

**The Seasonal and Environmental Effects on Photosynthetic Efficiency of Deciduous and Evergreen  
Tree Species during Winter and Spring Season in Vancouver**

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**University of British Columbia**

**BIOL 448**

**July 24, 2014**

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**BIOL 448 – DIRECTED STUDIES IN BIOLOGY**

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**July 24, 2014**

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## **Abstract**

The carbon sequestration efficiency of five tree species, Red maple (*Acer rubrum*), Red Oak (*Quercus rubra*), Western Red Cedar (*Thuja plicata*), Lawson Cypress (*Chamaecyparis lawsoniana*), and Juniper (*Juniperus communis*), was being investigated during springtime in Vancouver. The purpose of this study was to observe which tree species could be a more efficient and sustainable species at reducing the global warming effects. Photosynthesis, respiration, transpiration rates and photosynthetic protein expression level of the tree leaves were being measured and compared. Western Red Cedar, an evergreen tree species, was found to have the best overall ability at reducing atmospheric carbon dioxide – carbon sequestration. Deciduous trees in general, was found to have better conservation of water - transpiration rate.

## **1.0 Introduction**

Into the 21st century, global warming, greenhouse gas, and habitat destruction have quickly become parts of the urgent global issues. Constantly appearing on newspapers, movies, and Internet, they have successfully captured millions of people's attention. More efforts have therefore been put into understanding, preventing, and reversing the effects of these environmental damages. In an article by Vitousek (1994), the author mentions that the increasing carbon dioxide concentration, one of the dominant greenhouse gases, in the atmosphere is a major contributor to the global warming issue. In Root *et al.*'s (2003) review, the authors use two types of meta-analyses methods to combine and examine a broad spectrum of findings on global warming's impact on animals and plants. They conclude that the recent climatic warming has already had a significant impact and may continue to have a long-term, large-scale impact on the animal and plant populations (Root *et al.*, 2003). Along with many other reports, these reports stress the importance and urgency to resolve the global warming issue.

Many methods have been discussed and put to action to slow the progress of global warming such as using plants. Since plants are able to sequester carbon dioxide from the atmosphere, they have always been considered as part of the solution to global warming. Vitousek (1994) has also noted that some plant species could adapt to the increasing carbon

dioxide level with a faster growth rate meaning more atmospheric carbon dioxide can be fixed into biomass. Understanding the capacity that plants have for contributing to the reduction of atmospheric carbon dioxide level can help to increase the efficiency in forest or city tree planning in order to combat global warming.

Plants fix and store the atmospheric carbon dioxide into biomass by the process of photosynthesis with the utilization of light energy (Nowak and Crane, 2001). Nowak and Crane's (2001) study focuses mostly on assessing how much urban trees can sequester and store carbon dioxide from the atmosphere. They explain that because urban areas are expanding, urban forests can play a significant role in reducing the carbon dioxide emission from urban areas (Nowak and Crane, 2001). Their idea is encouraging since the main source of carbon dioxide emission comes from the urban area, as there are more fossil fuel usage and human activities. Another study done by McPherson (1998) evaluates the carbon storage and sequestration of urban forest as well, but in addition the author also evaluates the carbon dioxide releases by tree maintenance and decomposition. Tree species can also release carbon dioxide through a process called respiration, which utilizes nutrients and releases carbon dioxide as a by-product. These processes will be undesirable because they increase the atmospheric carbon dioxide level.

Studies have been conducted to show that climate change can have significant effects on the morphological, biochemical, as well as physiological aspects of tree species. One obvious effect is the leaf senescence and abscission of deciduous tree species during fall-winter season. Chen *et al.* (1999) has done their three-year study on the deciduous forest and found factors like spring air temperature can have a strong impact on leaf emergency timing and thus on carbon sequestration. Nonetheless, climate change can also influence evergreen tree species. For example, in Weger *et al.*'s study (1993), they indicate that low temperature has a negative effect

on the photosynthesis characteristics of Western Red Cedar and they examine different aspects such as chlorophyll fluorescence and carbon dioxide exchange of the tree species. Another study by Allard *et al.* (2008) assesses the influence of more environmental factors such as humidity, rainfall, and soil heat flux on the evergreen tree species in France. They have observed climatic changes could influence the net carbon dioxide exchange of the evergreen tree forest (Allard *et al.* 2008).

