UBC Social Ecological Economic Development Studies (SEEDS) Student Report

The Effects of Environmental changes on the Photosynthesis and Transpiration Rates for the Evergreen and Deciduous trees during the Spring and Summer Season

Harleen Sekhon

University of British Columbia

BIOL 448

July 02, 2012

[Academic work for this project completed August 31, 2011]

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BIOL 448 – DIRECTED STUDIES IN BIOLOGY

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Abstract

In this study, the photosynthesis and transpiration rates of two evergreen tree species: *Thuja plicata* (Western Red Cedar), *Chamaecyparis lawsoniana* (Lawson Cypress); and two deciduous tree species: *Acer rubrum* (Red Maple), *Quercus rubra* (Red Oak) were compared using the Licor photosynthesis System and an Infra-red Gas Analyzer (IRGA). The morphological, physiological and biochemical analyses were conducted during the spring and summer season. Environmental factors such as light intensity, temperature and precipitation were also recorded. The leaf samples were also subjected to gel electrophoresis for protein profiling, and the SDS gels for all the samples showed significant expression in the protein Rubisco (ribulose-1,5 bisphosphate carboxylase/oxygenase) and moderate expressions in the light-harvesting complex proteins. The average photosynthesis rate was highest in the Red Oak species and typically lowest in the Evergreen Lawson Cypress species. The average transpiration rate was just the opposite. These research findings will contribute information on plant sustainability based on the species, and the role of plants in CO_2 sequestration.

Introduction

The primary objective of this project is to determine whether environmental changes, such as light intensity, precipitation and temperature have an impact on the photosynthesis and transpiration rates of evergreen and deciduous trees. The four species being studied are the two evergreen species [*Thuja plicata* (Western Red Cedar) and *Chamaecyparis lawsoniana* (Lawson Cypress)] and the two deciduous species [*Acer rubrum* (Red Maple) and *Quercus rubra* (Red Oak)]. Other objectives include comparing the morphological data between the trees over the respective seasons of this study, such as leaf color/size during the spring and summer season. The seasonal changes result in variation of the photosynthesis and transpiration rates (Siegwolf *et al* 2010). Transpiration rate is the uptake of carbon dioxide which occurs when the stomata are opened, but also results in water loss (Kloeppel *et al* 1995). Plants do not have membranes that are both permeable to CO_2 and impermeable to water, so transpiration is a consequence of photosynthesis (Taiz and Zeiger, 2002). Furthermore, comparisons will be made between the photosynthesis and transpiration rates between the four trees as well as analyzing the biochemical changes in proteins such as Rubisco and light-harvesting complex proteins. The expression of these proteins varies with the changes in environmental conditions and processes such as senescence of plants.

Rubisco is a vital enzyme in the process of the fixation of carbon dioxide in the Calvin cycle (Tans 2006). The protein Rubisco is also known as Ribulose-1,5 bisphosphate carboxylase/oxygenase. Light harvesting complex proteins of the appropriate photosystems within the chloroplasts, such as LHC-IIa, LHC-IIb, or LHC-IId, concentrate the energy from the photons so it can be used as needed for the process of photosynthesis (Keeling and Sundquist 2008). Photosynthesis is the process whereby plants uptake atmospheric carbon dioxide and water to yield oxygen and glucose (Tans 2006). It is by this process that plants are able to sustain themselves and reduce atmospheric carbon dioxide levels (Wingler *et al* 1998). Similarly, through the process of photosynthesis, green plants use solar energy absorbed by light harvesting complex proteins, in chlorophyll to turn atmospheric carbon dioxide into carbohydrates (Taiz and Zeiger, 2002).

Carbon dioxide levels in the atmosphere are continuously increasing over the years. Global atmospheric CO_2 levels have increased from 315.98ppm in (1958) to 385.75ppm in (2008) according to data taken from the Mauna Loa Observatory in Hawaii (Keeling and Sundquist, 2008). The average annual increase has increased to 2.04ppm per year (Keeling and Sundquist, 2008). These increased levels lead to effects such as global warming. Global warming occurs when “greenhouse gases” such as carbon dioxide accumulate in the atmosphere of the earth and do not allow much heat to radiate away from the earth; most heat is trapped within the atmosphere, resulting in an overall increase in temperature of the planet (Gunderson *et al* 2004). Increased carbon dioxide levels in the environment will have profound effects on the photosynthesis rates of the plants (Shirke 2001). An example of known global warming effect on plants is that plants are flowering earlier, which will have an impact on the plant's biochemical, processes (Kloeppel et Al 1995). These are known short-term immediate effects, and the long term effects are expected to be drastic and are currently being studied (Miyazawa *et al* 2001). Plants are essential to maintaining appropriate carbon dioxide levels in the atmosphere and thus, plants are vital for the earth's sustainability.

The two species of trees being compared for their photosynthetic and transpiration rates are the evergreen trees and deciduous trees. Deciduous trees are typically very large in size and have leaves larger than evergreen trees (Bassow & Bazzaz 1998). Leaves of the deciduous trees are broad and large, hence, have a larger surface area than the needle-shaped leaves of the evergreen species (Siegwolf 2010). Deciduous trees follow typical patterns of tree abscission (Siegwolf 2010). Leaves are shed in fall, no leaves in water, and leaves grow back during the spring season (Warren 2004). By summer, the leaves are full of the respective colour of the trees species (typically a solid green colour) due to the large amount of chlorophyll present along with the increased presence of photosynthetic proteins (Yoshiyuki 2006). As it becomes time for the leaves to shed in fall, the photosynthetic proteins automatically decrease in amount in response to the change in temperature and light intensity (Hikosaka *et al* 2004). Chloroplasts are also destroyed in this process and regained in the spring season (Gunderson *et al* 2004).

