

UBC Social Ecological Economic Development Studies (SEEDS) Student Report

The Effect of Environmental Changes on the Photosynthesis and Transpiration of Rates of Evergreen and Deciduous Trees during the Summer, Autumn, and Winter Periods

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Directed Studies in Biology (BIOL 448)

June 5, 2011

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Biology 448 - Directed Studies Research in Biology

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ABSTRACT:

By analyzing the seasonal changes in the photosynthesis (CO₂ uptake and fixation) and transpiration (water loss) rates of evergreen and deciduous trees, we can determine which trees should be promoted for future plantation projects, to develop a greener and more sustainable future. Trees exhibiting higher photosynthesis rates under various environmental conditions and growing seasons could reduce the rapidly increasing concentration of CO₂ in our atmosphere, and global warming. Evergreen and deciduous trees differ in their leaf senescence patterns through seasonal variations. Photosynthesis and transpiration rates were measured using the LI-COR LI-6200 Portable Photosynthesis System. Samples of evergreen and deciduous leaves under study were taken from 4 different tree species; 2 Evergreen Species: *Thuja plicata* (Western Red Cedar), *Chamaecyparis lawsoniana* (Lawson Cypress), and 2 Deciduous Species: *Acer rubrum* (Red Maple), *Quercus rubra* (Red Oak) on UBC's Sustainability Street, for a span of 9 months including summer, autumn, and winter (July 2010 - February 2011). Environmental factors such as light intensity, temperature, and the amount of precipitation were also recorded with observational data provided from Environment Canada's Historical Weather Database. Results showed higher photosynthetic and transpiration rates amongst the evergreen species during the fall/winter period, when compared to the deciduous species, as deciduous leaves undergo a senescence process due to environmental changes. Furthermore, specific protein gel analysis data through SDS-PAGE profiling and Western Blotting also showed higher levels of key photosynthesis proteins, light harvesting complex of photosystem II (LHCIIB), and the ribulose-1,5 biphosphate carboxylase/oxygenase (RUBISCO) in evergreen species when compared to deciduous ones during the fall/winter. Photosynthesis and transpiration data will be discussed in relation to environmental parameters such as light intensity, temperature, and the amount of precipitation. The findings of this study will contribute to our knowledge about the role of these trees in CO₂ absorption and sustainable development.

CONTENTS

ABSTRACT.....	3
LIST OF ILLUSTRATIONS	5
1.0 INTRODUCTION	6
2.0 MATERIAL AND METHODS.....	10
3.0 RESULTS	13
3.1 Section A: Morphological Data.....	13
3.2 Section B: Photosynthesis Rate	14
3.3 Section C: Transpiration Rate.....	15
3.4 Section D: Environmental Data.....	16
3.5 Section E: Biochemical Data	18
4.0 DISCUSSION	20
5.0 FUTURE STUDIES.....	25
6.0 ACKNOWLEDGEMENTS	25
7.0 LITERATURE CITED	26
8.0 APPENDIX	27

LIST OF ILLUSTRATIONS

List of Figures:

Figure 1: Changes in atmospheric carbon dioxide (CO ₂) levels from 1958-2010. Data collected from Mauna Loa Observatory Hawaii. (Keeling and Sundquist, 2008)	7
Figure 2: Experimental Setup of the LI-COR 6200 Portable Photosynthesis System.	11
Figure 3: Morphological changes occurring in leaves of deciduous and evergreen trees from July 2010 – February 2011.....	13
Figure 4: Average Photosynthesis Rates (μmol/m ² /s) in deciduous and evergreen samples from June 2010 – February 2011 measured by the LI-COR 6200.	14
Figure 5: Average Transpiration Rates (μmol/m ² /s) in deciduous and evergreen samples from June 2010 – February 2011 measured by the LI-COR 6200.	15
Figure 6: Light Intensity (μmol/m ² /s) VS Time (date).	16
Figure 7: Temperature (°C) VS Time (date).	16
Figure 8: Photoperiod (hr) VS Time (date).	17
Figure 9: Precipitation (mm) VS Time (date).	17
Figure 10: A-D) SDS-PAGE Coomassie Brilliant Blue gels of 4 tree species (A-B) Evergreen, (C-D) Deciduous, showing the protein-profile of the leaf extracts. Two leaf samples were taken each month from July 2010 – January 2011. 10 μL of each sample were loaded on to the gel.....	18
Figure 11: A-D) Western Blots of 4 tree species (A-B) Evergreen, (C-D) Deciduous, showing amounts of specific proteins LHCIIB and RUBISCO. Two leaf samples were taken each month starting from July 2010 – January 2011. 5 μL of each sample were loaded on to the gel.....	19
Figure 12: Photosynthesis Rates & Stomatal Conductance of 2 Evergreen Species (Syzyglum, Hopea) VS 2 Deciduous Species (Vitex, Xylia) from a study done by Ishida <i>et al.</i> (2010).	22

1.0 INTRODUCTION

Atmospheric carbon dioxide (CO₂) levels are increasing in our atmosphere and this of considerable concern in global climate change because of its greenhouse gas warming potential. CO₂, like many other greenhouse gasses is capable of trapping heat radiating away from the earth, leading to an effect known as "Global Warming" (Tans, 2006). As human civilization progresses, advances in technology due to the industrial revolution, has lead to an increase in carbon emissions. Inventions such as the internal combustion engine, which burns fossil fuels in automobiles and other vehicles, and an increase in the number of coal burning factories has lead to increased levels of CO₂ (Lehr, 1992). Global atmospheric CO₂ 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).

Trees are one of the most important agents in reducing the concentration of CO₂ in our atmosphere as they play an important role in atmospheric gas exchange through the processes of photosynthesis and respiration. Plants take in carbon dioxide (CO₂) from the atmosphere during photosynthesis, and release oxygen (O₂) back into the atmosphere, reducing atmospheric carbon dioxide levels. Through 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). Other photosynthetic proteins, such as

RUBISCO, or ribulose-1,5 bisphosphate carboxylase/oxygenase catalyze the initial carbon fixing steps during this process (Taiz and Zeiger, 2002).