In this study, the physiological, morphological, and biochemical characteristics of deciduous and evergreen tree species were being investigated. This study was a continuation of University of British Columbia's (UBC) ongoing sustainability project, which aimed to observe the carbon sequestration efficiency of tree species yearly. This study took place during a spring season in Vancouver, which was characterized by cold temperature, moderate precipitation, and low light intensity. The climate of this season was deemed unfavorable for the photosynthesis by trees, but from comparing the photosynthesis efficiency of these trees during this season to previous records can show how much the seasonal climate can have an impact on these trees. By comparing the photosynthesis rate, transpiration rate, respiration rate, and protein expression levels, the study also aimed to see which tree species had a better potential at reducing the atmospheric carbon dioxide level and at water use during a colder climate. The protein expression level was evaluated based on two proteins: rubisco, a major photosynthesis protein, and light-harvesting-complex IIb (LHC-IIb), the first protein in the photosynthesis pathway. The results could thus give insights to the importance of trees in resisting global warming and to see which tree species could be a better candidate for future forest tree planning.

## **2.0 Materials and Methods**

### **Species**

- Red maple (*Acer rubrum*)
- Red Oak (*Quercus rubra*)
- Western Red Cedar (*Thuja plicata*)
- Lawson Cypress (*Chamaecyparis lawsoniana*)
- Juniper (*Juniperus communis*)

All species are located on UBC Vancouver campus.

### **Sampling**

The tree species were being sampled once a week from January to March 2012. Two leaf samples were collected from each tree species and their petioles were kept in water before the Li-Cor reading. The morphology of the tree species as well as the leaf samples was documented using a digital camera. The light intensity reading was also recorded the same time as the samplings in the same spots every time near the tree species. The other environmental data such as temperature and humidity was obtained from weather websites (TheWeatherNetwork, 2012). Furthermore, A general observation of the weather and environment condition around the tree species was noted during the sampling process.

### **Li-Cor Photosynthesis System**

The Li-Cor Photosynthesis System 6200 was used to record the samples' transpiration rate and net photosynthetic rate (NPR). Detailed instruction on Li-Cor operation could be found in the BIOL 351 lab manual (2012). The sample filled half of the recording chamber (19cm<sup>2</sup>). The photosynthesis rate was measured after the samples were placed in front of a light box which shined a light source with 1000-1100cd intensity onto the leaf samples. A temperature adjuster was placed between the light box and the recording chamber so the temperature around the leaf sample was monitored and kept constant. On the other hand, the respiration rate was measured



when the leaf samples were in a dark condition in which the light box was switched off and a black cloth was placed around the recording chamber. This ensured that no light energy could be obtained by the leaf samples during the data collection. Throughout the data collection process, the leaf sample petioles were always immersed in water, so the sample could still actively undergo transpiration and photosynthesis.

After each week's Li-Cor reading, some leaves from the samples were collected and wrapped into an aluminum foil, then finally immersed in liquid nitrogen for at least 30sec. These samples were then further preserved in freezer for later analysis.

### **SDS Gel Electrophoresis and Western Blot**

SDS Gel electrophoresis and Western Blot were performed during the last week of the project. Two samples from each month and each tree species, a total of six samples from each tree species, were used in the SDS Gel electrophoresis analysis. The samples were first grinded into fine powder form, and then 50mg was used to mix with buffer. Each tree species' protein extract ran on one individual gel, so a total of three gels were run. The molecular markers were placed in lane 2 and 9, and the samples' protein extract were placed in lane 3 to 8. More details on the preparation and procedure of the SDS gel please refer to BIOL 351 lab manual (2012).

Western blot was performed only on the LHC-IIb of Western Red Cedar. Instruction on Western blot could be found in the BIOL 351 lab manual (2012).

### **Data Analysis**

The readings, net photosynthetic rate and transpiration rate, from the Li-Cor system were loaded onto Microsoft Excel for mathematical and graphical analysis. The Li-Cor system took three readings for each sample, and these readings were averaged. Since for each tree species two samples were used each week, the averaged reading values from each sample were again

averaged. The Li-Cor system only measured the net photosynthetic rate, so the respiration rate was obtained from taking the negative value of the averaged reading for samples in dark condition. The photosynthesis rate was obtained from the actual averaged values from samples in light condition. Each week's data was plotted using an excel line graph function.

### 3.0 Result

#### 3.1 Morphology

The two deciduous tree species Red Maple (*Acer rubrum*) and Red Oak (*Quercus rubra*) did not have any leaves during the month of January to March 2012 (Figure 1 and 2).



Figure 1. Red Maple in January, February, and March (left, middle, right).



