +0.737 mmol/m^2/s during the weeks of June 14th and June 29th. The following weeks' transpiration rate ranged from +0.150 to +0.363 mmol/m^2/s. From late August to early October, there were heavy fluctuations in the transpiration rates. From October 19th to November 2nd, the transpiration rates steadied at +0.515, +0.461 and +0.626 mmol/m^2/s.

For *Q. rubra*, the average photosynthetic rates are shown in Figure 6 and are represented by the blue line. The first two weeks of May 4th and May 11th show the highest rates for *Q. rubra* at +6.340 and +6.325 μmol/m^2/s respectively. The rate drops to +1.076 μmol/m^2/s on May 31st before suddenly rising to +4.347 μmol/m^2/s the following week and then returning to +1.230 μmol/m^2/s on June 14th. From June 22nd, the photosynthetic rate of +3.620 μmol/m^2/s slowly declines to -0.850 μmol/m^2/s on August 9th. The rate for July 13th seems to be an outlier with a dramatically low rate of -0.430 μmol/m^2/s. Following August 9th, there is a steady increase to +2.355 μmol/m^2/s over eight weeks of time. On October 19th, the photosynthetic rate drops to -0.195 μmol/m^2/s. The week of October 26th is the last week for *Q. rubra* and thus, the leaves give a final photosynthetic rate of -0.618 μmol/m^2/s.

The average transpiration rates for *Q. rubra* are shown in Figure 7 and are also represented by the blue line. The highest transpiration rates of +1.101 and +1.278 mmol/m^2/s were seen in the weeks of May 4th and May 11th, respectively. Then, the average transpiration rates ranged between values of +0.113 and +0.486 mmol/m^2/s from May 19th to October 3rd. There were a few exceptionally high transpiration values. On June 8th, a value of +0.566 mmol/m^2/s was recorded and on June 29th, a value of +0.612 mmol/m^2/s was recorded. From the onset of senescence, the weeks of October 19th and 26th had +0.196 and +0.191 mmol/m^2/s values for transpiration rates.

For *T. plicata* (1), the average photosynthetic rates are presented in Figure 6 and are indicated by the green line. The first photosynthetic rate was +0.336 μmol/m^2/s on May 4th. The next four weeks averaged to a value of +5.089 μmol/m^2/s with a slight drop to +3.662 μmol/m^2/s on June 8th. There was an increase in the photosynthetic rate to +6.573 μmol/m^2/s on June 14th but the following four weeks experienced a slight decline reaching +4.813 μmol/m^2/s on July 13th. The rest of July was a particularly profitable month, reaching values of +11.192 and +11.481 μmol/m^2/s on July 20th and July 27th respectively. The peak in photosynthetic rate for *T. plicata* (1) during this experiment was a value of +25.600 μmol/m^2/s on August 9th. This is the highest recorded rate of all the trees studied in this experiment. The following weeks showed varying levels of photosynthetic rates, the lowest being +3.940 μmol/m^2/s and the highest being +9.302 μmol/m^2/s.

The average transpiration rates for *T. plicata* (1) are shown in Figure 7 and are indicated by the green line as well. The first week of May showed a value of +0.174 mmol/m^2/s. Then, from May 11th to June 8th, there was a gradual decrease in transpiration rates from +0.683 to +0.041 mmol/m^2/s. There was a sudden peak on June 14th where the rate was +0.474 mmol/m^2/s

before dropping to +0.087 mmol/m^2/s the next week. The next two weeks (June 29th and July 6th) showed similar values to June 14th at +0.360 and +0.444 mmol/m^2/s respectively but the following two weeks (July 13th and 20th) showed values of +0.048 and +0.116 mmol/m^2/s respectively. The week of July 27th gave a transpiration rate of +0.814 mmol/m^2/s. On August 9th, the highest transpiration rate of +1.322 mmol/m^2/s was observed. The remaining weeks (until November 2nd) showed relatively consistent transpiration rates between +0.060 and +0.208 mmol/m^2/s with the only exception being October 19th where the transpiration rate was +0.427 mmol/m^2/s.

For *T. plicata* (2), the average photosynthetic rates are presented in Figure 6 and are indicated by the purple line. While the first week showed a low photosynthetic reading of only +0.213 μmol/m^2/s, the second week had a considerably higher value of +4.348 μmol/m^2/s. Then, there was a slight decline over the next four weeks until the photosynthetic rate was +2.658 μmol/m^2/s on June 14th. The following week of June 22nd showed another dramatic increase to +6.005 μmol/m^2/s before declining to +3.226 μmol/m^2/s on July 13th. On July 27th, the highest photosynthetic rate for *T. plicata* (2) was observed at +21.478 μmol/m^2/s. Then, there was a drop in the following week to +7.188 μmol/m^2/s. After this first peak, the rate declined to an average of +2.578 μmol/m^2/s from August 29th to September 14th. There was a second peak seen on September 28th with a value of +10.140 μmol/m^2/s. Then, in the following weeks, the photosynthetic rate dropped to a relatively constant level at around +5.002 μmol/m^2/s.

The average transpiration rates for *T. plicata* (2) are shown in Figure 7 and are indicated by the purple line as well. The month of May had values of +0.072, +0.541, +0.383 and +0.724 mmol/m^2/s for its average transpiration rates in that chronological order. Then, the transpiration rate consistently averaged around +0.097 mmol/m^2/s from June 8th to July 20th. On July 27th, the

transpiration rate was +3.646 mmol/m^2/s. This transpiration rate was the highest seen in the entire experiment. The weeks from August 9th to September 9th had transpiration rates that narrowly averaged +0.055 mmol/m^2/s. Then, there was a slight increase from +0.0513 mmol/m^2/s on September 9th to +0.605 mmol/m^2/s on September 19th. There was a drop in the transpiration rate the following week to +0.308 mmol/m^2/s but it increased to +0.737 mmol/m^2/s on October 3rd. The rate dropped to +0.15 mmol/m^2/s on October 19th and a slight decline persisted into early November.

The protein profiles of leaf samples are shown in Figure 9, 10, 11 and 12 for *A. rubrum*, *Q. rubra*, *T. plicata* (1) and (2) respectively. Each protein has a unique band on a protein profile which corresponds to its size. The thickness and intensity of a band indicate the amount of the protein that was present in the leaf sample.

The bands within the top red box of Figure 9, 10, 11 and 12 represent the relative amount of RubisCO. In *A. rubrum*, the band is relatively stable from late May to early September as indicated by Figure 9. The band becomes extremely thin on October 19th and then, after October 26th, the band completely disappears. In *Q. rubra*, Figure 10 shows that the RubisCO bands were also relatively stable throughout the study, although the intensity of the bands was much lighter than the other trees. By October 26th, the bands are also nonexistent. The bands for RubisCO in both the *A. rubrum* and *Q. rubra* disappear once their leaves turn yellow and brown, as shown in Table 1. On the other hand, Figure 11 and 12 shows that RubisCO bands in *T. plicata* are relatively constant in thickness throughout the study. A few exceptions include the slightly thinner bands for RubisCO on May 4th and July 13th in *T. plicata* (1) and the thinner band for RubisCO on May 31st in *T. plicata* (2).

The bottom red box in Figure 9, 10, 11 and 12 shows the bands for the LHC IIb. As shown in Figure 9, the thickness of the LHC IIb band in *A. rubrum* is relatively constant from early May to early August. On October 19th, when the colour change in *A. rubrum* occurred, the band is almost nonexistent and by October 26th, there is no band at all for LHC IIb. In *Q. rubra*, Figure 10 shows that the LHC IIb band is very light for much of the study, except for the last three weeks where it is nonexistent. The only weeks where the band for LHC IIb is prominent in *Q. rubra* is during the week of July 13th and the week of August 9th. The LHC IIb bands for *T. plicata* (1) and (2) are quite different from *A. rubrum* and *Q. rubra*. In Figure 11, the LHC IIb bands are thicker in *T. plicata (1)* during the weeks of May 31st and June 22nd. In Figure 12, the band in *T. plicata* (2) for the week of May 4th is slightly thicker as well.

In Figure 11 and 12, the middle red box contains two bands: the upper one represents the D2 protein and the lower one represents the D1 protein. In *T. plicata* (1), these bands are very faint until the week of August 9th where the bands show medium thickness. On September 14th, the bands are of maximal thickness. Then, in October, the bands disappear again. In *T. plicata* (2), the thickness of the bands for the D1 and D2 proteins are relatively constant with a few exceptions. The bands are absent on June 22nd. On July 13th and August 9th, only the band for the D2 protein is thicker than usual. On September 14th, the bands for the D1 and D2 proteins are the thickest relative to the other weeks. Please note that the bands for D1 and D2 proteins in *A. rubrum* and *Q. rubra* were not significant.

Discussion

Acer rubrum

The Red Maple, *A. rubrum*, showed relatively low rates of photosynthesis and transpiration throughout the length of this study. These results directly contradicted my hypothesis.

The chlorophyll levels in *A. rubrum* steadily increased until the end of September as shown in Figure 5. A study by Sibley et al. showed a similar increase in chlorophyll content in *A. rubrum* from May to September (1996). Because chlorophyll pigments are an integral component of capturing sunlight, it would seem reasonable to expect an increased capacity for photosynthesis. Yet, this is not what was seen in this study. To the best of my knowledge, there no studies testing the reason behind low photosynthesis rates and high chlorophyll levels.