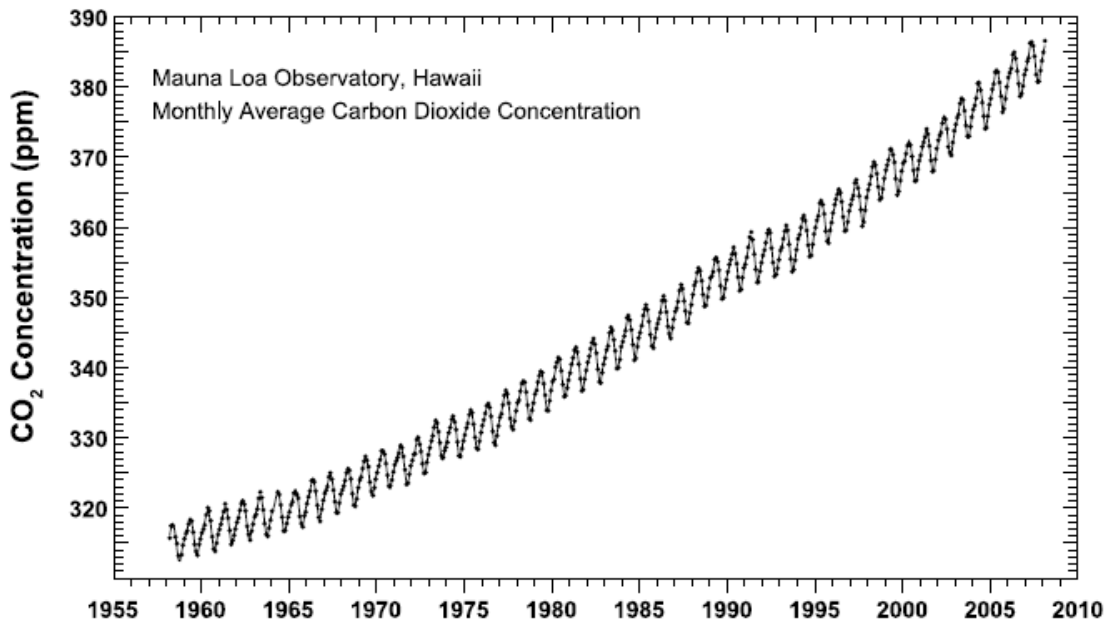


Fig. 1) Changes in atmospheric carbon dioxide (CO₂) levels from 1958-2010. Data collected from Mauna Loa Observatory Hawaii. (Keeling and Sundquist, 2008)

Photosynthesis consists of two stages; the light reactions and the dark reactions. The light reactions convert light into energy (ATP and NADHP) and the dark reactions use the energy and carbon dioxide to produce carbohydrates (Taiz and Zeiger, 2002).

Expression of these specific proteins, and others, change through seasonal variations, as environmental stresses cause certain senescence processes to begin in certain species (Smart, 2006). This consequently results in altered gas exchange as levels of photosynthesis and transpiration vary. Transpiration influences gas exchange as stomatal pores remain open,

allowing water to leave plants, and CO₂ to enter by passing guard cells to be used for photosynthesis (Taiz and Zeiger, 2002). Since plants do not have membranes that are both permeable to CO₂ and impermeable to water, transpiration is an inevitable consequence of photosynthesis (Taiz and Zeiger, 2002).

The two types of trees under study are evergreen and deciduous trees. Evergreen trees conserve energy and nutrients by slowly growing new foliage, or leaves, year-round (Goldstein *et al.*, 1989). This can be advantageous in regions where nutrients are limited, as an evergreen's needle shaped structure aids in transpiration and allows them to endure a rough season, whereas a deciduous tree might fail (Taiz and Zeiger, 2002). Deciduous trees on the other hand have broad-shaped, color changing, leaves and shed their leaves during the autumn period of every year (Taiz and Zeiger, 2002). The shedding or genetically programmed death of the leaves, known as leaf senescence, is a highly ordered process regulated by approximately 30 genes known as senescence associated genes (SAGs) (Smart, 2006). The leaf when young and mature, accumulates nutrients and exports them to growing parts of the plant during the initial stages of senescence (Yoshiyuki and Kihachiro, 2006). The chloroplast is the first organelle to deteriorate during the onset of leaf senescence, with the destruction of thylakoid protein components and stromal enzymes including LHCIIB and RUBISCO (Wingler *et al.*, 1998).

Previous research conducted by Eamus (1999) on the seasonal changes in photosynthesis and transpiration rates of evergreen and deciduous species, analyzes the cost/benefit ratio of deciduous leaf production, in comparison to having year-long evergreen foliage. This particular study shows that during the drier periods of the summer, deciduous

species become drought avoiders as they drop their entire canopy and; therefore, do not transpire at significant rates in the dry season as their foliage avoids drought (Eamus, 1999). Deciduous species invest large amounts of nitrogen in leaves to support a very large assimilation rate each day of the wet season; therefore their short-lived leaves fix large amounts of carbon for a short time (Eamus, 1999). Ishida *et al.* (2010) and Golstein *et al.* (1989) both show that evergreen species do not invest large amounts of nitrogen into their leaves, and assimilation rates are consequently lower, but they fix carbon for 12 months and hence the return to the tree occurs over a longer time.

The purpose of this experiment was to analyze the effect of environmental changes on the photosynthesis and transpiration rates of the two different tree species. By analyzing the seasonal changes in the photosynthesis (CO₂ uptake and fixation) and transpiration (water loss) rates of evergreen and deciduous trees, we can determine which trees should be promoted for future plantation projects, to develop a greener and more sustainable future. Trees exhibiting higher photosynthesis rates under various environmental conditions and growing seasons could reduce the rapidly increasing concentration of CO₂ in our atmosphere, and global warming. Other more specific objectives of this research was: to observe and analyze morphological changes in leaf color/structure through seasonal variation; to compare and contrast photosynthesis and transpiration rates amongst the 4 tree species; and to analyze/interpret biochemical changes in expressions of photosynthetic proteins, such as the light harvesting complex of photosystem II (LHCIIB), which focus energy from photons, and ribulose-1,5 biphosphate carboxylase/oxygenase (RUBISCO), which is a key enzyme catalyzing the CO₂ fixation reaction in the Calvin Cycle.

2.0 MATERIALS AND METHODS:

Samples of evergreen and deciduous leaves under were taken from 4 different tree species; 2 Evergreen Species: *Thuja plicata* (Western Red Cedar), *Chamaecyparis lawsoniana* (Lawson Cypress), and 2 Deciduous Species: *Acer rubrum* (Red Maple), *Quercus rubra* (Red Oak) on UBC Sustainability Street, for a span of 9 months including summer, autumn, and winter (July 2010 - February 2011). The samples were taken on a weekly basis, with 3 samples coming from each tree species, combining for a total of 12 samples per day. The samples were taken from various, lower, reachable, areas on the trees and were approximately 20 cm² in area. The light intensity was also recorded using a light meter and pictures were taken from specific locations for each tree.

The samples were carefully taken back to the lab in a 1000 mL beaker ensuring water contact is continuous to the stem of the leaf. This will keep functional processes in the leaf to continue without being disturbed, due to lack of water. After the samples were taken back to the lab, the photosynthesis and transpiration rates were measured by LI-COR LI-6200 Portable Photosynthesis System (Refer to **Appendix: Section A** for detailed LI-COR instructions). Close-up pictures of the samples were also taken to analyze morphological changes occurring with seasonal variation. The light intensity was preset to 500 $\mu\text{mol}/\text{m}^2/\text{s}$ and the flow rate was also around 500 $\mu\text{mol}/\text{m}^2/\text{s}$. After recording data from the samples, the LI-COR was taken to the lab's main computer to transfer the data into Microsoft Excel format.



Fig. 2) Experimental Setup of the LI-COR 6200 Portable Photosynthesis System.