Figure 2. Red Oak in January, February, and March (left, middle, right).

The three evergreen species Western Red Cedar (*Thuja plicata*), Lawson Cypress (*Chamaecyparis lawsoniana*), and Juniper (*Juniperus communis*) did not show much morphological changes in their leaves nor the trees themselves (Figure 3, 4, and 5). The size, shape, and color of the leaves stayed consistent throughout the data collection period. Moreover, there were noticeable brown spots in some leaves in the Western Red Cedar and Juniper, but not as much in the Lawson Cypress.



January

February

March



January

February

March

Figure 3. Western Red Cedar tree and leaf morphology. Pictures were taken from two different times during each month.



January

February

March



January

February

March

Figure 4. Lawson Cypress tree and leaf morphology. Pictures were taken from two different times during each month.



Figure 5. Juniper tree and leaf morphology. Pictures were taken from two different times during each month.

### **3.2 Photosynthesis Rate (Figure 6):**

The photosynthesis rate of Western Red Cedar did not vary as much as the other two species during the data collection period. The highest photosynthesis rate for Western Red Cedar was on March 2<sup>nd</sup>, which was  $1.10 \mu\text{mol}/\text{m}^2/\text{s}$ ; the lowest was on January 25<sup>th</sup>, which was  $-0.19 \mu\text{mol}/\text{m}^2/\text{s}$ . During the month of January and March, the photosynthesis rate for Western Red Cedar decreased and during the month of February, the rate increased. The overall trend of the photosynthesis rate for the Western Red Cedar stayed above zero. The photosynthesis rate of Lawson Cypress started as increasing from January then in the early February, the general trend started to decrease. On February 23<sup>rd</sup> and Mar 7<sup>th</sup>, there was a noticeable increase in photosynthesis rate for Lawson Cypress compared to the previous and subsequent recorded data. The general trend for photosynthesis rate for the Juniper was similar to Lawson cypress.

However, during the January to February increase, Juniper evidently increased more than Lawson Cypress and even Western Red Cedar. The photosynthesis rate of Juniper also stayed higher longer than Lawson Cypress and this could be seen on the Feb 13<sup>th</sup> data point, where the photosynthesis rate for Lawson Cypress already starting to decrease but for Juniper the rate still stayed high and positive. During the month of March, the photosynthesis rate for all three species decreased with Juniper decreased the most.

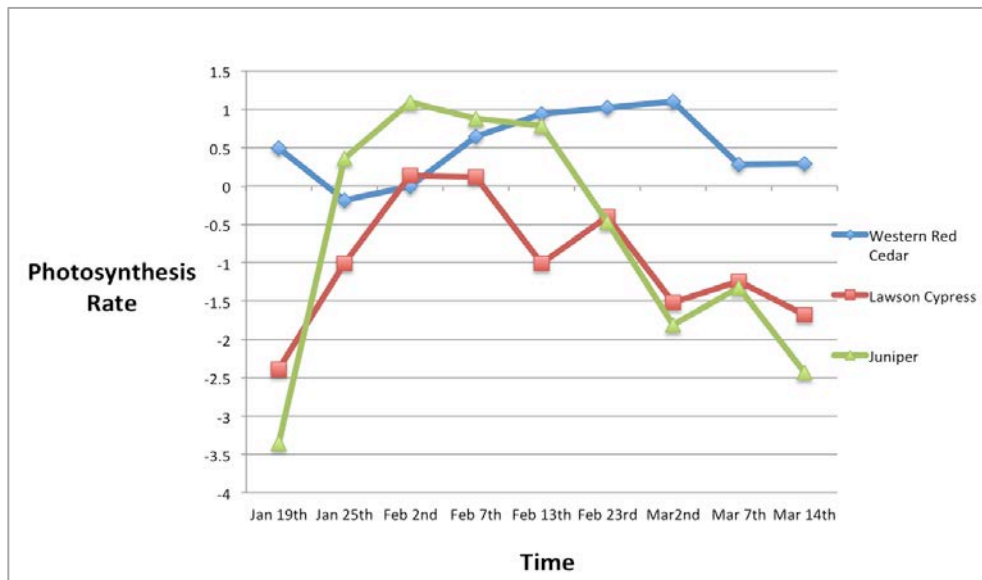


Figure 6. Photosynthesis rate of Western Red Cedar, Lawson Cypress, and Juniper from January to March. Each data point shown was the average of two samples' net photosynthesis rate measurements.