Plants take in carbon dioxide during the process of photosynthesis by which oxygen is released to the environment (Gunderson *et al* 2004). In evergreen species, they have needle-shaped leaves which have decreased surface area, however these leaves allow for evergreen trees to be able to accommodate to stressful environments more easily in comparison to deciduous trees (Bassow & Bazzaz 1998). Stressful environments include lack of nutrients, extreme weathering, heavy winds, etc. These needle-shaped leaves also have waxy foliage, which allows for decreased loss of water from the leaves (Shirke 2001). Water is conserved more for needle-shaped leaves of evergreen versus the broad leaves of deciduous trees (Shirke 2001). The transpiration rate is typically higher for evergreen than deciduous in colder climates, such as the winter season (Warren 2004). The leaves of evergreen trees grow slowly year-round, and cannot be pinpointed to a particular season (Warren 2004). During summer however, evergreen trees have the largest leaves from all year-round and are at the prime of their growth (Yoshiyuki 2006). Photosynthetic rates are the highest for both evergreen and deciduous species in the summer season versus the winter season (Yoshiyuki 2006).

The increase in light intensity and temperature in the spring and summer season results in an increased amount of ATP synthase present and active, as well as photosynthetic proteins (light-harvesting complexes) which increases the photosynthetic rates of both types of trees (Hikosaka *et al* 2004). This will result in the trees both using more nutrients as well to accommodate for this growth period (Gunderson *et al* 2004). Eamus (1999) has conducted studies on the effects of the seasonal changes on the photosynthesis and transpiration rates of the evergreen and deciduous trees. The two species are compared for their variation in leaf shapes and sizes (deciduous leaf production and year-long evergreen foliage). The conclusion most relatable to this study is that evergreen trees fix carbon for a full year and therefore the intake of carbon dioxide is much slower than deciduous trees which invest very large amounts of nitrogen into their leaves to promote high intake of carbon dioxide for carbon fixation. The short-lived leaves of deciduous trees have very high rates of carbon fixation and photosynthesis for a short period of time, while the year-long needle-like leaves of the evergreen trees have a relatively moderate rate of carbon fixation and almost constant rate of photosynthesis.

By analyzing the photosynthesis and transpiration rates of the evergreen and deciduous species in the seasonal changes and various environmental stressors, we can conclude which trees should be encouraged for future plantation purposes. To create a more sustainable future would entail planting the species of trees which have efficient photosynthesis rates (including carbon dioxide intake and fixation) and transpiration (the loss of water through the intake of carbon dioxide via the stomata). Proteins such as Rubisco and the light-harvesting complex proteins were also studied to observe their role in the determining of the photosynthesis and transpiration rates.

Materials and Methods:

Samples were collected on a weekly basis. This occurred from the period of May 2011 – August 2011 (4 months). The leaves of the 4 different species of trees were subjected to . The two evergreen species are *Thuja plicata* (Western Red Cedar) and *Chamaecyparis lawsoniana* (Lawson Cypress). The two deciduous tree species are *Acer rubrum* (Red Maple) and *Quercus rubra* (Red Oak). These four species are located on the UBC "*Sustainability Street*". At least three leaf samples from each tree were analyzed for photosynthesis and transpiration processes.

The light intensity was measured from two different locations, which are referred to as 'Location 1' (close by the Evergreen Lawson Cypress tree) and 'Location 2' (close by the Deciduous Western Red Cedar tree). The average of both recordings was calculated and noted down. Pictures for each of the four species were also taken from consistent locations. The temperature and precipitation for the day were also noted down.

Triplicate samples from each tree were studied, and placed in the Li-cor Photosynthesis machine. The samples were also analyzed at least once by the Infra-Red Gas Analyzer (IRGA) method - Refer to Singh, 2008, Biology 351: Lab 2. The photosynthesis and transpiration rates were determined using the LI-COR LI-6200 Portable Photosynthesis System (Refer to *Appendix: Section A* for detailed LI-COR instructions). Pictures of all 12 samples were also taken indoors. These close-up pictures of the samples were taken to analyze morphological changes occurring with the seasonal variation. The light intensity was preset to 500 µmol/m^2/s and the flow rate was also around 500 µmol/m^2/s. After recording data from the samples, this was added to the computer LI-COR was taken to the lab's main computer to transfer the data into Microsoft Excel format. The pictures were also transferred to the computer. The data was printed out and calculated.

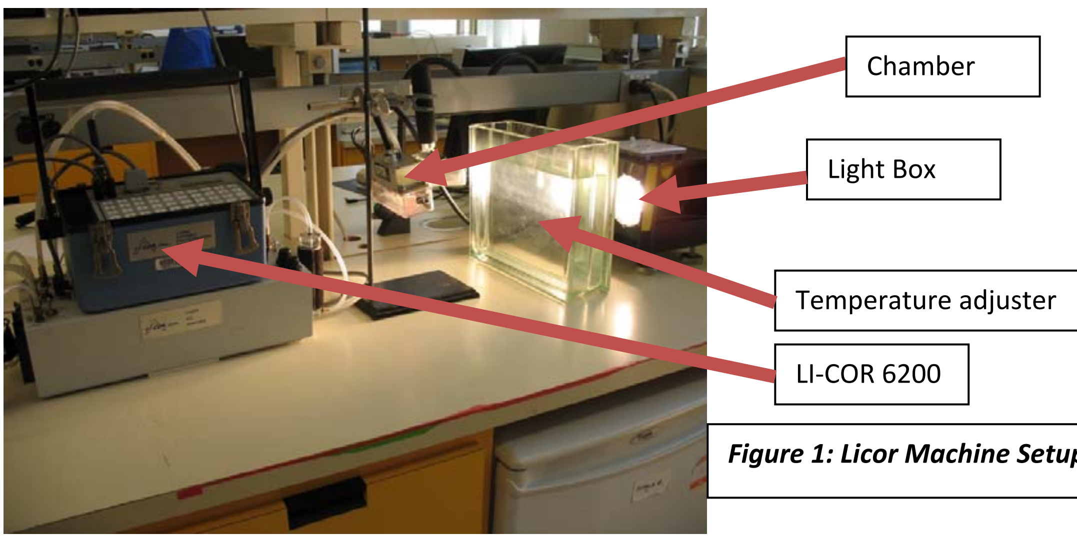

Figure 1: Licor Machine Setup

All of the samples were then placed into labeled aluminum foil for each of the species and then frozen in liquid nitrogen. After a couple of minutes, they were then taken out and placed into a freezer for further protein analysis.