Furthermore, the relative abundance of RubisCO and LHC IIb are equal to, if not slightly greater than, the evergreen trees *T. plicata* (1) and (2). Having similar levels of these proteins should mean that *A. rubrum* can support rates of photosynthesis that are like *T. plicata*. However, a study by Salvucci and Crafts-Brandner showed that RubisCO can actually inhibit photosynthesis in cotton plants when the temperature is above 30°C (2004). While the temperature during this study did not pass 30°C (as shown in Figure 2), there is a possibility that the RubisCO in *A. rubrum* is inhibitory at a lower temperature. To the best of my knowledge, there are no studies as of yet that can give more information regarding the specific temperature. This would explain the high levels of photosynthetic proteins and the contrasting low rates of photosynthesis. Because the inhibition of RubisCO is reversible, it would also explain the slight recovery of the photosynthetic rate in some weeks (Salvucci and Crafts-Brandner, 2004). On another note, the absence of a RubisCO band in the last weeks can be explained by the degradation of this highly abundant protein and then, redirecting the resulting nitrogen to new and developing organs (Ono et al., 2013).

According to Figure 2, days like May 31st and August 29th should have produced the highest levels of transpiration because transpiration rates increase when the temperature increases and decrease when the relative humidity increases (Taiz et al., 2015). The absence of

these expected results could be explained by water stress. Bauerle et al. showed that the level of transpiration and the amount of soil moisture are positively correlated (2002). If *A. rubrum* were water stressed, the stomata would stay closed to preserve water and thus, the transpiration rates would be low. This is exactly what is shown in Figure 7. The transpiration rates in *A. rubrum* were consistently low throughout this entire study. This suggests that there was a decrease in capture of CO_2 and therefore, photosynthetic rates were negatively affected. The lack of stomata aperture could explain why *A. rubrum* shows high levels of chlorophyll and photosynthetic proteins but does not display the corresponding high photosynthetic rates.

Perhaps the most interesting change that occurred in *A. rubrum* during this study was the lack of its' characteristic red foliage. Figure 3 shows the very limited reddening that occurred in the foliage. However, when most leaves abscised, they were yellow or brown. This is in stark contrast to Anderson and Ryser's study that showed *A. rubrum* began its' colour change to red as early as August and that by mid-September, most the foliage was a deep, red colour (2015). This suggests that the physiological processes behind senescence were not functioning normally. Another thing to consider is the presence of mechanical damage on the leaves as shown in Figure 4. *A. rubrum* could have been in a state of defense, synthesizing secondary metabolites or doing RNA silencing (Taiz et al., 2015). This could have resulted in the abnormal colour change and/or low photosynthetic rates. Nonetheless, the morphology of the *A. rubrum* tree suggests irregular functioning.

Quercus rubra

The Red Oak, *Q. rubra*, showed similar photosynthesis and transpiration rates to *A. rubrum*. However, because the rates were relatively low and showed no significant decline with senescence, these results also contradicted my hypothesis.

The chlorophyll levels (as shown in Figure 5) in *Q. rubra* increased steadily until late July and began to decrease well into October. A similar study by Holland et al. showed that many species in *Quercus* maintained chlorophyll levels relatively well before dropping dramatically in late October and early November (2013). In my *Q. rubra*, there is a slight decline in the chlorophyll levels before the dramatic drop due to senescence. This suggests that chlorophyll degradation began to occur much earlier than the other *Quercus* species (Holland et al., 2013).

During the summer, *Q. rubra* had photosynthesis rates of up to +20 μmol/m^2/s in the Harvard Forest, Massachusetts (Hadley et al., 2007). The maximum rate reached by *Q. rubra* in this study was only +6.325 μmol/m^2/s and was during the spring. Another study showed a similar rate of +20 μmol/m^2/s in non-stressed *Q. rubra* leaves but a rate of +8 μmol/m^2/s when leaves were under drought conditions (Weber and Gates, 1990). While the temperature was considerable higher (30°C) and the photon density was 1000 μmol/m^2/s in this study, the resulting photosynthetic rate in the drought treatment is surprisingly close to the results in this study (Weber and Gates, 1990). This suggests that the photosynthetic rates in *Q. rubra* could be due to water stress. In support of this, Figure 7 shows the consistently low transpiration rates in *Q. rubra*. Furthermore, these rates match values given when a similar species, *Quercus ilex*, was experiencing a seasonal drought (Sala and Tenhunen, 1996). Specifically, the values in Sala and Tenhunen's study showed transpiration rates between +0.0 and +1.0 mmol/m^2/s when the leaves were in shade and drought. The possibility of shade contributing to the low photosynthetic rates could also be a factor because leaves taken for sampling during this experiment were taken from the bottom branches. Then, like *A. rubrum*, the stomata in *Q. rubra* may not have opened under water stress conditions to allow sufficient CO_2 capture for photosynthesis.

Unlike *A. rubrum* however, the protein profiles for *Q. rubra* were astonishingly weak. The bands for RubisCO were not even half of the maximal thickness seen in other trees in this study. This suggests that the low levels of photosynthetic proteins are correlated to the low rates of photosynthesis. Something to note is that despite the increased thickness on August 9th, the photosynthetic rate actually dropped from the week before. A study by Haldimann and Feller tracked the effect of temperature on RubisCO's activity and they found that RubisCO in *Quercus* had maximal activity at 25°C and this produced the highest photosynthetic rates (2004). During this study, the highest temperature seen was 23.5°C as shown in Figure 2 and this could suggest that the low levels of photosynthesis seen were due to suboptimal temperatures and thus, decreased functioning of RubisCO. The LHC IIb showed faint and blurry bands, indicating low amounts of the protein and possible degradation as well. The only bands that showed thickness of the LHC IIb protein did not display higher photosynthetic rates. The lack of a RubisCO band in the last two weeks indicates degradation and relocation of the nitrogen in this enzyme to new and developing organs in the plant body (Ono et al., 2013). This is to be expected during senescence.

Furthermore, deciduous species (both *Q. rubra* and *A. rubrum*) tend to have more stomatal sensitivity to soil drought because of their less elastic cell wall and increased susceptibility to turgor loss than other species like evergreens (Eamus, 1999). It may be interesting to note that both the *A. rubrum* and *Q. rubra* trees were in areas with heavily paved, concrete pathways and buildings. The poor soil and drainage may have been a contributing factor to the low photosynthesis and transpiration rates.

Thuja plicata

The Western Red Cedar, *Thuja plicata,* showed relatively consistent rates for photosynthesis and transpiration except for the dramatic peaks in both rates during July and August. Thus, these results weakened my hypothesis because it did not account for any increases in the rates.

Compared to *Q. rubra* and *A. rubrum*, *T. plicata* showed consistently higher photosynthetic rates and consistently lower transpiration rates for much of the duration of this study. Like my results, multiple other studies have suggested that deciduous species do not fare as well as their evergreen counterparts (Goldstein et al., 1989; Eamus, 1999). Furthermore, evergreen species, like *T. plicata*, have a much higher energetic cost to maintain their extensive root systems and waxy, scleromorphic leaves (Eamus, 1999; Goldstein et al., 1989). Thus, to support its morphology, evergreen trees must maintain their leaves and photosynthesize over a longer time (Goldstein et al., 1989).

Other studies showed similar photosynthesis rates in *T. plicata* that ranged from +4.0 to +7.0 μmol/m^2/s (Pepin et al., 2001; Grossnickle and Russell, 2009). However, *T. plicata* (2) achieved a rate of +21.478 μmol/m^2/s, which could be explained by high temperature (23.5°C) and the low relative humidity (71%) on this date. Both variables favour a high transpiration rate and as a matter of fact, this day has the highest transpiration rate in the whole study (Taiz et al., 2015). This possibly allowed maximal CO_2 capture and thus, maximal carbon fixation. But, interestingly, the day of the highest photosynthesis rate in *T. plicata* (1) and in the entire study, the temperature was only 15.7°C and the relative humidity was 95%. These results suggest that transpiration, which is linked to how long stomata are open, may not be the only factor in determining photosynthetic rates. In fact, variations in photosynthetic rate occurred throughout

the day and the highest rates are observed between 10 am to 12 pm (Goldstein et al., 1989). Despite controlling for time, if the *T. plicata* samples were collected and analyzed slightly earlier than the other weeks, it could explain the high peaks seen in July and August.

The low transpiration rates seen in *T. plicata* during this study are no surprise to scientific literature. One study that measured the transpiration rates in *T. plicata* had values that ranged from +0.4 to +0.6 mmol/m^2/s (Pepin et al., 2001). The transpiration rates seen in my *T. plicata* were predominately less than +0.5 mmol/m^2/s. However, due to the strong suggestions of water stress in *A. rubrum* and *Q. rubra* and the proximity of *T. plicata*, the lower transpiration rates could be due to water stress as well. Nonetheless, the difference between the transpiration rates in evergreen and deciduous species are not to due to differences in the structure of xylem (ie. wider and more efficient vessels) but rather the relatively low leaf surface area to vascular tissue ratio (Goldstein, 1989). This water transport efficiency in evergreens allows them to maintain high stomatal conductance without turgor loss (Goldstein et al., 1989).