### **3.3 Respiration Rate (Figure 7)**

The general trends for respiration rate in three tree species were similar in that they stayed relatively constant during early February and started to increase during late February to March period. Of the three tree species, the respiration rate for Juniper was the highest, and for Western Red Cedar was the lowest. The trends for Western Red Cedar and Juniper were very similar; they both stayed relatively consistent in early February, then started to increase in late February (Feb23rd) and decrease after Mar 2<sup>nd</sup>. One exception was on Mar 14<sup>th</sup>, the respiration

rate of Western Red Cedar increased comparing to previous recorded data, but for Juniper, the rate was still decreasing. As for Lawson Cypress, its respiration rate stayed relatively constant in early February and started to show mild increase in late February to early March (Feb 13<sup>th</sup>-Mar2<sup>nd</sup>). The strongest increase in respiration rate was after Mar 2<sup>nd</sup> to the end of recorded data; interestingly, there was no considerable decrease in respiration rate for Lawson Cypress.

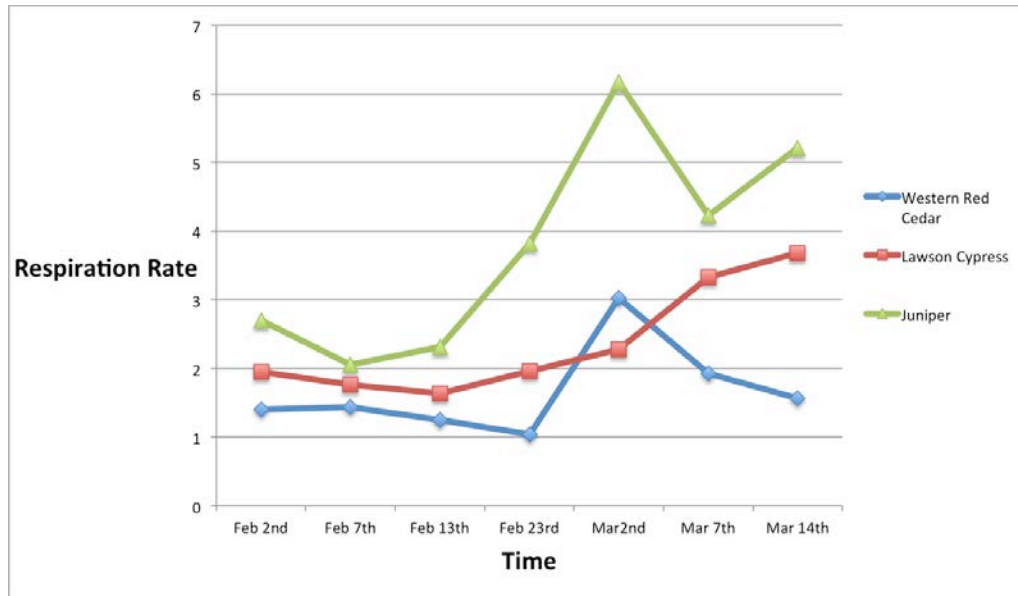


Figure 7. Respiration rate of Western Red Cedar, Lawson Cypress, and Juniper during January to March. Each data point was calculated by taking the negative of the averaged net photosynthetic rate from two samples measured in dark condition.

### **3.4 Transpiration Rate (Figure 8)**

The transpiration rate for Western Red Cedar was in general the lowest among the other species, and that for the Lawson Cypress was the highest. There was a noticeable increase in transpiration rate from Jan 19<sup>th</sup> to Jan 25<sup>th</sup> for all three tree species then the rate slowly decreased after Jan 25<sup>th</sup>. Both Western Red Cedar and Juniper’s transpiration rates had remained relatively constant during the month of February compared to Lawson Cypress because the Lawson Cypress transpiration rate decreased all the way from Jan 25<sup>th</sup> to Feb 13<sup>th</sup>, then sharply increased

after Feb 13<sup>th</sup>. From Feb 23<sup>rd</sup> to Mar 2<sup>nd</sup>, the transpiration rate for Western Red Cedar had a significant increase, while the rates for Lawson Cypress and Juniper only had a mild increase and decrease, respectively. The transpiration rate for Juniper, however, had its significant increase from Mar 2<sup>nd</sup> to Mar 7<sup>th</sup>, while the rates for the other two tree species had only mild changes. During Mar 7<sup>th</sup> to Mar 14<sup>th</sup>, the transpiration rate for Western Red Cedar had an obvious increase whereas the rate for Lawson Cypress had an opposite observation.