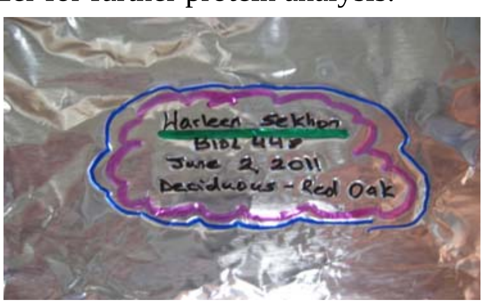

Figure 2: Generic Label for Samples

Figure 3: Freezer in which Samples Kept

Ribulose-1,5 bisphosphate carboxylase/oxygenase (Rubisco) and the light harvesting complex proteins (LHC-IIa, LHC-IIb, LHC-IIc and LH-IId) from the photosystems were studied using SDS gel electrophoresis. These photosynthetic proteins were analyzed in mid-August 2011. Refer to Singh, 2008, Biology 351: Lab 5 for the preparation of the samples, creating the separating and stacking gels, and for the setup of the gel electrophoresis apparatus. A total of four gels were run and were Coomassie blue stained. A total of 28 samples were studied, with 7 samples per gel. Each gel run was for a respective species of the plants. The 28 samples selected were May 31 (4 samples of species), June 2 (4 samples of species), June 28 (4 samples of species), July 7 (4 samples of species), July 26 (4 samples of species), August 2 (4 samples of species), and August 19 (4 samples of species). Approximately 50 µL of each of the plant samples was grinded for use. From that 50 µL, 7.5 µL of each of the samples was loaded onto the gels and 6 µL of the molecular weight markers were added as well. After they finished running, the gels were stained. Refer to AgriSera Educational Tool Kit for making the Coomassie blue stained gels. The Coomassie blue stain assisted in protein profiling.

Results

Morphological Data:

Red Oak and Red Maple are the two deciduous trees and Lawson Cypress and Western Red Cedar are the two evergreen trees. The deciduous species are increasing in leaf size as well as surface area. The leaves are also becoming greener over time. The evergreen species are relatively the same in leaf size and surface area over the prolonged period, except for the Western Red Cedar tree increases the number of branches coming off the main branch over the time. These two trees also remain the same shade of green over the months. This data corresponds to Figure 5, which shows that the Red Oak tree has the highest photosynthesis rate and both evergreen trees have lower photosynthetic rates according to the size of leaf and colour of leaf.

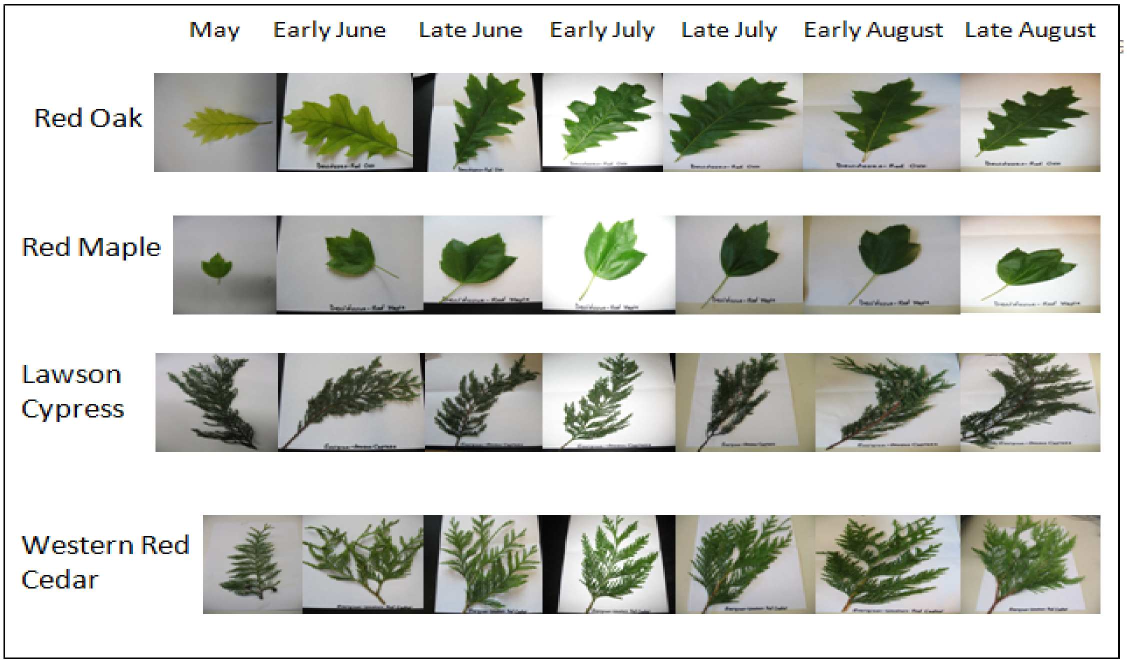

Figure 4: Morphological changes in Deciduous & Evergreen

Photosynthesis Data:

According to Figure 5, Red Oak seems to have the highest photosynthetic rate. It is mainly higher throughout both seasons, except during July. In July, however, another deciduous species (the Red Maple) has the highest photosynthetic rate with Red Oak being second. The Evergreen species generally have lower photosynthetic rates and appear to be constant throughout the spring and summer season. The photosynthetic rates amongst all the species are relatively the same difference amongst one another through the four months. Furthermore, as it appears to be warmer in temperature (Figure 7), the photosynthetic rates actually appear to be negative values.