The protein levels for *T. plicata* (1) and *T. plicata* (2) are shown in Figure 11 and 12 respectively. The bands for RubisCO and the LHC IIb are relatively constant through the duration of this study. These results are consistent with the fact that evergreen trees maintain their needle-like leaves all year round and thus, maintain their leaf function as well (Eamus, 1999; Goldstein et al., 1989). Furthermore, the relatively higher levels of these proteins than *Q. rubra* can be a possible explanation for the significantly higher photosynthetic rates but do not work for well in comparison with *A. rubrum*. These two species seem to have similar levels of RubisCO and LHC IIb although *T. plicata* surpasses *A. rubrum*'s photosynthesis rates. On another note, an interesting pattern that was seen in *T. plicata* was the banding of the D1 and D2 proteins that make up the reaction core in PSII. There is a seemingly random pattern in their

banding. On July 13th and August 9th, only the D2 protein showed thickness and on June 22nd, both bands seemed to disappear. A few studies have showed that there is degradation of the D1 protein (more than the D2 protein) under 800 μmol/m^2/s of strong light and/or 1000 μmol/m^2/s of photosynthetically active radiation (Liu, 2006; Babu et al., 1999). This may explain the strong bands of only the D2 protein. To the best of my knowledge, no other studies have tracked the changes in the D1 and D2 protein and this may be the first study to do so.

Concluding Notes

Overall, the photosynthetic rates observed in the deciduous species *A. rubrum* and *Q. rubra* were lower than expected and the evergreen species, *T. plicata*, produced an expected peak in its photosynthetic rates (Pepin et al., 2001; Grossnickle and Russell, 2009; Hadley et al., 2007; Weber and Gates, 1990). While the aforementioned reasons are valid enough to explain these results, here are two more.

Photoinhibition is the inhibition of PSII under strong light conditions (Taiz et al., 2015; Murata et al., 2006). Because photosynthesis cannot occur without light, plants deal with photoinhibition by rapidly and efficiently repairing PSII (Murata et al., 2006). Usually, photoinhibition-causing light can range from 1000 to 2000 μmol/m^2/s but these values depend on the species (Niinemets and Kull, 2000; Kitao et al., 2000). It has also been shown that photoinhibition at low temperatures can cause inactive, although intact PSII centers (Shipton and Barber, 1993). Perhaps, the 250 μmol/m^2/s of light used during analysis was enough to cause photoinhibition in this study. To my knowledge, there are no studies confirming or denying that this level of light intensity can cause photoinhibition. Another possibility is that the trees were exposed to strong light before collection but because sun irradiances levels were not measured on

the days of sample analysis, this is not something I can conclude with certainty. Nevertheless, photoinhibition can cause extremely low photosynthetic rates.

Injury to plant tissue can cause a wide array of defensive mechanisms. For instance, cells close to the wound can proliferate and excrete water impermeable substances like lignin and suberin to seal off the wound (Rittinger et al., 1986; Delessert et al., 2003). The plant effectively prevents water loss and indirectly prevents transpiration by inhibiting the pull of water upwards. If a wound response caused waterproofing of the petiole when it was cut during this study, the transpiration rates would be low and the resulting photosynthesis rates would also be affected. Thus, the measurements calculated inside the CI-340 Handheld Photosynthesis Machine may not be indicative of actual values.

If this study were to be repeated in the future, I would recommend taking photosynthesis and transpiration rates when the leaves are still joined to the tree and/or measuring the light intensity near the leaf before cutting it. Also, committing to a strict schedule of collection and analysis would help to eliminate the difference in diurnal photosynthesis rates.

Acknowledgements

I would like to express my utmost gratitude to Dr. Santokh Singh and Mr. Jarnail Chandi for their supervision, support and continuous guidance throughout this project. Also, a special thank you to Aman Chera and Kartik Arora for their appreciated assistance.

Literature Cited

Anderson, R. and Ryser, P. 2015. Early Autumn Senescence in Red Maple (*Acer rubrum* L.) Is Associated with High Leaf Anthocyanin Content. Plants. 4: 505-522.

Babu, T.S., Jansen, M.A.K., Greenberg, B.M., Gaba, V., Malkin, S., Mattoo, A.K. and Edelman, M. 1999. Amplified Degradation of Photosystem II D1 and D2 proteins under a Mixture of Photosynthetically Active Radiation and UVB Radiation: Dependence on Redox Status of Photosystem II. Photochemistry and Photobiology. 69: 553-559.

Bauerle, W.L., Post, C.J., McLeod, M.F., Dudley, J.B. and Toler, J.E. 2002. Measurement and modeling of the transpiration of a temperate red maple container nursery. Agricultural and Forest Meterology. 114: 45-57.

Buchanan-Wollaston, V., Earl, S., Harrison, E., Mathas, E., Navabpour, S., Page, T. and Pink, D. 2003. The molecular analysis of leaf senescence – A genomics approach. Plant Biotechnology Journal. 1: 3-22.

Delessert, C., Wilson, I.W., Van Der Straeten, D., Dennis, E.S. and Dolferus, R. 2004. Spatial and temperal analysis of the local response to wounding in *Arabidopsis* leaves. Plant Molecular Biology. 55: 165-181.

Dreyfuss, B. and Thornber, J.P. 1994. Assembly of the Light-Harvesting Complexes (LHCs) of Photosystem II. Plant Physiology. 106: 829-839.

Eamus, D. 1999. Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. Trends in Ecology & Evolution. 14: 11-16.

Environment of Canada. 2016. *Historical Data – Climate*. Nov 10, 2016. https://www.canada.ca/en/services/environment/weather/past.html.

Goldstein, G. 1989. Gas exchange and water relations of evergreen and deciduous tropical savanna trees. Annales des sciences forestieres. 46: 448-453.

Grossnickle, S.C. and Russell, J.H. 2010. Physiological variation among western redcedar (*Thuja plicata* Donn ex D. Don) populations in response to short-term drought. Annals of Forest Science. 67: 506.

Hadley, J.L., Kuzeja, P.S., Daley, M.J., Phillips, N.G., Mulcahy, T. and Singh, S. 2007. Water use and carbon exchange of red oak- and eastern hemlock-dominated forests in northeastern USA: implications for ecosystem-level effects of hemlock woolly adelgid. Tree Physiology. 28: 615-627.

Haldimann, P. and Feller, U. 2004. Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant, Cell and Environment. 27: 1169-1183.

Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.W. and Medina-Elizade, M. 2006. Global temperature change. Proceedings of the National Academy of Sciences of the United States of America. 103: 14288-14298.

Holland, V., Koller, S. and Bruggemann, W. 2013. Insight into the photosynthetic apparatus in evergreen and deciduous European oaks during autumn senescence using OJIP fluorescence transient analysis. Plant Biology. 4: 801-808.

Kamiya, N. and Shen, J.R. 2005. Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.6-A resolution. Proceedings of the National Academy of Sciences. 1: 98.

Kammann, C., Ratering, S., Eckhard, C. and Muller, C. 2011. Diochar and Hydrochar Effects on Greenhouse Gas (Carbon Dioxide, Nitrous Oxide, and Methane) Fluxes from Soils). Journal of Environmental Quality. 41: 1052-1066.

Kawamori, A., Ono, T.A., Ishii, A., Nakazawa, S., Hara, H., Tomo, T., Minagawa, J., Bittl, R. and Dzuba, S.A. 2005. The functional sites of chlorophylls in D1 and D2 subunits of Photosystem II identified by pulsed EPR. Photosynthesis Research. 84: 187-192.

Kim, J., Chung, K. and Woo, H. 2011. Three positive regulators of leaf senescence is *Arabidopsis*, ORE1, ORE3 and ORE9, play roles in crosstalk among multiple hormone-mediated senescence pathways. Genes & Genomics. 33: 373-381.

Kitao, M., Lei, T.T., Koike, T., Tobita, H. and Maruyama, Y. 2000. Susceptibility to photoinhibition of three deciduous broadleaf tree species with different successional traits raised under various light regimes. Plant, Cell and Environment. 23: 81-89.

Lal, R. 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science. 304: 1623 - 1627.

Li, Z., Peng, J., Wen, X. and Guo, H. 2013. ETHYLENE-INSENSITIVE3 Is a Senescence-Associated Gene that Accelerates Age-Dependent Leaf Senescence by Directly Repressing *miR164* Transcription in *Arabidopsis*. The Plant Cell. 25: 3311-3328.

Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta. 1767: 414-421.

Niinemets, U. and Kull, O. 2000. Sensitivity of photosynthetic electron transport to photoinhibition temperate deciduous forest canopy: Photosystem II center openness non-radiative energy dissipation and excess irradiance under field conditions. Tree Physiology. 21: 899-914.

Ono, Y., Wada, S., Izumi, M., Makino, A. and Ishida, H. 2013. Evidence for contribution of autophagy to Rubisco degradation during leaf senescence in *Arabidopsis thaliana*. Plant, Cell & Environment. 36: 1147-1159.

Pepin, S., Livingston, N.J. and Whitehead, D. 2001. Responses of transpiration and photosynthesis to reversible changes in photosynthetic foliage area in western red cedar (*Thuja plicata*) seedlings. Tree Physiology. 22: 363-371.