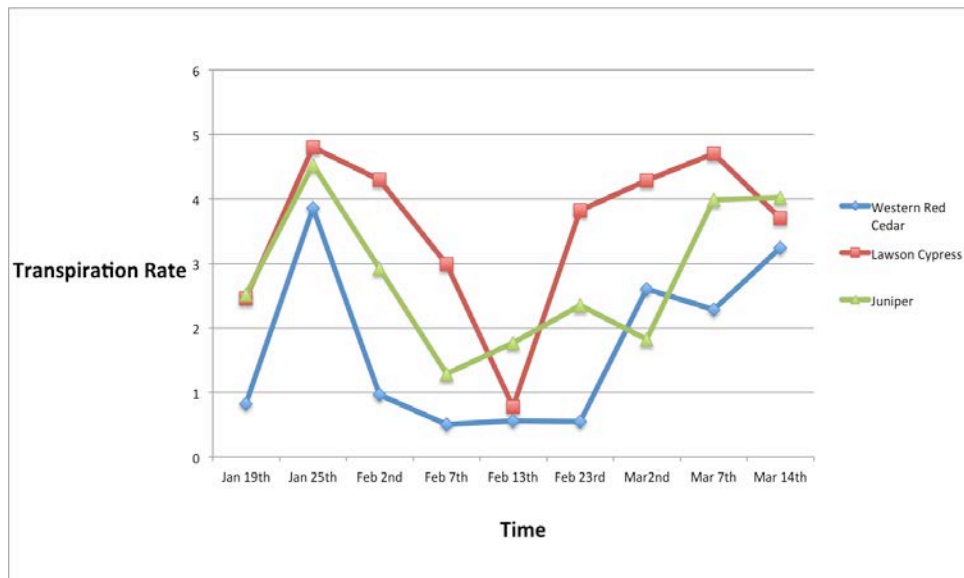


Figure 8. Transpiration rate of Western Red Cedar, Lawson Cypress, and Juniper during January to March. Each data point shown was the average of two samples' transpiration rate measurements.

### **3.5 Protein Expression Level**

In Juniper, the rubisco level was higher during late January to February compared to the expression level in early January and March (Figure 9). The trend for expression level of light-harvesting-complex IIb (LHC-IIb) was similar to that for the rubisco, but the overall expression level of LHC-IIb was lower than that for rubisco, which was evidenced by the overall lighter bands. In Figure 10, the rubisco and LHC-IIb expression level for Western Red Cedar stayed

relatively consistent throughout the collection period. Moreover, the rubisco expression level was higher than the LHC-IIb expression level, indicated by the more intense colored bands for rubisco. The rubisco expression level for Lawson Cypress was higher in the January to early February period, then decreased in the late February to March period (Figure 11). The LHC-IIb expression level for Lawson Cypress was generally very low, only the early February and late March sample seemed to be a little higher than the rest (Figure 11). Within the three evergreen species, the Western Red Cedar samples had the highest and most consistent protein expression level in both proteins.