The remaining samples were then frozen in liquid nitrogen, folded in aluminum foil, and placed in the freezer for further protein analysis on ribulose-1,5 bisphosphate carboxylase/oxygenase (RUBISCO) and light harvesting complex of photosystem II (LHCIIB). Frozen samples were taken out of the freezer during late February of 2011 for protein profiling and analysis. Refer to Singh, 2008, Biology 351: Lab 5 for the preparation of the samples. Also, refer to Singh, 2008, Biology 351: Lab 5 for the synthesis of the separating and stacking gels, and for the gel electrophoresis apparatus setup. Two samples were taken for each month from the first and fourth week of the month, from July 2010- January 2011, resulting in a total of 14 samples per gel. A total of 8 gels were run, 4 for the Coomassie blue stain, and 4 for the Western Blot to identify specific proteins RUBISCO and LHCIIB. For Coomassie blue stained gels 10 μL of each sample and 6 μL of the molecular weight markers were loaded. For the Western Blots 5 μL of each sample and 5 μL of the molecular weight markers were loaded. Refer to

AgriSera Educational Tool Kit for the synthesis of the Coomassie blue stained gels and Western Blots. RUBISCO and Light Harvesting Complex IIB antibodies were used as the 1° antibody and Grown anti-Rabbit Horseradish Peroxidase as the 2° antibody. Refer to the Antibody Titration Table in the Appendix for sample dilutions, 1° antibody dilutions, and 2° antibody dilutions. After subsequent 20 minute washes using TBST, the blots were loaded onto the X-Ray film apparatus and taken to the dark room for results. 30 second films produced for each protein, displaying excellent clarity.

3.0 RESULTS

3.1. Section A: Morphological Data

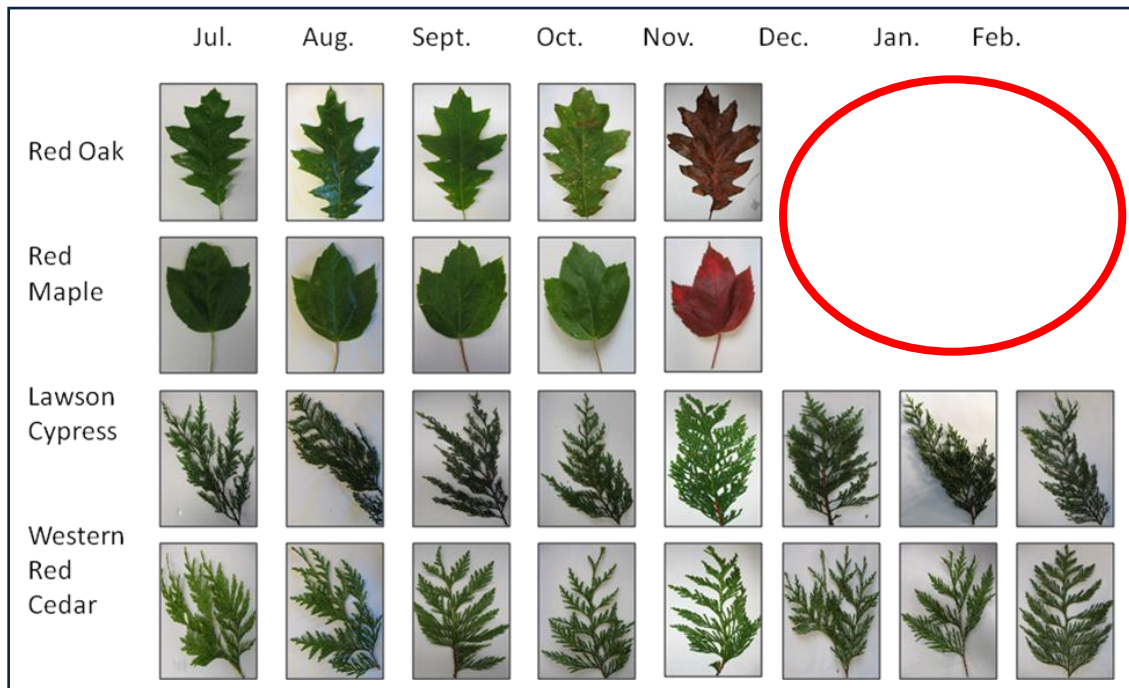


Fig. 3) Morphological changes occurring in leaves of deciduous and evergreen trees from July 2010 – February 2011.

Deciduous (Top) species are undergoing an autumnal senescence process as we can observe color changes/degradation of the leaves occurring in Oct./Nov. and no data available for Dec./Jan./Feb. (See red circle on Fig. 3). Evergreen (Bottom) species do not show autumnal senescence occurring. Data from the photosynthesis rate graph (See Fig. 4) correlate with morphological data as we see a rapid decline in the photosynthesis rates of deciduous species in Oct./Nov. Coomassie blue stain data and Western Blot data from (Fig. 8, Fig. 9) is also

concurrent with morphological data as we see protein degradation and lighter bands for deciduous species in Oct./Nov.

3.2. Section B: Photosynthesis Rate

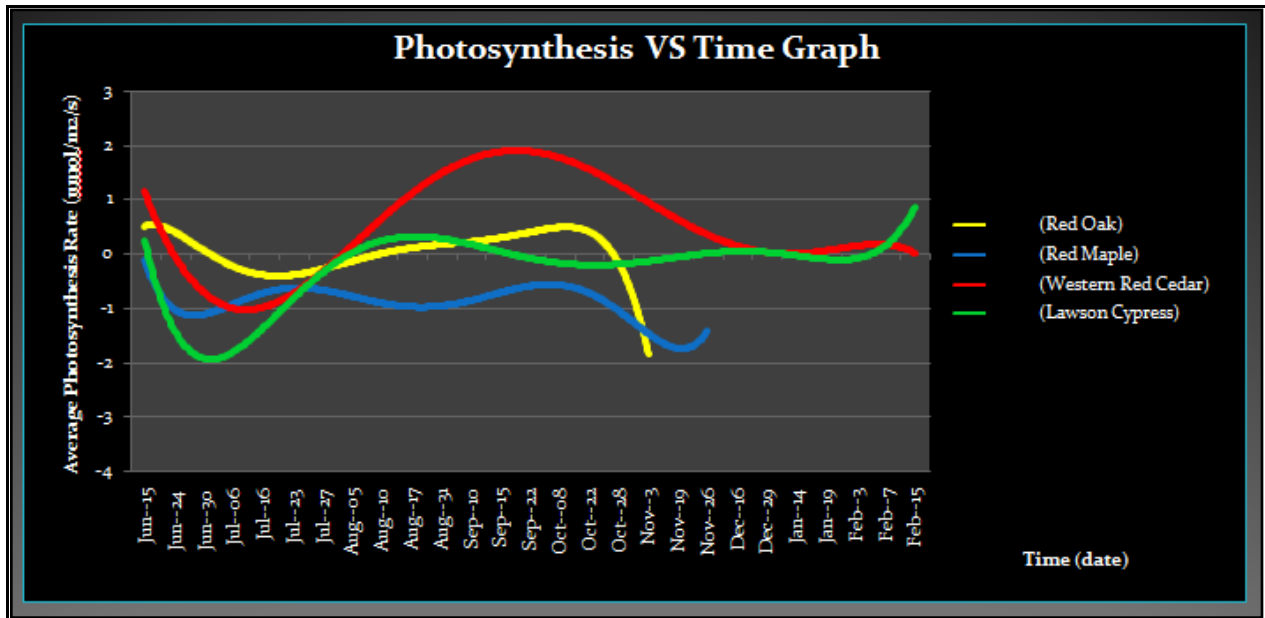


Fig. 4) Average Photosynthesis Rates ($\mu\text{mol}/\text{m}^2/\text{s}$) in deciduous and evergreen samples from June 2010 – February 2011 measured by the LI-COR 6200.