Rittinger, P.A., Biggs, A.R. and Peirson, D.R. 1986. Histochemistry of lignin and suberin deposition in boundary layers formed after wounding in various plant species and organs. Canadian Journal of Botany. 65: 1886-1892.

Sala, A. and Tenhunen, J.D. 1996. Simulations of canopy net photosynthesis and transpiration in *Quercus ilex* L. under the influence of seasonal drought. Agricultural and Forest Meteorology. 78: 203-222.

Salvucci, M.E. and Crafts-Brandner, S.J. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiologia Plantarum. 120: 179-186.

Sanchez, A., Hughes, N. and Smith, W. 2013. Water-use efficiency declines during autumn leaf senescence in three deciduous tree species. International Journal of Plant Biology. 4: 26-28.

Shipton, C.A. and Barber, J. 1994. *In vivo* and *in vitro* photoinhibition reactions generate similar degradation fragments of D1 and D2 photosystem-II reaction-centre proteins. European Journal of Biochemistry. 220: 801-808.

Sibley, J.L., Eakes, D.J., Gilliam, C.H., Keever, G.J., Dozier, W.A. and Himelrick, D.G. 1996. Foliar SPAD-502 Meter Values, Nitrogen Levels, and Extractable Chlorophyll for Red Maple Selections. HortScience. 31:468-470.

Singh, S. 2016. BIOLOGY 352 Plant Physiology II: Plant Development Laboratory Manual. Lab Unit 3: Senescence and Regrening of Cucumber Cotyledons: Light and Hormonal Regulation of Proteins and Chlorophyll. 40-53. Feb 8 2016, www.elearning.ubc.ca/connect/.

Solomon, S., Plattner, G., Knutti, R. and Friedlingstein, P. 2008. Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences of the United States of America. 106: 1704-1709.

Taiz, L., Zeiger, E., Møller, I. M. and Murphy, A. 2015. Plant. Sinauer Associates, Inc., Sunderland. 681-684.

University of British Columbia. 2016. *Campus Maps + Wayfinding*. Nov 10, 2016. http://planning.ubc.ca/sites/planning.ubc.ca/files/images/planning-services/UBCMap-Portrait.pdf

Warren, C.R. and Adams, M.A. 2004. Evergreen trees do not maximize instantaneous photosynthesis. Trends in Plant Science. 6: 270-274.

Weber, J.A. and Gates, D.M. 1990. Gas exchange in *Quercus rubra* (northern red oak) during a drought: analysis of relations among photosynthesis, transpiration, and leaf conductance. Tree Physiology. 7: 215-225.

Appendices

The figures and tables in this study have been split into the following: Experimental Setup, Temperature and Relatively Humidity, Morphological Data, Chlorophyll Data, Photosynthesis and Transpiration Data, Protein Analysis and Raw Data.

Appendix 1: Experimental Setup

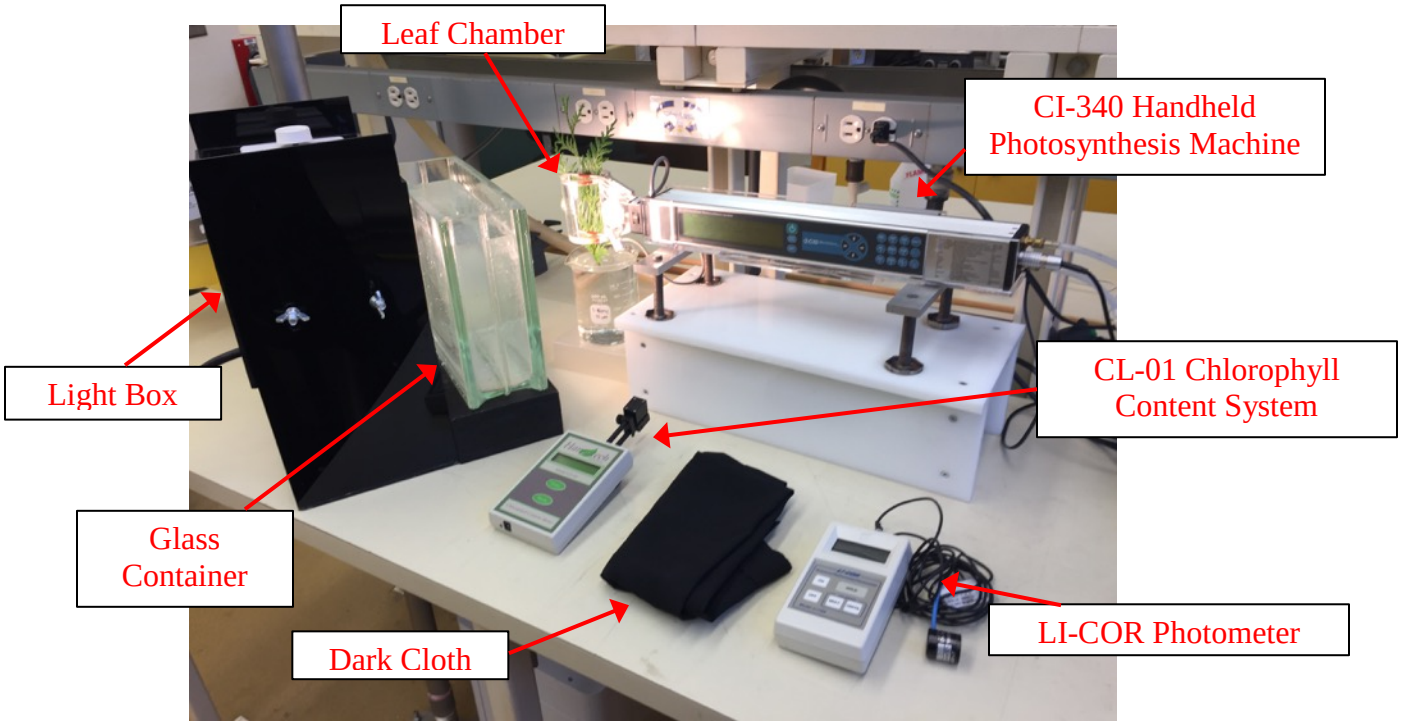


Figure 1. The experimental setup of the CI-340 Handheld Photosynthesis Machine, CL-01 Chlorophyll Content System and LI-COR Photometer is shown above. Please note that the glass container and the light box are normally positioned directly in front of the leaf chamber but are arranged differently here to effectively show the setup.

Appendix 2: Temperature and Relative Humidity

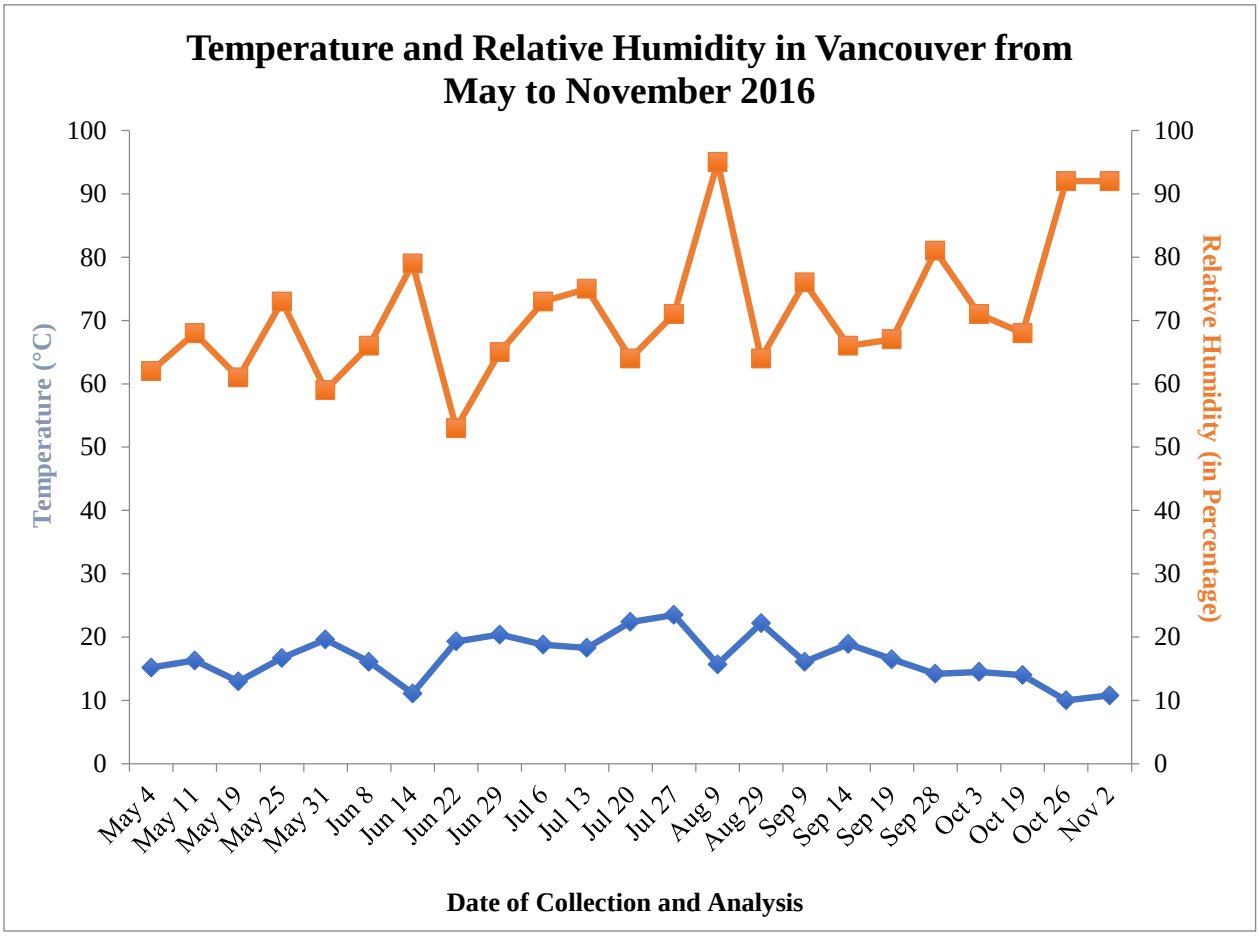


Figure 2. The average temperature (blue line) and relative humidity (red line) on the day of collection and analysis in this study are shown above. The location was the campus of the University of British Columbia, Vancouver. Samples were collected between 11:00am and 12:00pm and then, were analyzed from 12:00pm to 2:00pm. (Environment Canada, 2016)