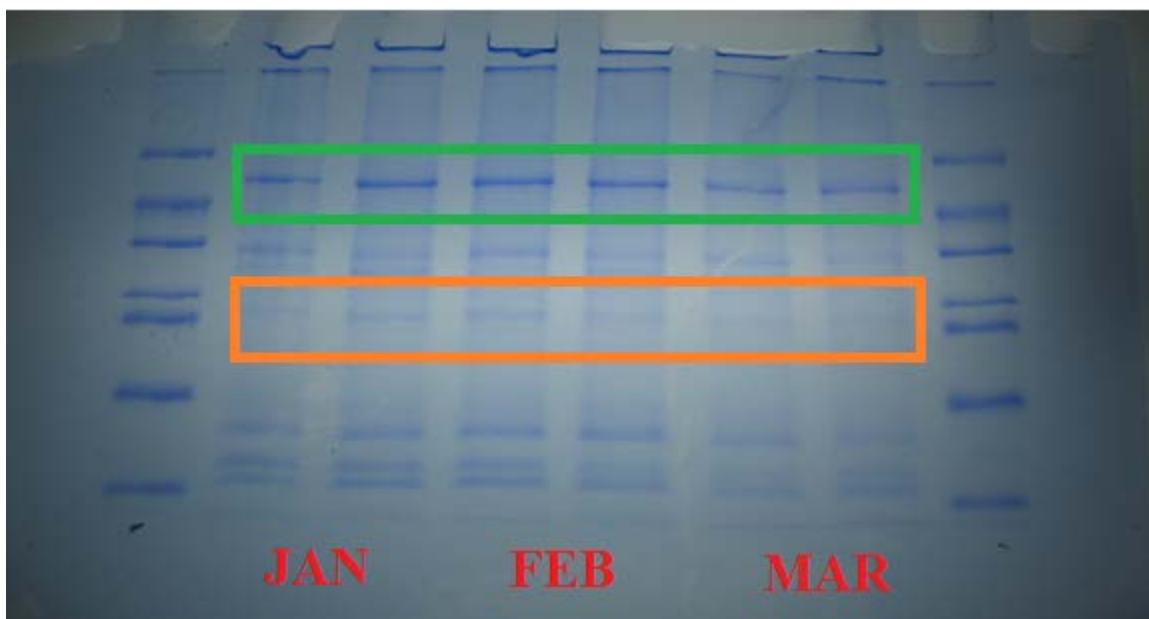


Figure 9. SDS-PAGE gel product of Juniper. Green box represents rubisco level and orange box represents LHC-IIb level.



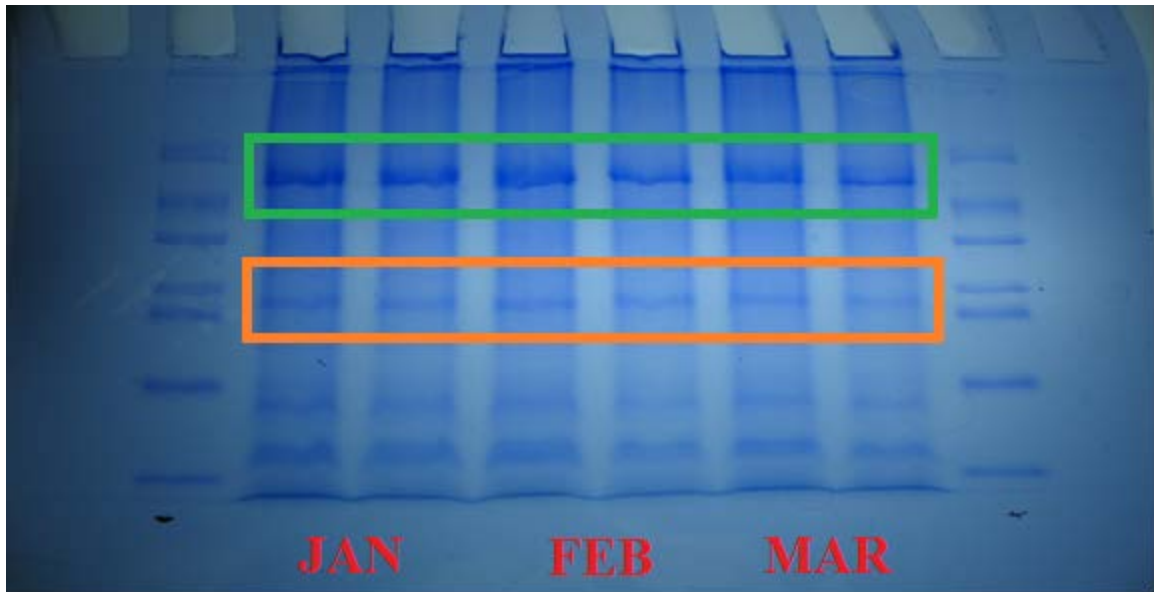


Figure 10. SDS-PAGE gel product of Western Red Cedar. Green box represents rubisco level and orange box represents LHC-IIb level.