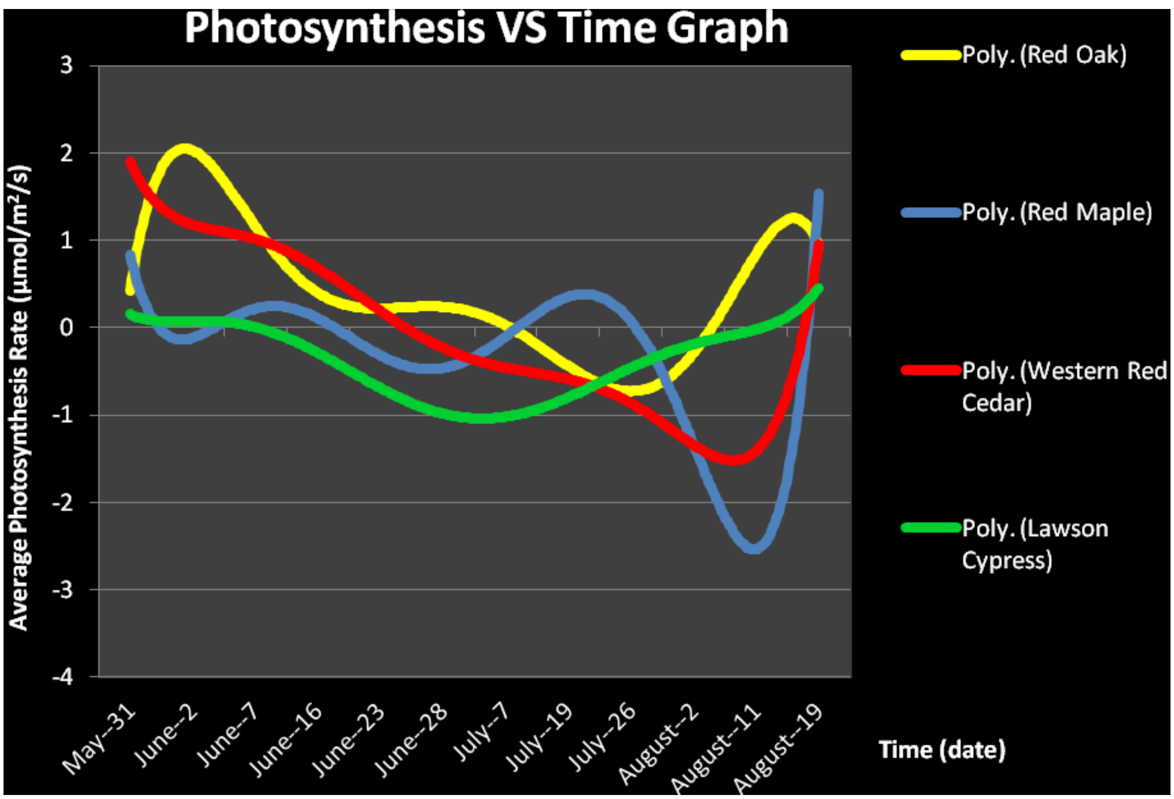

Figure 5: Average Photosynthesis Rates (μmol/m^2/s) *of the four different species of trees*

Transpiration Data:

Lawson Cypress has the highest transpiration rates amongst the species and Western Red Cedar is the second highest (both Evergreen trees). The rates all appear to have an equal difference amongst the four species. Both Red Maple and Red Oak have the lower transpiration rates. The transpiration rate remains primarily positive throughout the seasons.

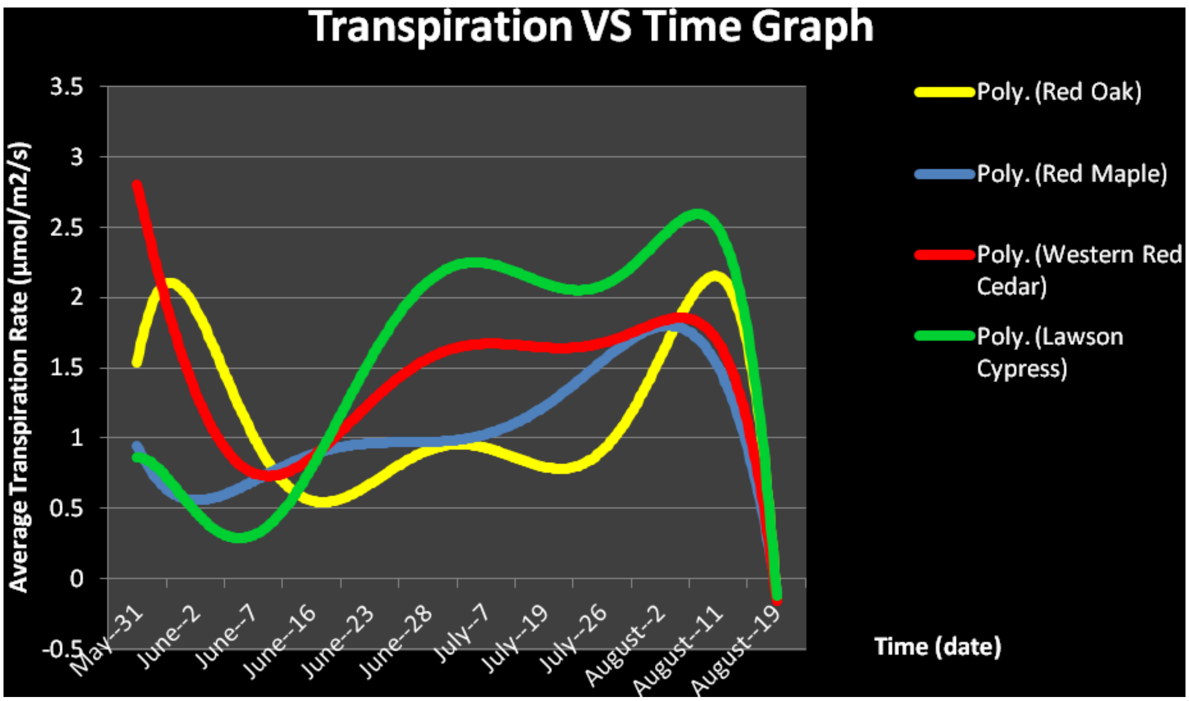

Figure 6: Average Transpiration Rates (µmol/m^2/s) *for the four species of trees*