Appendix 3: Morphological Data

Table 1. The leaves of the four trees, *Quercus rubra*, *Acer rubrum* and *Thuja plicata*, are shown in the following table. Boxes with "NO IMAGE" indicate missing/unavailable pictures of the sample. The note "NO MORE LEAVES" indicate the point at which the leaves of the tree had fully senescenced and abscised and thus, were unable to be collected and analyzed. Please note that the pictures are not scaled to size.

Date	***Quercus rubra***	***Acer rubrum***	***Thuja plicata (1)***	***Thuja plicata (2)***
04-May				NO IMAGE
19-May				
25-May				
31-May	NO IMAGE		NO IMAGE	NO IMAGE

14-Jun				
22-Jun				
29-Jun				
06-Jul				
13-Jul				

20-Jul				
27-Jul				
09-Aug				
29-Aug				
09-Sep				

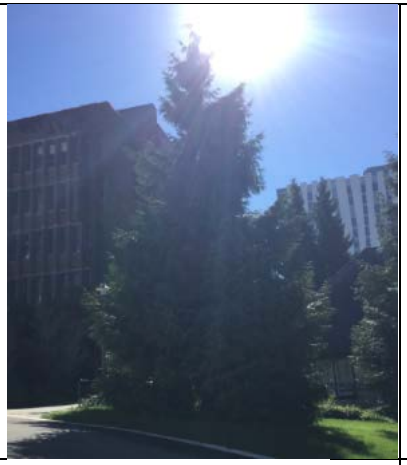
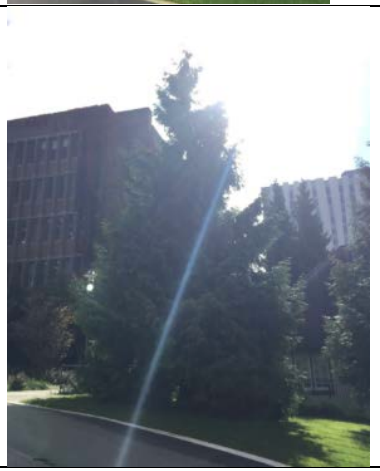
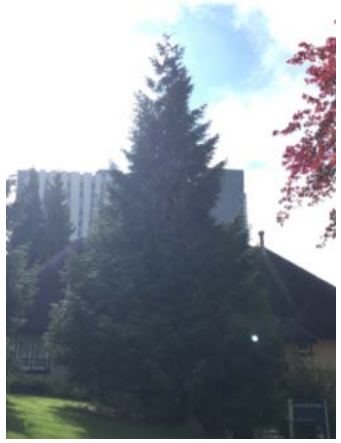
14-Sep				
19-Sep				
28-Sep				
03-Oct				
19-Oct				

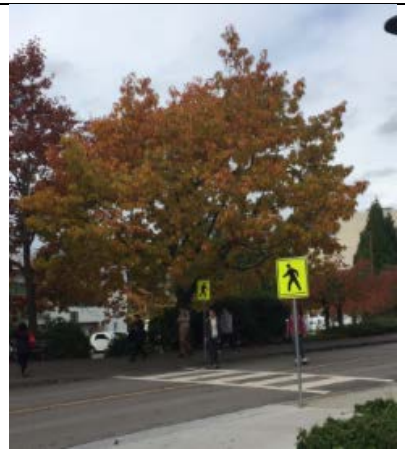
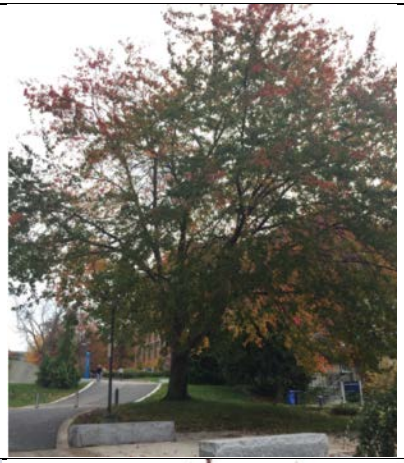
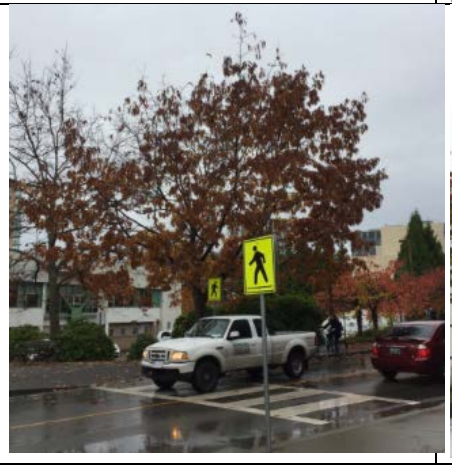
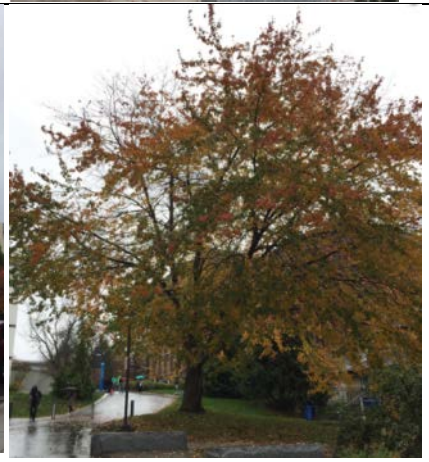
26-Oct				
02-Nov	NO MORE LEAVES			
09-Nov	NO MORE LEAVES			
15-Nov	NO MORE LEAVES	NO MORE LEAVES		

Table 2. *Quercus rubra*, *Acer rubrum* and *Thuja plicata* trees, from May to November 2016, are shown in the following table. Please note that one representative sample set is shown for each month until the month of September 2016, after which each week's sample set is shown to demonstrate the morphological changes of senescence.

Date	***Quercus rubra***	***Acer rubrum***	***Thuja plicata* (1)**	***Thuja plicata* (2)**
25-May				
14-Jun				

13-Jul				
09-Aug				
09-Sep				

14-Sep				
19-Sep				
28-Sep				

03-Oct				
19-Oct				
26-Oct				

02-Nov				
09-Nov				
15-Nov				

Figure 3. The above picture shows a portion of the *Acer rubrum* tree on October 19, 2016. The leaves at the edge have turned red while the rest of the foliage is a mix of yellow and green leaves.

Figure 4. The leaves above belong to the *Acer rubrum* tree on July 27, 2016. There is significant damage to the leaves but the cause of this damage is unknown. While this image only shows a few leaves, the damage was evident on leaves throughout the entire foliage.