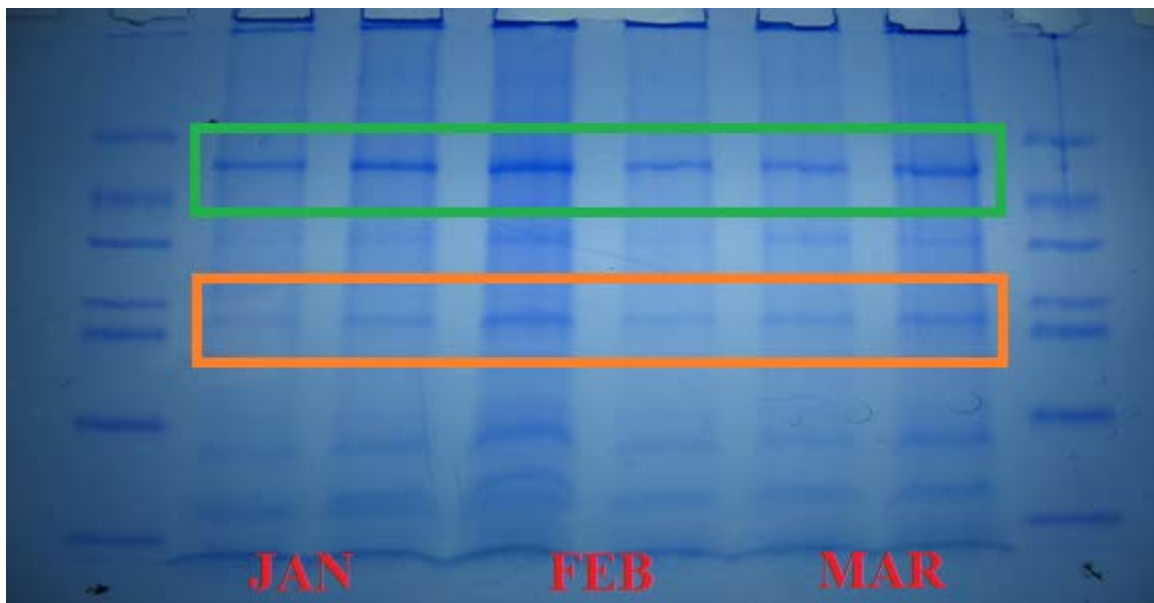


Figure 11. SDS-PAGE gel product for Lawson Cypress. Green box represents rubisco level and orange box represents LHC-IIb level.

## **4.0 Discussion**

### **Morphology**

The deciduous tree species did not grow out any leaves during the January to March period. This might due to the cold weather in the Vancouver area during the spring season because in Chen *et al.*'s research (1999), they concluded the spring air temperature could affect the leaf emergence timing in deciduous trees. The morphology of the trees and leaves for the evergreen species stayed consistent during the spring season. Nevertheless, there were brown spots noted on the leaf samples for all three evergreen species, which suggesting the three evergreen species were undergoing slight leaf senescence during the spring season in Vancouver.

### **Photosynthesis rate**

Of the three evergreen species, the Western Red Cedar had the best overall net photosynthesis rate (Figure 6). For most of the data collection days, Western Red Cedar engaged in positive photosynthesis whereas the other two species had at least half of the times having negative net photosynthesis rate (Figure 6). The net photosynthesis rate was calculated by subtracting the respiration rate from photosynthesis rate. Photosynthesis meant reduction in atmospheric carbon dioxide level and respiration meant increase in atmospheric carbon dioxide level. Having a positive net photosynthesis rate showed the species was photosynthesizing more than respiring and having a negative net photosynthesis rate showed the species was respiring more. Therefore, the Lawson Cypress and Juniper were engaging in respiration for at least half of the data collection period. Furthermore, the conclusion on whether Lawson Cypress or Juniper has a better photosynthesizing ability during spring season was hard to decide because both species had very similar trend and values in net photosynthesis rate (Figure 6).

All of the evergreen species had a relatively higher net photosynthesis rate during Jan 25<sup>th</sup> to Feb 23<sup>rd</sup> (Figure 6), which might be resulting from warmer temperature, higher light intensity, and high air humidity during this time period (TheWeatherNetwork, 2012). This was in agreement with the observation in Matyssek's (1986) study that these environmental factors, in a nonlimiting climatic condition, could aid in the photosynthesis process for trees. During the other time periods, the Lawson Cypress and Juniper had decreased photosynthesis rates (Figure 6) this might be the result of lower temperature and lower light intensity during these time periods. The result that Western Red Cedar could maintain its relative photosynthesis rate during these times inferred that Western Red Cedar might be more resistant to colder climates than the other two evergreen tree species.

Since this study was the continuation of an ongoing project, the photosynthesis rate of the evergreen Western Red Cedar obtained was compared with the previous results in summer and fall season (Figure 12, Singh *et al.*, 2012). The February to March period was when the second highest photosynthesis rate (highest being the October period) occurred, and at the end of March, the photosynthesis rate was almost as high as the maximum photosynthesis rate of the deciduous species Red Oak (Figure 12). This suggested the evergreen Western Red Cedar could have a significant contribution to the year-round carbon sequestration, especially a highly efficient photosynthesis rate during the spring and fall. When comparing to the photosynthesis rate of deciduous species, the evergreen species Western Red Cedar did have a better efficiency than the deciduous species even during springtime.