The Western Red Cedar appears to be the best photosynthesizing tree species as it continues to photosynthesize strongly in the fall period where others photosynthetic ability tends to decline. Deciduous species show negative photosynthesis rates during the fall/winter period, where they are releasing CO_2 and actually contributing to atmospheric warming. There is also a period in July with intense sunshine and heat may have led to an effect known as photo-inhibition resulting in lower rates for all species. During the winter period beginning in Dec. and ending in Feb. we also see a major decline in the photosynthetic abilities of the two remaining evergreen species with the Western Red Cedar being impacted the greatest

potentially due to the colder, harsh temperatures (See Fig. 7), and lower amounts of precipitation (See Fig. 8).

3.3, Section C: Transpiration Rate

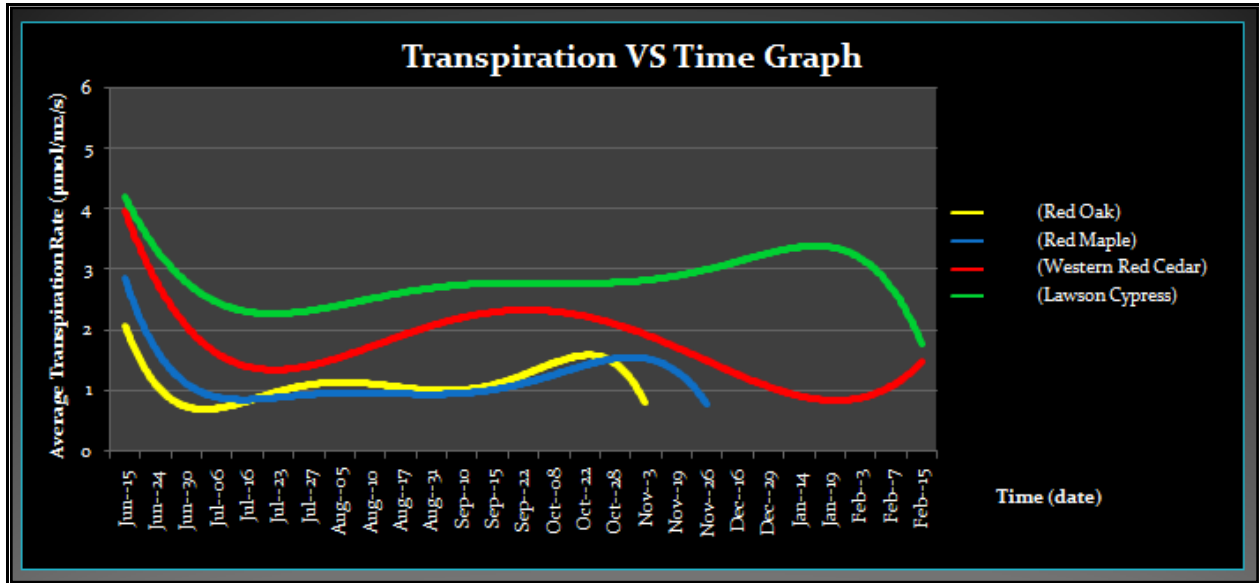


Fig. 5) Average Transpiration Rates ($\mu\text{mol}/\text{m}^2/\text{s}$) in deciduous and evergreen samples from June 2010 – February 2011 measured by the LI-COR 6200.

Evergreen species have shown to consistently have higher transpiration rates as compared to deciduous species throughout the entire time of the data taken (See red/green lines in Fig. 5). High transpiration rates indicate that their stomates remain open 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.

3.4. Section D: Environmental Data

- Weather Data provided by Environment Canada On-line Historical Database.

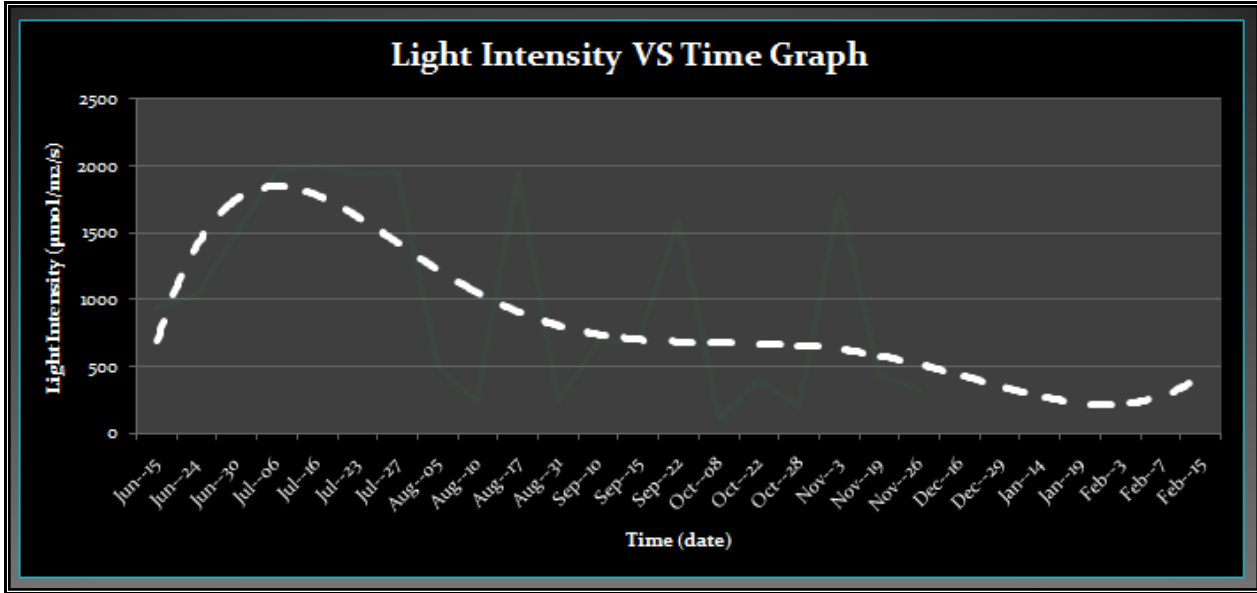


Fig. 6) Light Intensity ($\mu\text{mol}/\text{m}^2/\text{s}$) VS Time (date)