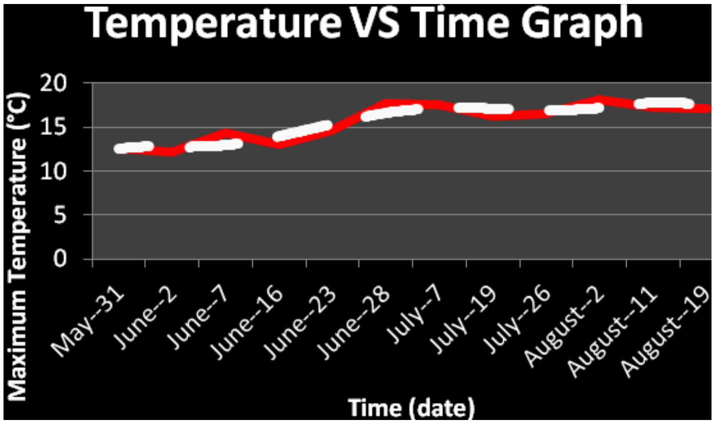

Figure 7: Temperature Graph

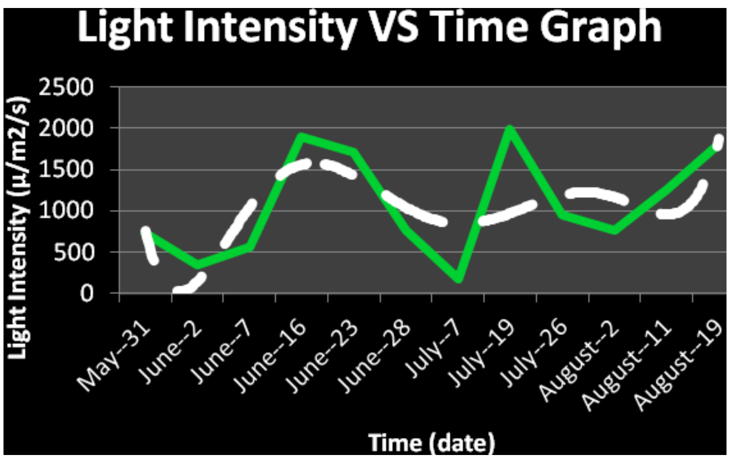

Figure 8: Light Intensity Graph

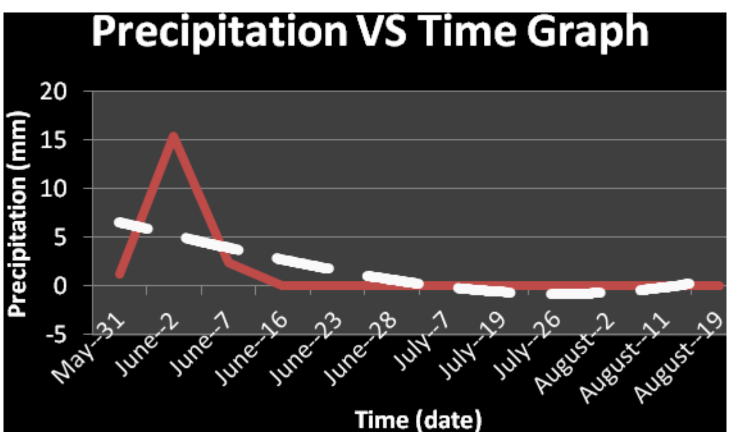

Figure 9: Precipitation Graph

Environmental Data:

The temperature (Figure 7) is steadily increasing. It has no dramatic decline or increase. The light intensity (Figure 8) has its peaks and drops, but it remains fairly constant with the weather (ex. Cloudiness, bright sunny day, etc.). The precipitation levels were moderate at the beginning of this study, but declined over the course of this research.