Appendix 4: Chlorophyll Levels

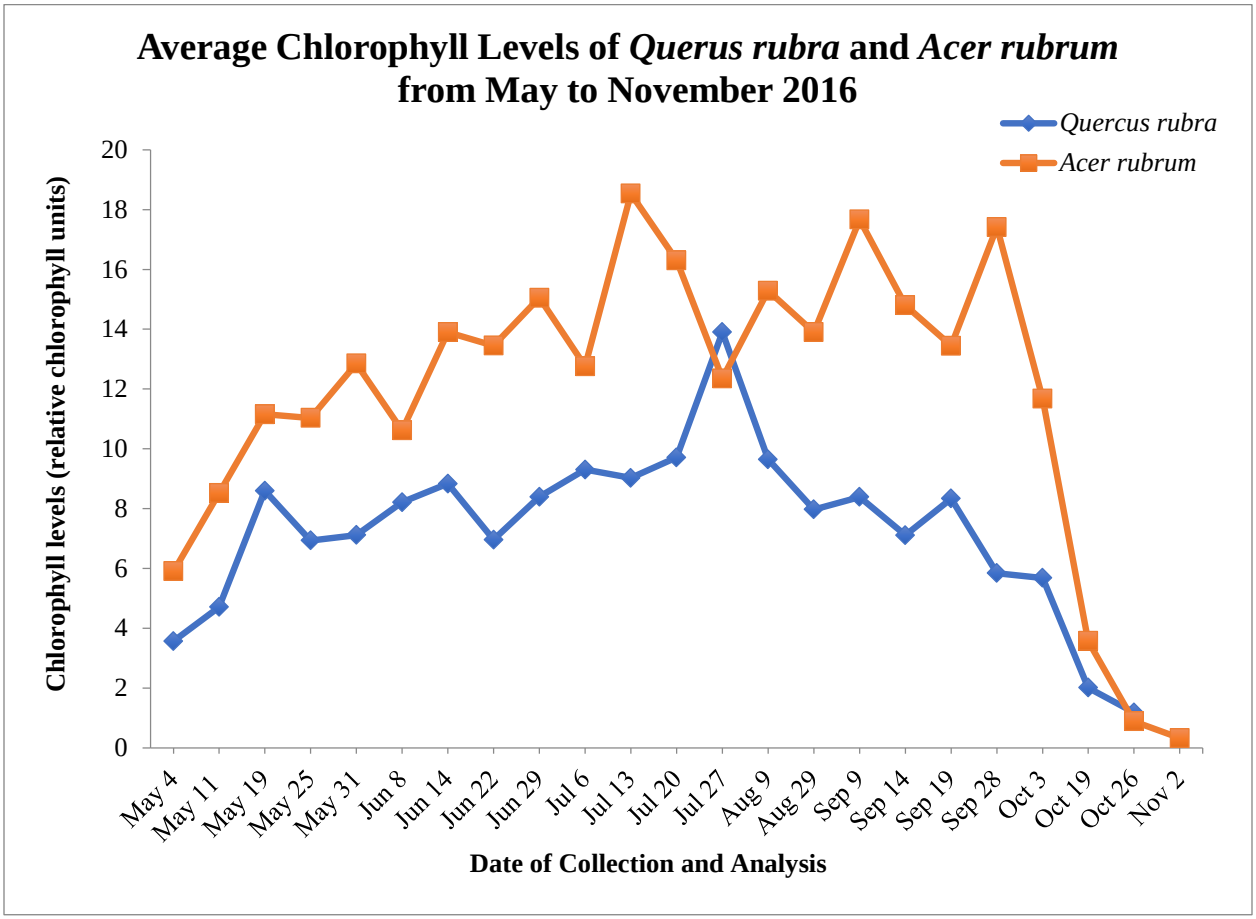


Figure 5. The average chlorophyll measurements for *Quercus rubra* and *Acer rubrum* are shown above. The machine used was the Hansatech CL-01 Chlorophyll Content System. Each average is a collection of three measurements from each of the two leaf samples on the day of collection and analysis. *Thuja plicata* samples were not included.

Appendix 5: Photosynthesis and Transpiration Data

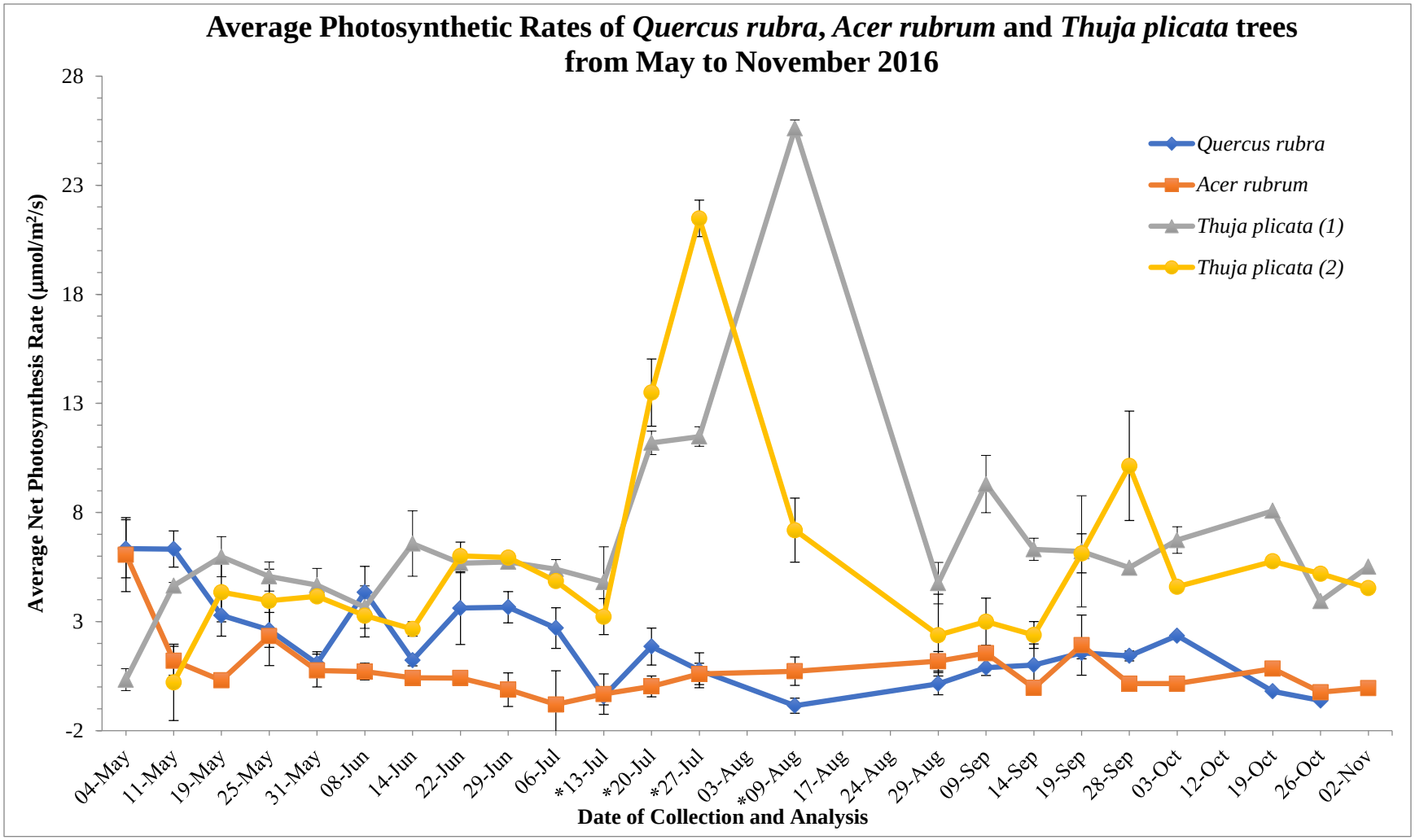


Figure 6. The average photosynthetic rates of the leaves of *Quercus rubra*, *Acer rubrum* and *Thuja Plicata* trees are shown above. Two leaves were taken from each tree and the average reflects both leaves. The asterisk (*) on the samples from July 13, 2016 to August 9, 2016 is to indicate technical issues with the CI-340 machine and the deciduous samples only. These results may not be indicative of actual values. The error bars represent one standard deviation from the mean.