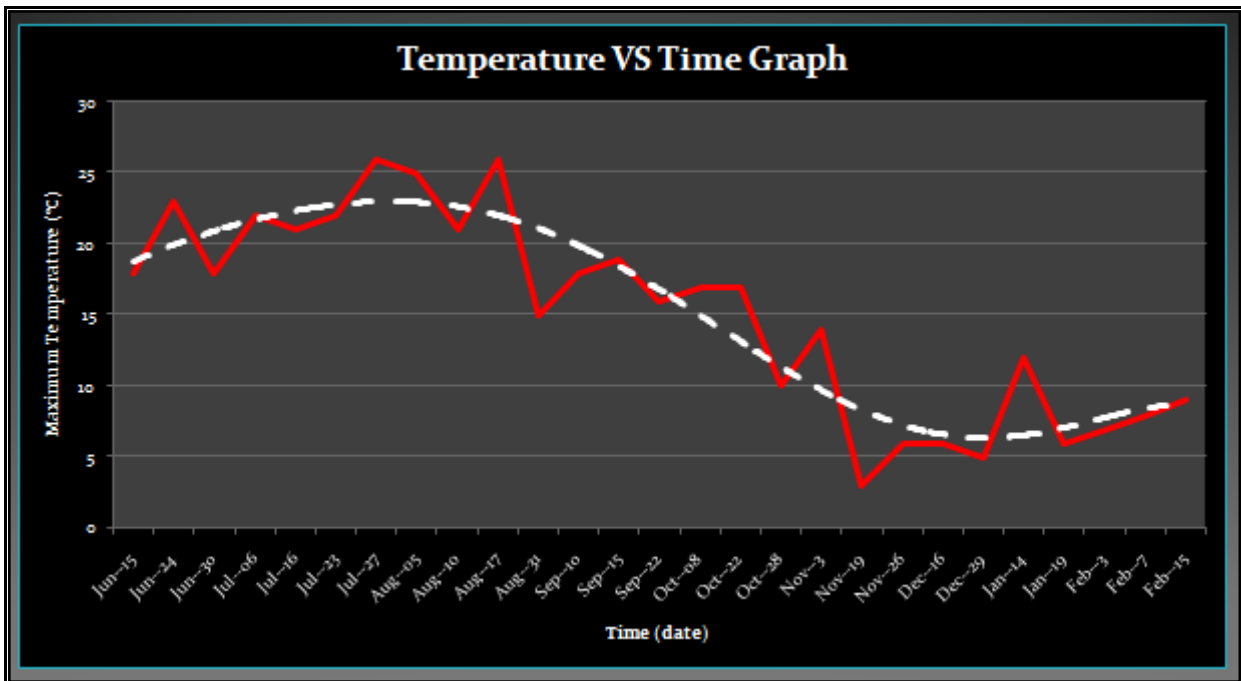


Fig. 7) Temperature ($^{\circ}\text{C}$) VS Time (date)

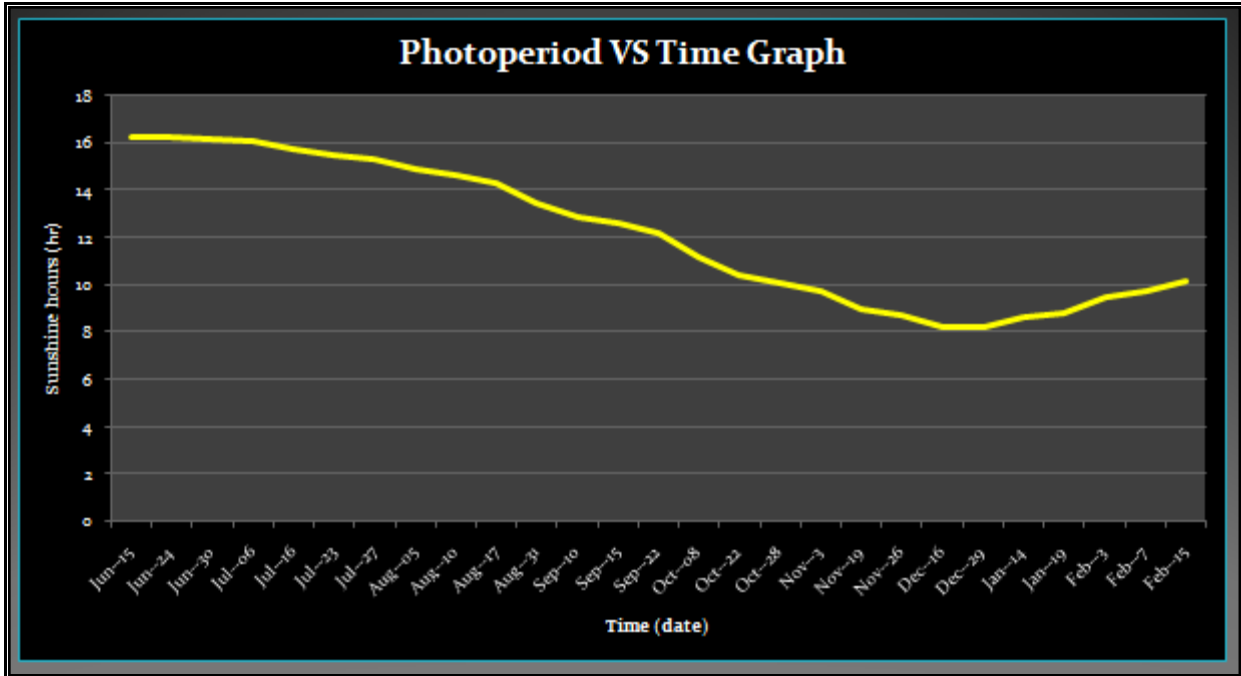


Fig. 8) Photoperiod (hr) VS Time (date)