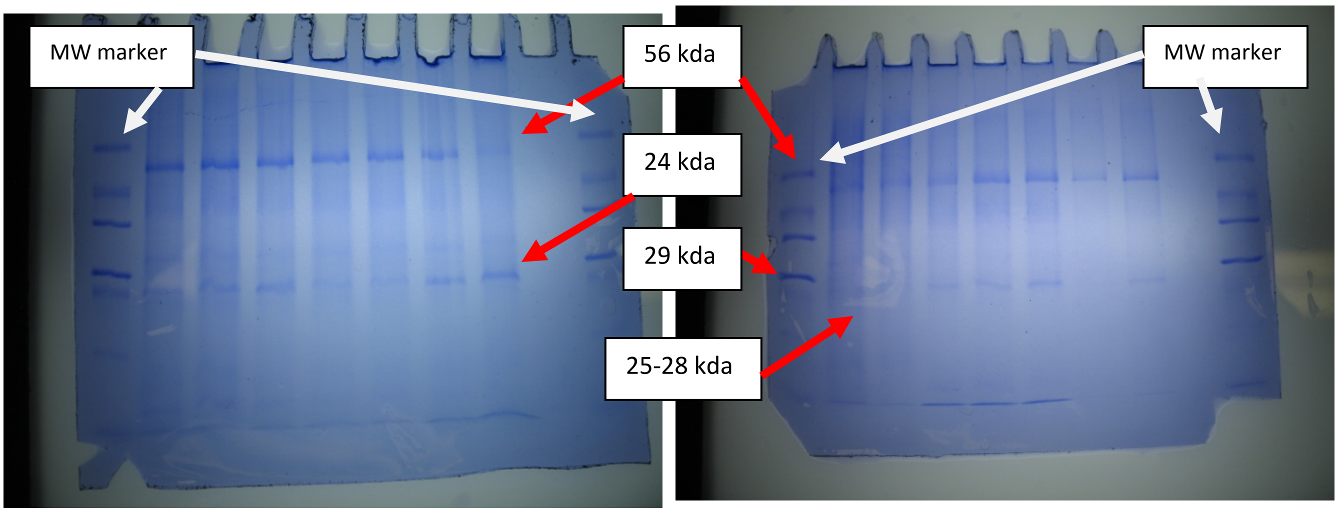

Figure 10: Gel 4 = A2 – ***Deciduous Red Maple***

Figure 11: Gel 3 = A1 – ***Deciduous Red Oak***

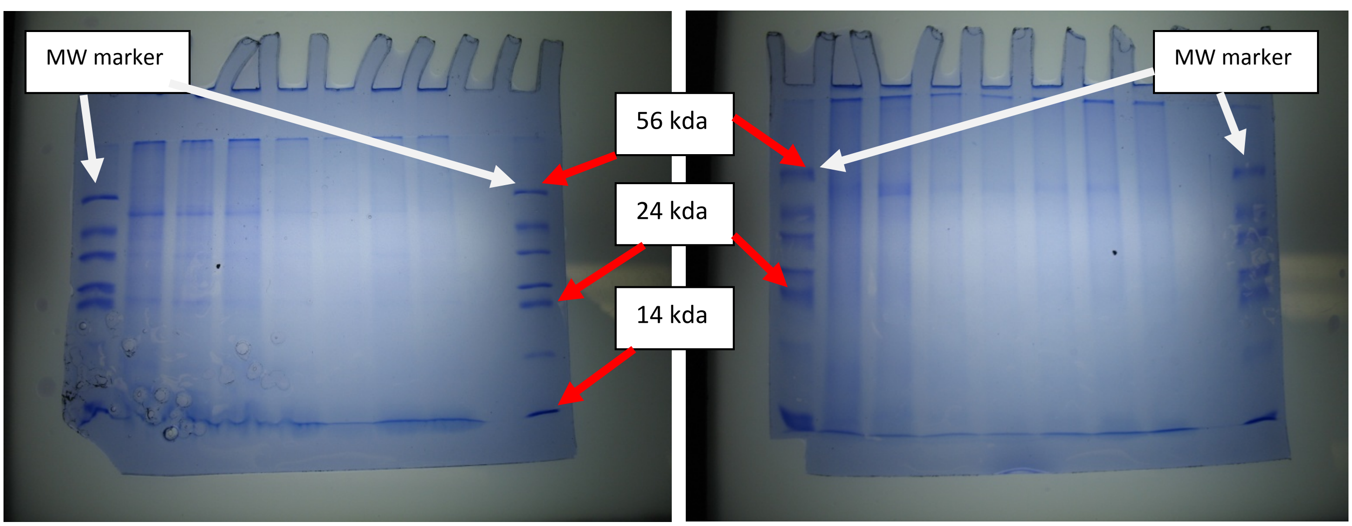

Figure 12: Gel 1 = C1 – ***Evergreen Lawson Cypress***

Figure 13: Gel 2 = C2 – ***Evergreen Western Red Cedar***

Biochemical Data on Leaf Proteins:

The SDS gels (electrophoresis) contained 7 samples on each gel for a total of 28 samples. The first lane of each gel is the Molecular Weight marker, second lane is May 31, third is June 2, fourth is June 28, fifth is July 7, sixth is July 26, seventh is August 2, eighth is August 19 and tenth is also the Molecular Weight Marker. In Gel 4 (A2), there is a large Rubisco subunit, which decreases in concentration over time of the study (protein expression declines). This was for the Deciduous Red Maple sample. The Deciduous Red Oak sample also has Rubisco at 56 kda, however, the concentration appears to be relatively consistent. Also on Gel 4, is a LHC – IId (light harvesting complex protein). Gel 3 (A 1) contains the following proteins: Rubisco and LHC – IIb (light harvesting complex protein. Gel 1 (C1) contains Rubisco (large subunit), LHC – IId (light – harvesting complex protein) and a small subunit of Rubisco as well. Gel 2 (C2) contains the Rubisco protein only (both small and large subunits).

Photosynthesis data from the IRGA analysis:

The IRGA data shows that as the temperature increases (Figure 7) the photosynthetic rate of deciduous red oak is higher than the evergreen trees (Figure 14). The Evergreen Lawson cypress photosynthetic values for both dates, dramatically declines and stays consistently low. Figure 15 shows the compensation point is lowest for the Deciduous Red Oak (June 7) and highest for Deciduous Red Oak (May 31) and also Evergreen Lawson Cypress (June 2).