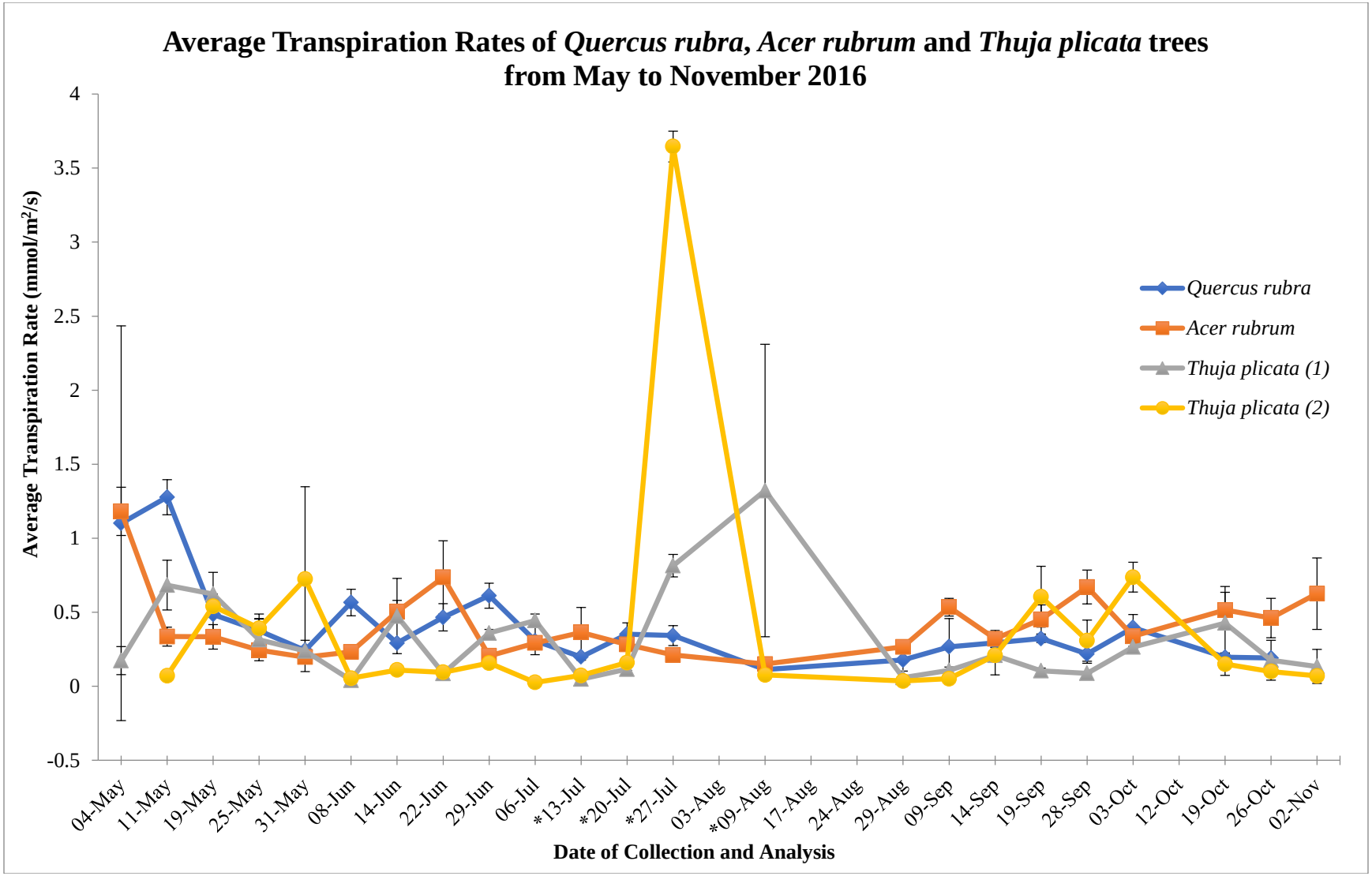


Figure 7. The average transpiration rates of the leaves of *Quercus rubra*, *Acer rubrum* and *Thuja Plicata* trees are shown above. Transpiration was measured during the light condition only. Two leaves were taken from each tree and the average reflects both leaves. The asterisk (*) on the samples from July 13, 2016 to August 9, 2016 is to indicate technical issues with the CI-340 machine and the deciduous samples only. These results may not be indicative of actual values. The error bars represent one standard deviation from the mean.

Appendix 6: Protein Analysis

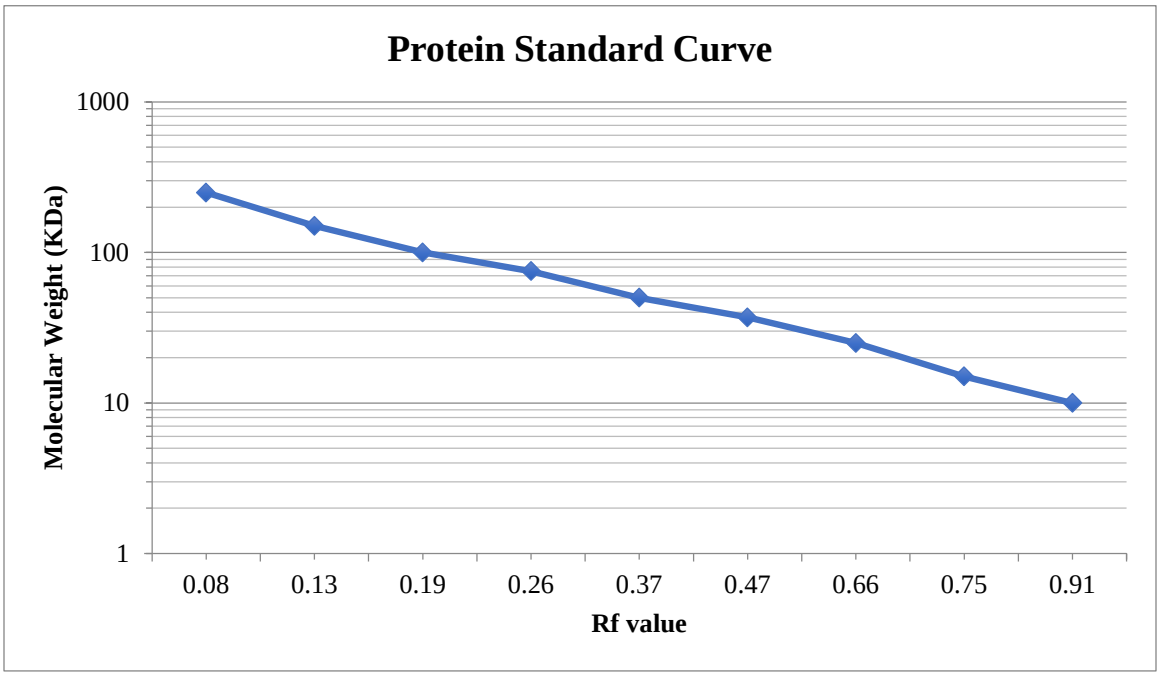


Figure 8. The protein standard curve for the protein standards used in the SDS-PAGE gel for *A. rubrum*, *Q. rubra* and *T. plicata* is shown above.

SDS-PAGE Gel of the Leaves of *Acer rubrum* from May to November 2016

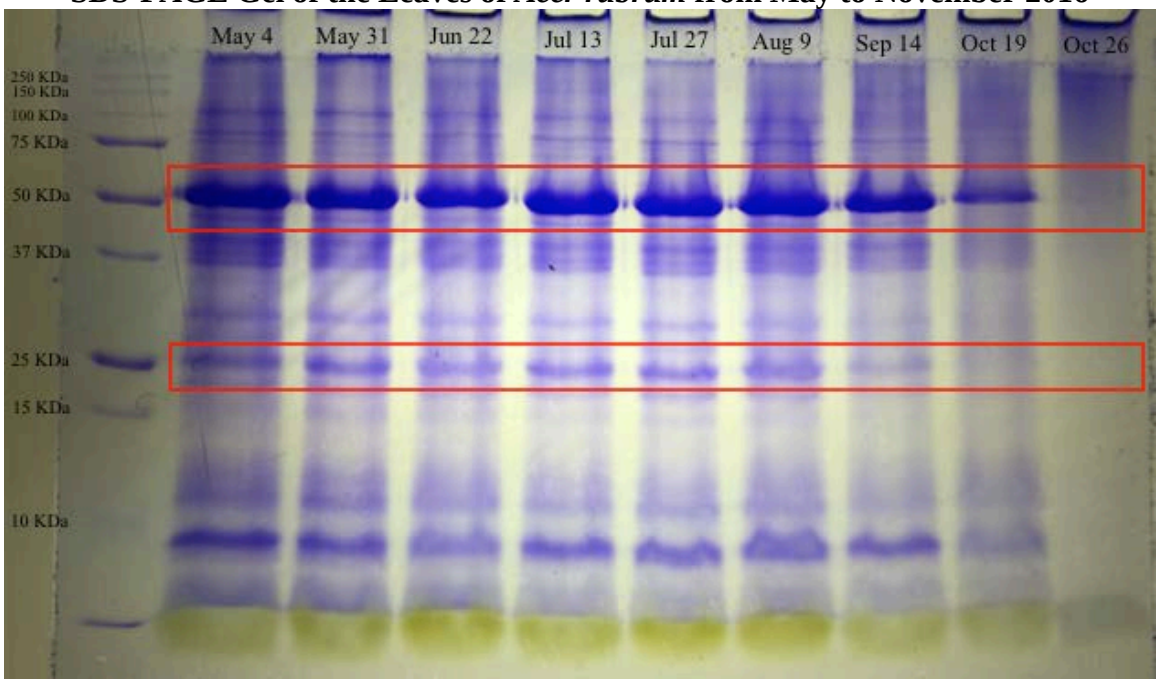


Figure 9. An image of the SDS-PAGE gel that was conducted on *Acer rubrum* leaves during various weeks from May to November 2016. Lane 1 shows protein standards (7 µL) while lanes 2-10 show 12 µL leaf protein samples. The corresponding weeks have been labeled above each lane. The top red box represents the protein bands for RubisCO (Large subunit, 50 KDa) and the bottom red box represents the Light Harvesting Complex IIb (LHCIIb, 25-28 KDa).

SDS-PAGE Gel of the Leaves of *Quercus rubra* from May to November 2016

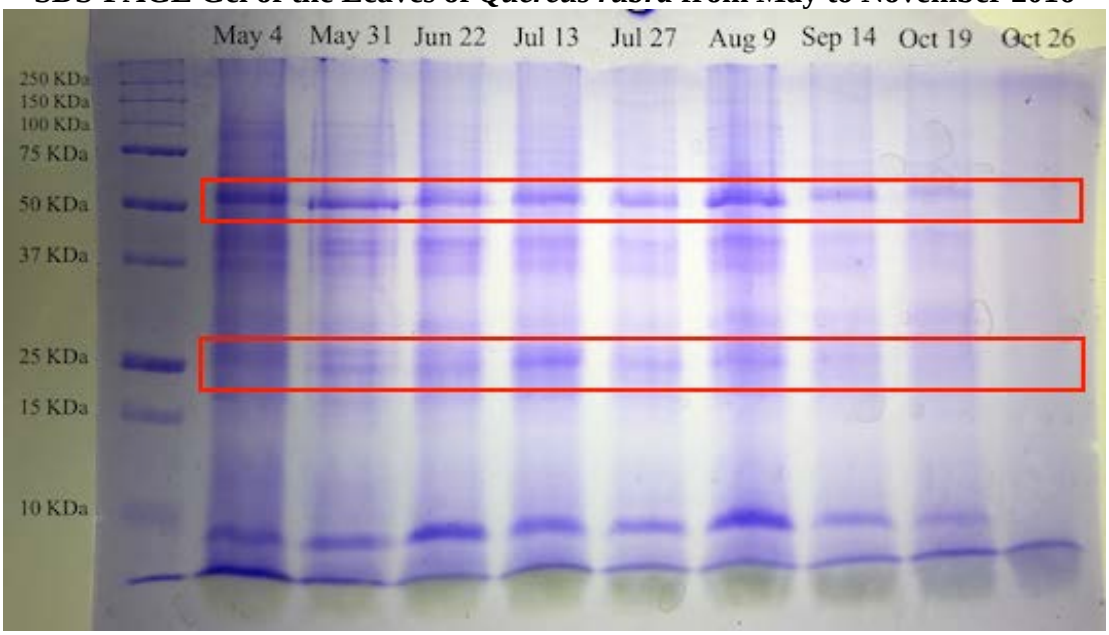


Figure 10. An image of the SDS-PAGE gel that was conducted on *Quercus rubra* leaves during various weeks from May to November 2016. Lane 1 shows protein standards (7 µL) while lanes 2-10 show 12 µL leaf protein samples. The corresponding weeks have been labeled above each lane. The top red box represents the protein bands for RubisCO (Large subunit, 50 KDa) and the bottom red box represents the Light Harvesting Complex IIb (LHCIIb, 25-28 KDa).