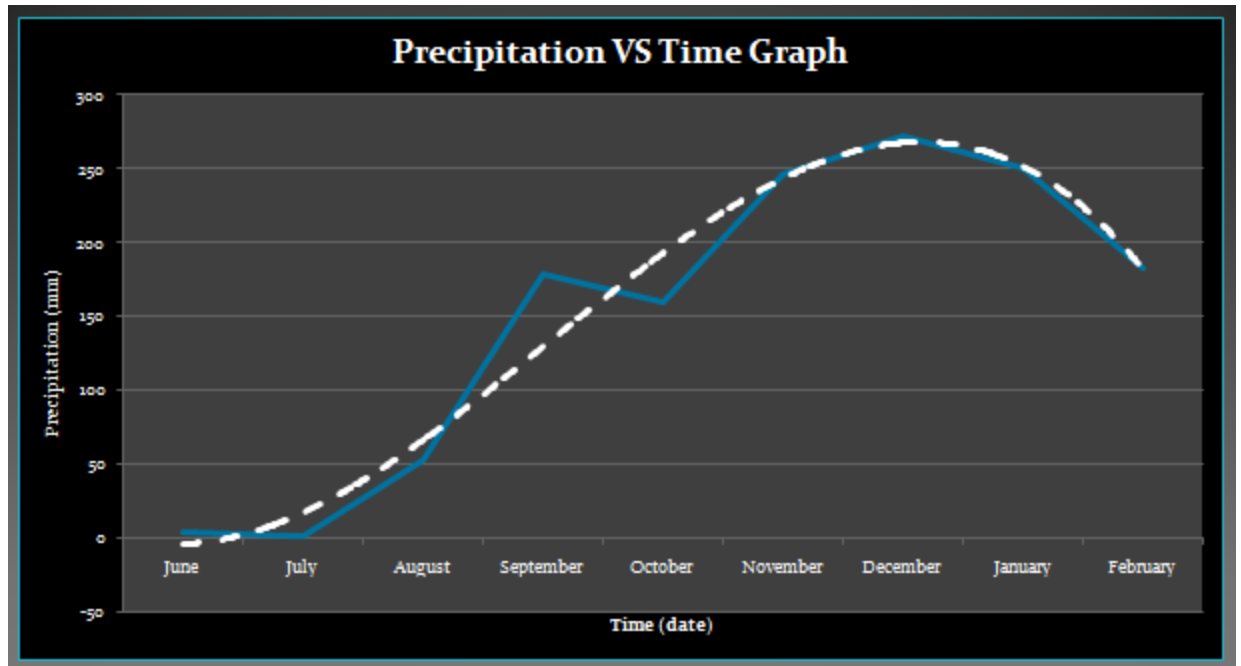


Fig. 9) Precipitation (mm) VS Time (date)

3.5. Section E: Biochemical Data

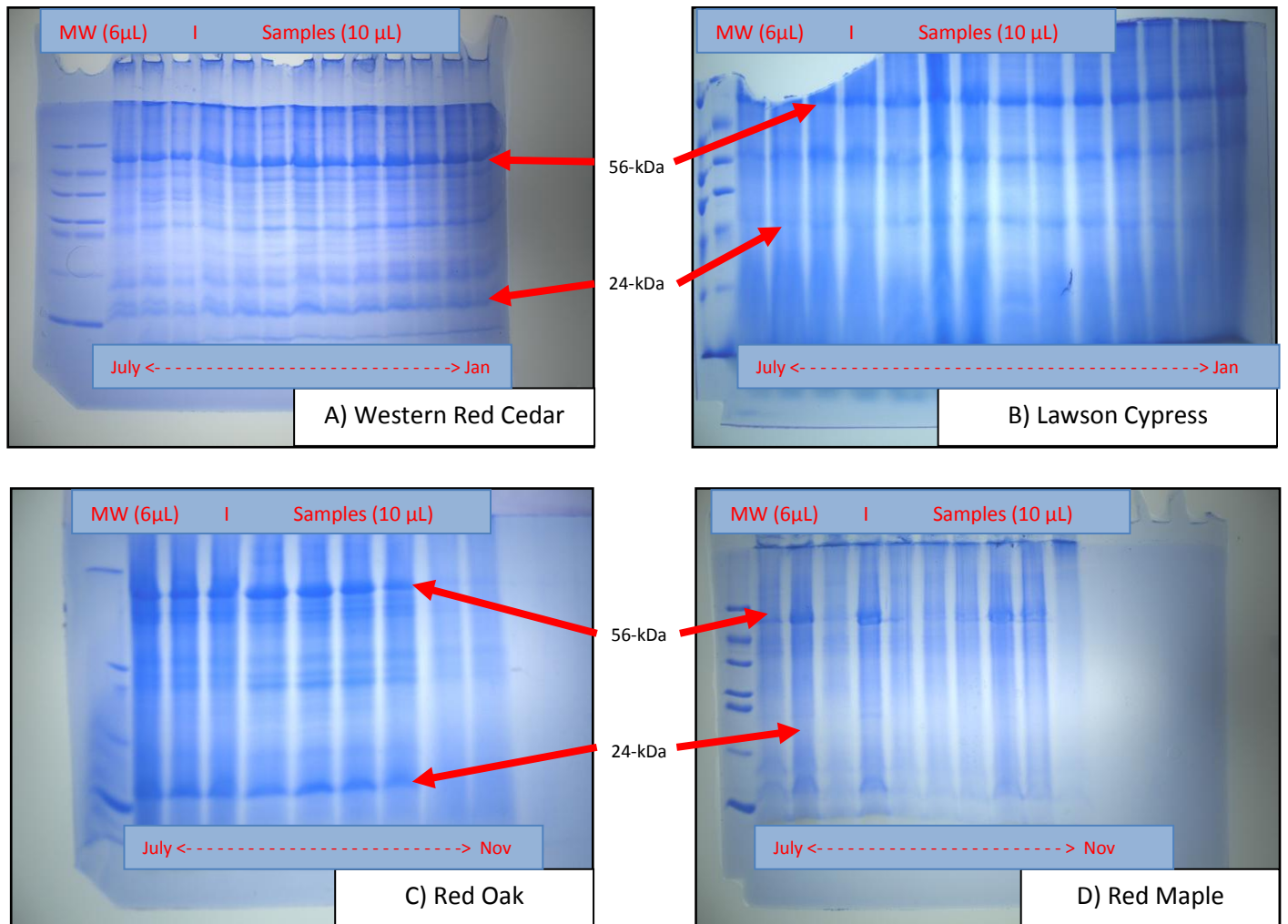


Fig. 10)A-D) SDS-PAGE Coomassie Brilliant Blue gels of 4 tree species (A-B) Evergreen, (C-D) Deciduous, showing the protein-profile of the leaf extracts. Two leaf samples were taken each month from July 2010 – January 2011. 10 μL of each sample were loaded on to the gel.

Protein degradation is present throughout the autumn period for the 2 deciduous species (Bottom) as we can see a decrease in the thickness of bands present on the stained gels during Oct./Nov. Evergreen species display more consistency with regards to protein expression as degradation is not as visible.