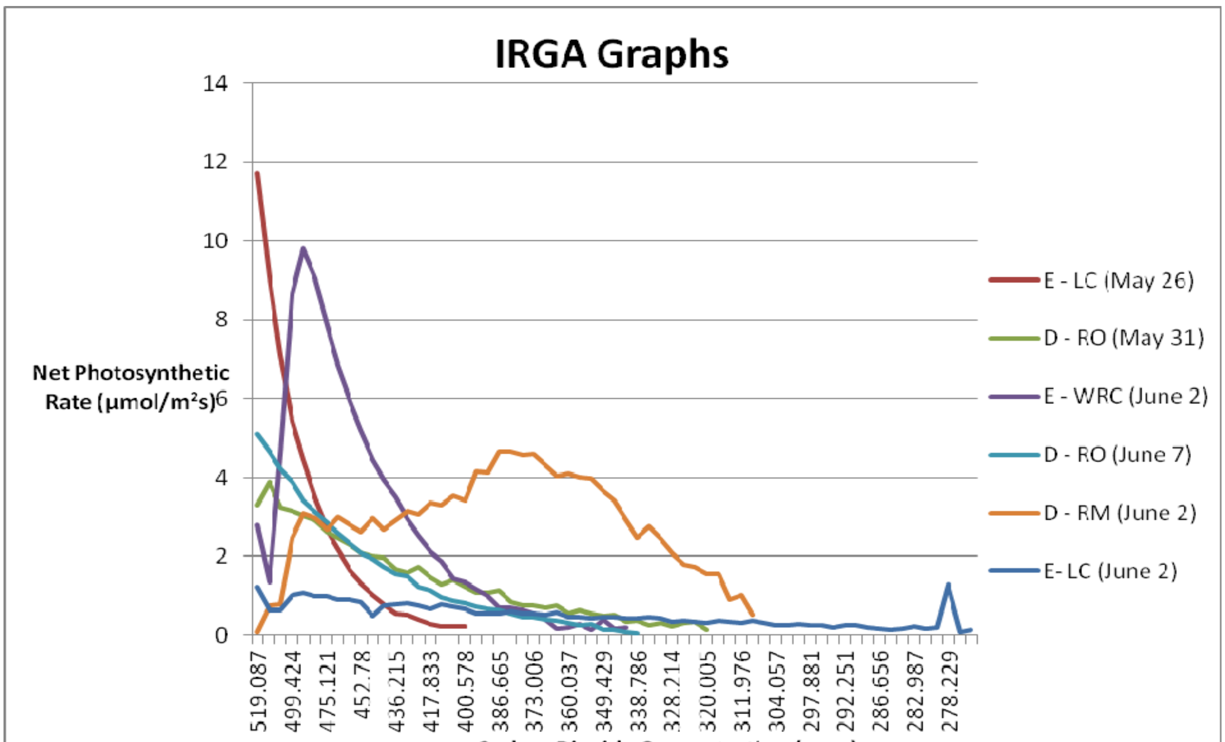

Figure 14: IRGA Graphs for all of the four species

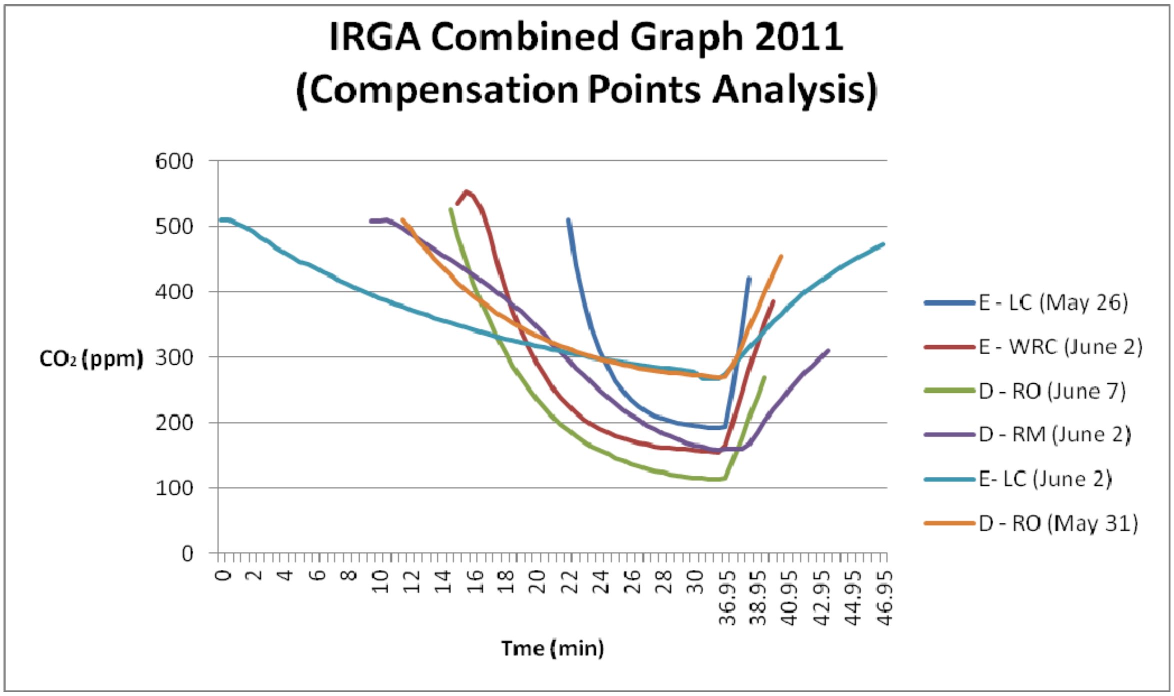

Figure 15: Comparison of the Compensation points of all 4 species

Discussion

This whole study is based on analyzing and comparing the evergreen and deciduous species for the photosynthesis and transpiration rates, as well as for levels of protein expression. Figure 4 shows the morphological aspects to the plants and the change in their physical appearance from May to August (spring to summer seasons). Deciduous species tend to have greater change in their leaf, shape size and colour because deciduous trees undergo senescence, the process whereby they drop their canopy of leaves in fall or autumn, and are bare branches during winter. During the spring season, they begin to grow their leaves again, and reaching their optimal prime size in the summer. The leaves also become darker from spring to summer. As it can be seen in Figure 4, the Deciduous Red Oak is a pale yellow in May and by the end of August, is a dark green colour. The leaves by the end of august contain higher levels of chlorophyll and also increased protein expression levels according to Figure 11. Figure 11 shows that over the prolonged period of time, the protein expression levels of Rubisco (ribulose-1,5 bisphosphate carboxylase/oxygenase) increase (56 kda) and light harvesting complex proteins (LHC-IIb) also slightly increase in concentration (25-28 kda). Deciduous Red Maple also increases in size over the months and also becomes a darker green colour in appearance (Figure 4). The reason for this is also because of the Deciduous trees patterns of leaf senescence and growth. According to Figure 10, the protein expression levels in the Red Maple are relatively consistent with the Red Oak (Figure 11). In the deciduous Red Oak, Rubisco is also expressed in significant levels (56 kda) and light harvesting complex proteins also increase in concentration over time (LHC-IId at 24 kda). The Evergreen Lawson Cypress and Evergreen Western Red Cedar's leaves remained fairly constant throughout this research. They did not show any significant growth, did not show any change in morphological structure nor change in colour (Figure 4). The leaves for the evergreen species, however, did appear to become slightly thicker and more branched off over time. This is also apparent in Figure 4, when comparisons are made between the leaves of May and late August. In Figures 12 and 13 respectively, it is shown that the levels of protein express decrease significantly over the period of time. In particular, the Rubisco protein decreases in protein expression levels. It can barely be seen on the gels by late August. Small levels of the Rubisco protein (small subunits, versus the regular large subunits) are apparent at 14 kda. In both the Lawson Cypress and Western Red Cedar species, initially, there was some expression of light harvesting complex proteins at 24 kda (LHC-IId), but these dramatically decline over the course of this research.