SDS-PAGE Gel of the Leaves of *Thuja plicata* (1) from May to November 2016

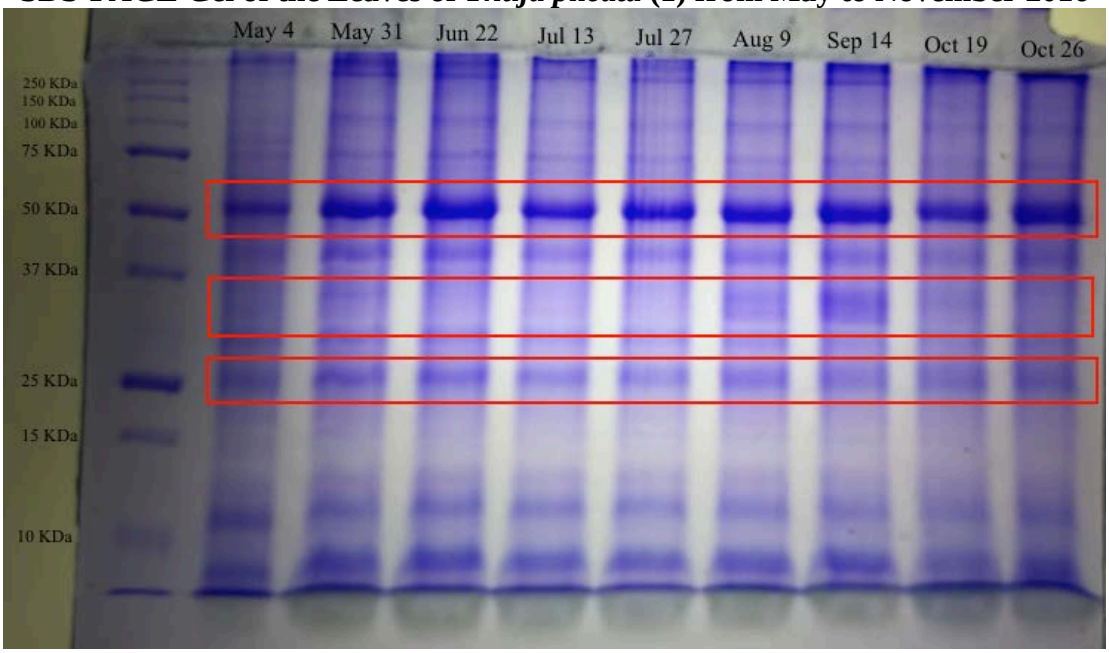


Figure 11. An image of the SDS-PAGE gel that was conducted on *Thuja plicata* (1) leaves during various weeks from May to November 2016. Lane 1 shows protein standards (7 µL) while lanes 2-10 show 12 µL leaf protein samples. The corresponding weeks have been labeled above each lane. The top red box represents the protein bands for RubisCO (Large subunit, 50 KDa). The middle box represents the two bands for D1 (BOTTOM) and D2 (TOP) proteins (Subunits of Photosystem II core complex, 32 and 34 KDa respectively). The bottom red box represents the Light Harvesting Complex IIb (LHCIIb, 25-28 KDa).

SDS-PAGE Gel of the Leaves of *Thuja plicata* (2) from May to November 2016

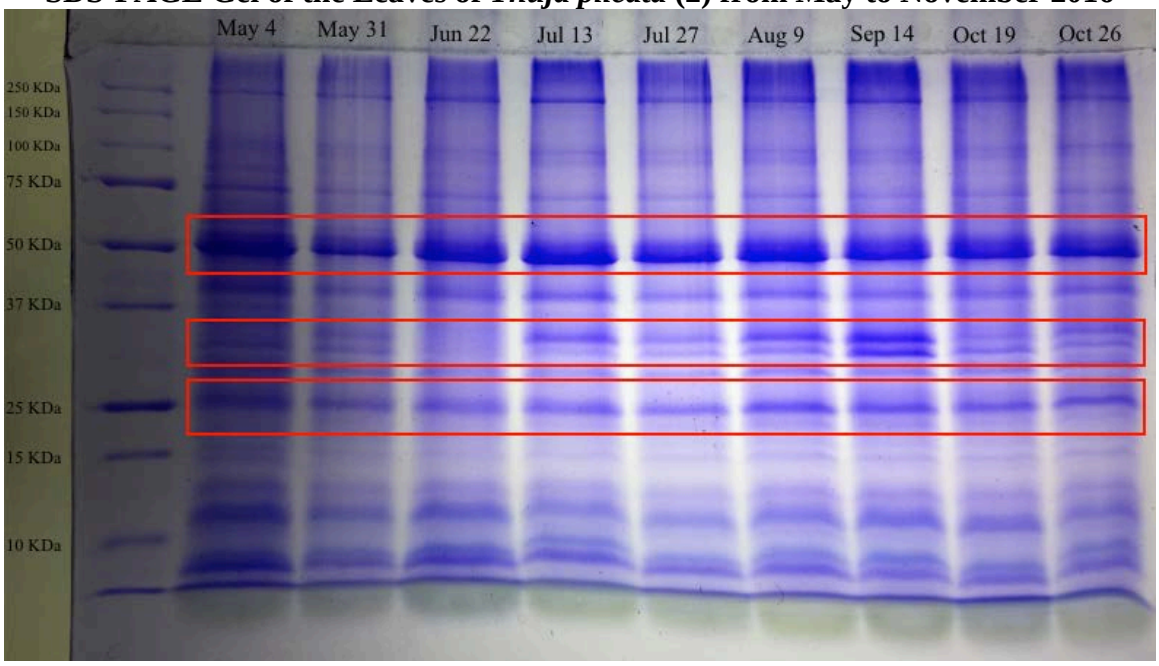


Figure 12. An image of the SDS-PAGE gel that was conducted on *Thuja plicata* (2) leaves during various weeks from May to November 2016. Lane 1 shows protein standards (7 µL) while lanes 2-10 show 12 µL leaf protein samples. The corresponding weeks have been labeled above each lane. The top red box represents the protein bands for RubisCO (Large subunit, 50 KDa). The middle box represents the two bands for D1 (BOTTOM) and D2 (TOP) proteins (Subunits of Photosystem II core complex, 32 and 34 KDa respectively). The bottom red box represents the Light Harvesting Complex IIb (LHCIIb, 25-28 KDa).

Appendix 7: Raw Data

With over 200 samples, the data from the CI-340 Handheld Photosynthesis Machine was too lengthy to be included in this paper. An example of the raw data is shown in Table 3 for one of the two *Q. rubra* leaves on May 11, 2016.

Original name: Z15																			
area	V	open/clsd	Delta T	Delta C															
11.1	0	1	0	0															
#	internal T	Flow	P	PAR	Tair	Tleaf	CO2in	CO2 out	H2O in	H2O out	W	Pn	E	C	RH in	RH out	int_CO_2	Flo	VPD
	°C	l/min	kPa	umol/m^2/s	°C	°C	ppm	ppm	kPa	kPa	mol/m^2/s	umol/m^2/s	mmol/m^2/s	mmol/m^2/s	%	%	ppm		kPa
113	32.9	0.3	100.65	244	25.4	26.4	498.1	486.6	1.1	1.86	0.18	2.13	1.42	91.69	33.8	57.1	460	0	1.6
114	32.9	0.3	100.65	246.5	25.4	26.7	494	482.3	1.09	1.73	0.18	2.15	1.19	68.37	33.5	53.1	442.6	0	1.79
115	33	0.3	100.65	248.4	25.4	26.6	497.3	480.8	1.09	1.7	0.18	3.06	1.14	64.9	33.3	52.1	420.5	0	1.81
116	33	0.3	100.64	249.4	25.4	26.5	500.1	471.8	1.09	1.7	0.18	5.21	1.15	66.77	33.3	52.2	372.7	0	1.77
117	33	0.3	100.65	247.2	25.5	26.4	509.2	480.7	1.09	1.74	0.18	5.26	1.22	73.18	33.2	53.2	391.7	0	1.71

Table 3. The raw data for *Q. rubra* on May 11, 2016 is shown above as an example.