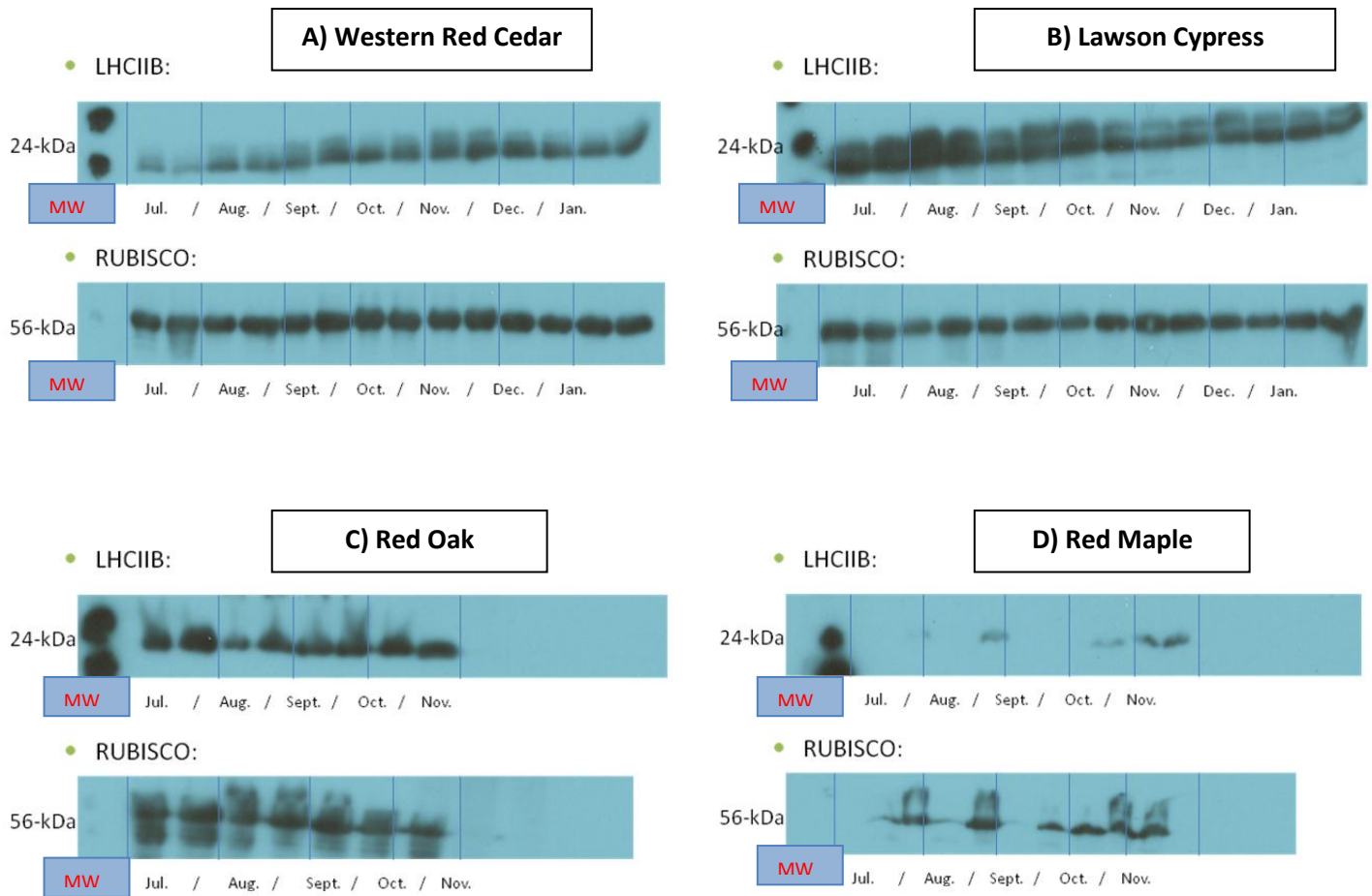


Fig. 11) A-D) Western Blots of 4 tree species (A-B) Evergreen, (C-D) Deciduous, showing amounts of specific proteins LHCIIB and RUBISCO. Two leaf samples were taken each month starting from July 2010 – January 2011. 5 μ L of each sample were loaded on to the gel.

We can clearly see higher levels of LHCIIB protein expression during the fall/winter periods for evergreen species (A-B) when compared to deciduous ones (C-D). RUBISCO levels are relatively high/constant, and greater than deciduous species throughout the entire time of the data taken for evergreens. Evergreen species clearly have higher levels of photosynthetic proteins during the fall/winter months, as deciduous species undergo a senescence process causing a decline in their relative amounts of photosynthetic proteins. Morphological data (See **Fig. 3**) also corresponds with these results.

4.0 DISCUSSION

After analyzing and comparing the photosynthesis and transpiration rates of the 4 different tree species (See **Fig. 4**, **Fig. 5**), from the period of July 2010 – February 2011, it can be concluded that the evergreen Western Red Cedar (*Thuja plicata*) was the most effective photosynthetic specie at reducing atmospheric carbon dioxide levels during the autumn/winter period. This was mainly due to the fact that the deciduous species underwent a senescence process, causing protein degradation and lower observed photosynthesis rates during this time (See **Fig. 3**). Studies done by Eaus (1999) on the seasonal changes in the photosynthesis and transpiration rates of evergreen and deciduous species support my results as similar findings were concluded. Eaus (1999) and Goldstein *et al.* (1989) found that evergreen species displayed higher photosynthetic ability throughout the year, whereas deciduous species were short-lived and fixed large amounts of carbon during early growth periods. This might be made possible by a root system that accesses deeper layers of soil than deciduous species (Eaus, 1999).

Photosynthesis rates for all 4 tree species display an initial rapid decline (See **Fig. 4**) in CO₂ uptake during the high temperature (See **Fig. 6**, **Fig. 7**, **Fig. 9** –July), drought-like conditions of July 2010. In high light intensities conditions, photosystem II (PSII) in chlorophyll can be damaged by the enhanced activity of electrons beyond that which it can process (Baker, 1997). PSII centers are continuously repaired via degradation and synthesis of the D1 protein in the photosynthetic reaction centre of PSII (Baker, 1997). Due to rapid repair, most PSII reaction centers are not photo-inhibited; however, extreme environmental stresses such as extreme temperature, salinity, or drought, limit the supply of CO₂ for carbon fixation which decreases

the rate of repair of PSII (Baker, 1997). Therefore less CO₂ is taken up & lower photosynthesis rates are observed. During periods of photo-inhibition we see lower levels of LHClIB present (See **Fig. 8, Fig. 9** -July), indicating that the deactivation of PSII is most likely the cause of the observed lower photosynthesis rates.

After July, photosynthesis rates tend to increase for all species as temperatures and light intensity decline, removing the effect of photo-inhibition (See **Fig. 4**). During this time, leaves are allocating resources for growth and photosynthesizing at high rates. Eventually during the later stage of autumn, lower temperatures, with reduced amounts of sunlight, and biological limitations on growth size based on nitrogen investment in photosynthetic machinery, leads to the release of photosynthesis inhibiting hormones marking the beginning of the senescence process for deciduous species (Sobrado, 1994). In contrast, evergreen species continue to photosynthesize as they have several adaptations including a covering of waxy foliage which helps prevent moisture loss and subsequent freezing (Taiz and Zeiger, 2002). These trends on **Fig. 4** are consistent with specific protein gel analysis (**Fig. 8, Fig. 9**) as evergreen species show consistently higher levels of photosynthetic proteins such as RUBISCO and LHClIB compared to deciduous ones during the autumn/winter. This, in part results in higher photosynthesis rates and increased uptake of carbon dioxide during the fall/winter periods for these trees. During the winter period, negative photosynthesis rates for all species are observed as freezing temperatures limit the circulation of water/nutrients inside the remaining evergreen trees (See **Fig. 4**). A very similar study conducted by Ishidia *et al.* (2010), which also used the LI-COR 6200 Portable Photosynthesis System had similar photosynthesis rate data for evergreen species: *Syzygium cumini*, *Hopea ferrea* and deciduous species: *Xylia xylocarpa* *Vitex peduncularis* (See

Fig 12). Ishida *et al.* (2010) also shows how stomatal conductance is linearly correlated with the net photosynthetic rate. This is because transpiration is an inevitable consequence of photosynthesis (Taiz and Zeiger, 2002).