The average photosynthesis rates of the four species are compared in Figure 5. During the spring season, the Deciduous Red Oak appears to have a higher photosynthesis rate than the other species. The lowest in rates is initially the Lawson Cypress leaves. The deciduous Red Maple has a higher photosynthesis rate than the Lawson Cypress all throughout the study, until August, when the Lawson Cypress overcomes the Red Maple. The Western Red Cedar species decreases in its photosynthesis rate all throughout the seasons, until late August, when it begins to increase. This could be a result of the needle foliage of the Evergreen leaves, which conserve more water and have relatively consistent photosynthesis levels throughout the year [Wingler et. al 1998). Lawson Cypress species do not decline as much as the Western Red Cedar, and appear to remain relatively constant at a particular range of the average photosynthesis rate. The Red Maple and Red Oak mainly have higher photosynthesis rates than the Evergreen species and this is because the Deciduous trees increase in size over the course of this season, until they reach their maximum size, with which leaf senescence occurs [Shirke 2001]. The leaves of deciduous trees also appear a darker green colour (Figure 4) over the four months, while the Evergreen trees remain the same colour. This implies increased in chlorophyll levels and protein expression levels in the deciduous species which is evident in both Figure 10 and 11. In Figures 12 and 13, it is shown that both Evergreen species decrease in their levels of protein expression over the spring and summer seasons. From this, it can be deduced that the photosynthesis levels of these species would also be appropriately lower. Deciduous and Evergreen species show negative photosynthesis rates primarily during the month of July, where they are releasing CO_2 and actually contributing to atmospheric warming. There is also a period in July with intense sunshine (increased light intensity as shown in Figure 8 where there are peaks of high light intensity) and increased heat (temperature increases over the course of this study as shown in Figure 7) may have lead to an effect known as photoinhibition resulting in lower rates for all species. Photoinhibition is the process whereby photosystem II (PS II) is more sensitive to light and this excessive light essentially causes damage to the photosynthesis machinery resulting in negative photosynthetic levels [Shirke 2001].

The Transpiration graph (Figure 6) shows the comparisons between the average transpiration rates of both the Evergreen and Deciduous species over the time of this study. Transpiration rate can be defined as the efficiency of the leaves of a plant to intake carbon dioxide and exchange gases with the atmosphere, all the while trying to obtain minimal water loss. High transpiration rates indicates that the stomata remains open longer during this time allowing water to be released, and carbon dioxide to come in. This also allows for better nutrient circulation as water is continuously lost by the process of a proton pump, which increases intracellular concentration, which results in water entering the cells via osmosis [Warren and Adams 2004]. The Western Red Cedar tree, initially has the highest transpiration rate and decreases to a level that all the species stay consistent at for the month of June. The precipitation levels for May and early June were also significant as shown in Figure 9. These levels resulted in minimal transpiration rates for all the species in Figure 6 because water was abundant for the plants to uptake. There was no significant losing of water to exchange gases in the stomata. In July, where temperature and light intensity levels were higher (Figure 7 and 8) the transpiration rates were also much higher. The precipitations levels were also very low during this period (Figure 9). This corresponds well with each other because the increase in temperature results in increased water loss and the stomata also remain open longer because of increased light intensity (photoperiods). The Evergreen Lawson Cypress has the highest transpiration rate which is because the evergreen leaves have less surface area exposed than the deciduous species, which results in less reduction of water by assimilating gases such as carbon dioxide. The deciduous Red Oak has the lowest transpiration rate because the deciduous species have more surface area exposed for water loss and do not have the waxy needle-like foliages, which evergreen species do.

The IRGA graph (Figure 14) shows the net photosynthetic rate of the four species with decreasing carbon dioxide concentration. The Evergreen Lawson Cypress species initially has the highest photosynthesis rate for both May 26 and June 2. The Deciduous Red Maple has the overall highest net photosynthetic rate and the evergreen species predominantly show decreased photosynthetic levels which correspond with decreased protein levels (Figure 12 and 13). The reason for this is the same as mentioned before because of morphological structures (Figure 4) and other environmental factors affecting the morphology such as temperature (Figure 7), light intensity (Figure 8) and precipitation levels (Figure 9). Figure 15 shows the IRGA compensation points. The Deciduous Red Oak has the lowest compensation point and the Evergreen Lawson

Cypress has the highest compensation point. The compensation point is the amount of light intensity on the light curve where the rate of photosynthesis exactly matches the rate of respiration. At this point, the uptake of CO_2 through photosynthetic pathways is exactly matched to the respiratory release of carbon dioxide, and the uptake of O_2 by respiration is exactly matched to the photosynthetic release of oxygen. At this point, the net assimilation of carbon dioxide is zero. Therefore, if the Evergreen Lawson cypress in May has a higher compensation point, it is able to take in more carbon dioxide and higher photosynthesis rates. Also the deciduous Red Oak species has the lowest compensation point, which means decreased photosynthetic levels because the amount of carbon assimilated is not high.

In Conclusion, the deciduous trees had a higher photosynthetic rate because of their broad leaf structure and senescence. They also had higher levels of protein expression. The Evergreen species had higher transpiration levels because of their ability to conserve water because of their year-long needle-like foliages and the protein levels were also decreasing over the course of the spring and summer season. The compensation point is also slightly higher for the Evergreen species versus the deciduous species, but there is not a significant difference.

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Appendix

Section A

Operation of the Licor Machine (used primarily for this study and research)

LI-COR 6200 Portable Photosynthesis System Procedure:

To calibrate the LI-COR, first the magnesium perchlorate dessicant ($MgClO_4$) dessicant must be replaced. Calibration of the LI-COR must also be done by establishing the "Zero" baseline and establishing the" Ambient CO_2 levels", by using the zero knob, and the span knob respectively, samples can then be placed into the chamber for analysis. The light intensity must be preset to 500 µmol/m^2/s and the flow rate must be around 500 µmol/m^2/s. The area of the sample should be measured and input into the LI-COR. After these requirements are completed, the PUMP will be turned on and measurements of the photosynthesis and transpiration rates will be taken in 3, 30 second intervals for a total sample period of 90 seconds. After recording data from the samples, the LI-COR can be taken to the lab's main computer to transfer the data into Microsoft Excel format.