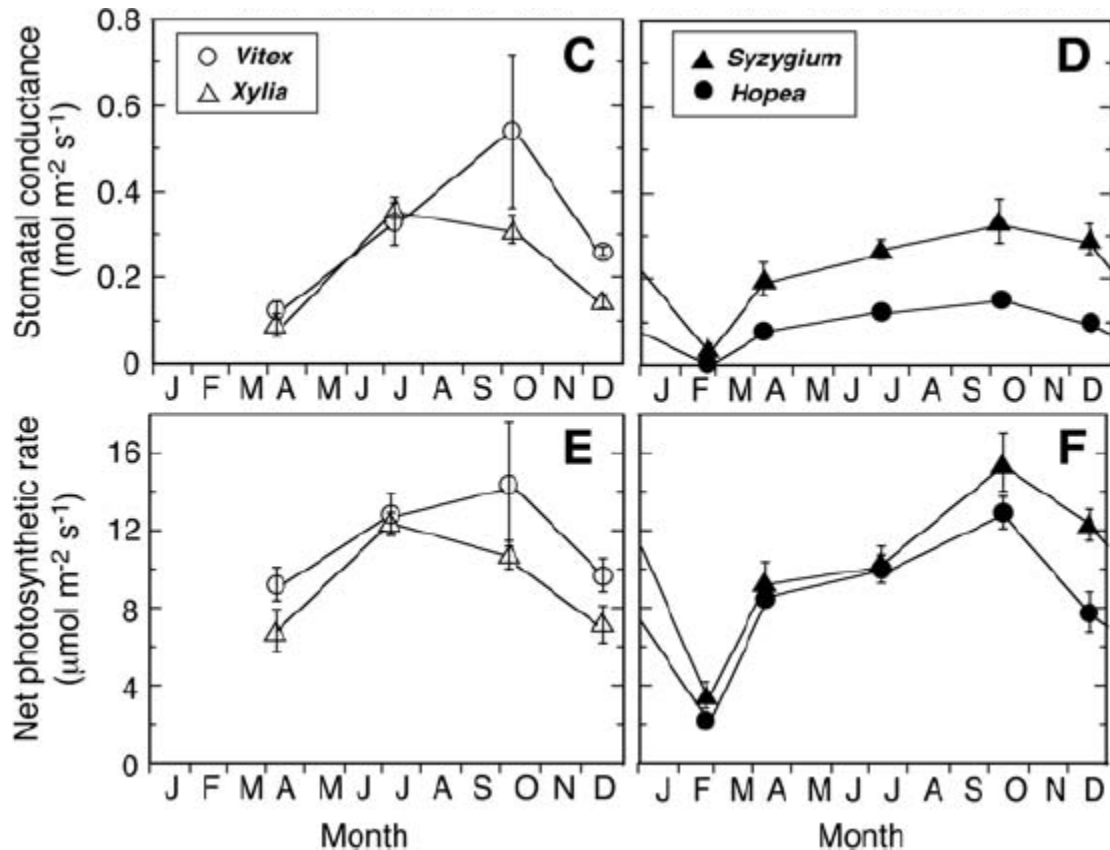


Fig. 12) Photosynthesis Rates & Stomatal Conductance of 2 Evergreen Species (Syzygium, Hopea) VS 2 Deciduous Species (Vitex, Xylia) from a study done by Ishida *et al.* (2010).

Evergreen species also show higher transpiration rates compared to deciduous ones (See **Fig. 5**) throughout the summer/autumn/winter periods indicating that their stomates remain open during these times to allow water to leave cells, and carbon dioxide to enter. Almost all of the CO_2 fixed by terrestrial plants and most of the water transpired pass through these stomatal pores. The degree of opening of these pores is modulated by variation in the turgor status of the two surrounding guard cells (Christodoulakis *et al.* 2002). The regulation of

stomatal aperture determines the compromise between increasing CO₂ fixation and reducing transpiration to prevent desiccation. Studies done by Goldstein *et al.* (1989) agree with my results as evergreen species in their experiments “generally exhibited higher rates of water loss than deciduous species”. Goldstein *et al.* (1989) concludes that evergreen species have more efficient systems for water transport compared to deciduous ones, which is advantageous as it allows for high stomatal conductance without turgor loss. This becomes particularly important during the drier periods of summer when evaporate demand is higher. Goldstein *et al.* (1989) also proposed that having relatively high stomatal conductances may be critical for the maintenance of a favorable carbon and nutrient balance in evergreen trees. Compared to deciduous trees, evergreen trees have a much higher maintenance cost due to the presence of an extensive root system and waxy leaves (Eamus, 1999). An increased leaf life span increases the time period for photosynthesis, compensating for higher maintenance costs; however, it appears that the increased life span cannot compensate for additional maintenance costs if photosynthesis rates of evergreen species are low (Eamus, 1999).

Some potential experimental errors which may have resulted in variations, or discrepancies in data could potentially be due to several factors. One factor is that our weather is constantly changing, with changes in humidity, visibility, etc occurring every second effecting photosynthesis and transpiration rates. Another factor may be that the time of the day that the data was taken was not 100% consistent throughout experiment as levels of photosynthesis change throughout the day due to a plants circadian rhythm.

Other studies and previous research conducted in Dr. Singh's Lab (Department of Botany, University of British Columbia) show that since deciduous leaves have a larger surface area to take up CO₂, it allows them to collectively have stronger photosynthesis during certain times of the year (ie. Spring). Studies done by Eamus (1999) agree with this data as he analyzes the cost/benefit relationships amongst deciduous and evergreen trees. Deciduous species invest large amounts of nitrogen in leaves to support a very large assimilation rate each day of the wet season; therefore their short-lived leaves fix large amounts of carbon for a short time (Eamus 1999).

5.0 FUTURE STUDIES

Based on the results of this investigation and previous studies in our laboratory, a combination of planting both photosynthetically-efficient and environmental stress-resistant deciduous and evergreen trees could help reduce atmospheric CO₂ levels. Future studies include conducting experiments on several other deciduous and evergreen tree species located on UBC's campus and exploring other environmental variables such as humidity and it's relation to photosynthetic and transpiration ability.

6.0 ACKNOWLEDGEMENTS

I would like to thank Dr. Singh and Mr. Mehroke for their continuous suggestions and guidance throughout the whole experiment. I would also like to thank Brenda Sawada of UBC SEEDS for coordination of this project. Finally, my special thanks to all the volunteer students in Dr. Singh's lab who helped me at the various stages of this project.

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8.0 APPENDIX

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 $500 \mu\text{mol}/\text{m}^2/\text{s}$ and the flow rate must be around $500 \mu\text{mol}/\text{m}^2/\text{s}$. 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.