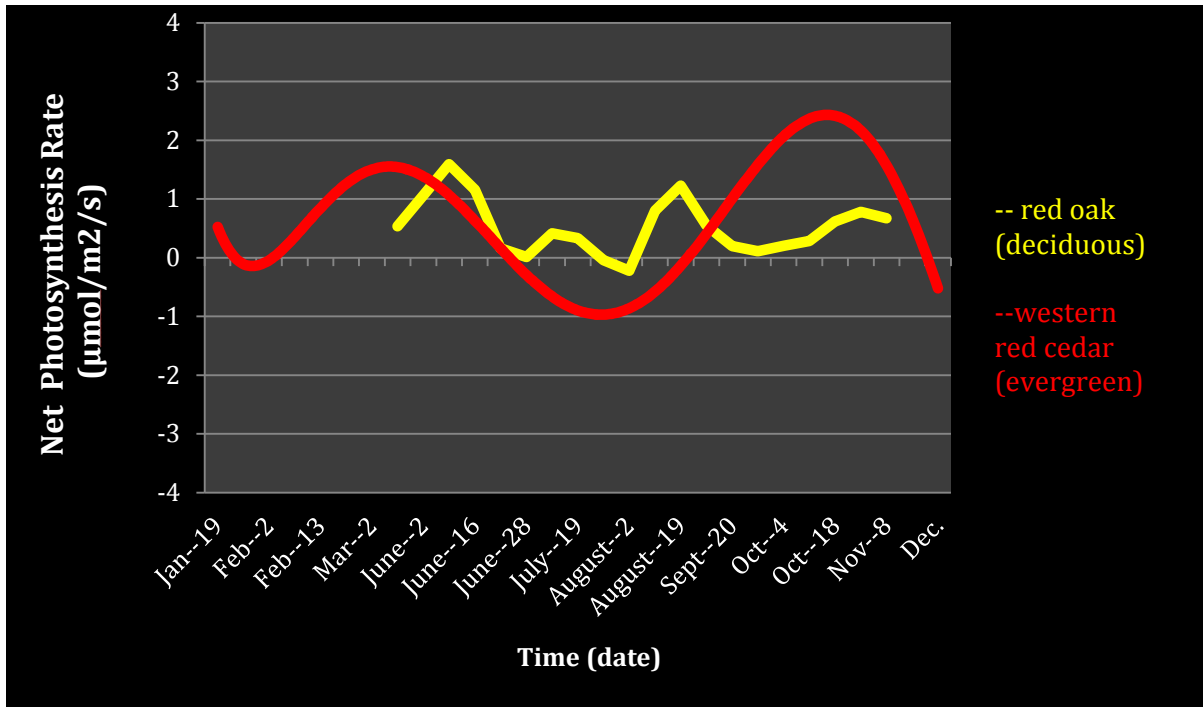


Figure 12. Net photosynthetic rate of Red Oak and Western Red Cedar on a one-year base. June 2 to December data collected from previous studies (Singh *et al.*, 2012).

### Respiration rate

The respiration rate of the Western Red Cedar in general was the lowest among the three evergreen species (Figure 7). The Lawson Cypress respiration rate trend was noted with a milder change from week to week comparing to the other two species which had rapid increase or decrease rates. This indicated that the respiration rate of Western Red Cedar and Juniper might be more sensitive to environmental changes. There was a significant increase in respiration rate on Mar 2<sup>nd</sup> for the Western Red Cedar and Juniper. This might be resulting from low temperature (4°C) and/or low light intensity (105.38cd) on Mar 2<sup>nd</sup> (TheWeatherNetwork, 2012). In Ryan's (1991) research, he found that tree species could have a higher respiration rate under low temperature environment; moreover, he also mentioned few other factors that can affect the respiration rate of tree species such as water stress, protein concentration, and air pollution.

### **Transpiration rate**

The transpiration rate on Jan 25<sup>th</sup> of all three tree species had increased remarkably compared to the previous date (Figure 8). This might be the result of warmer temperature (Jan 19<sup>th</sup>: -3.3 vs. Jan 25<sup>th</sup>: 6.4) because higher temperature could increase the evaporation rate. However, on Jan 25<sup>th</sup>, there was heavy rainfall compare to no rainfall in the previous or later dates which seemed to contrast with the higher water loss rate because the high ambient moisture should slow the water evaporation rate.

During February, Western Red Cedar and Juniper had relatively a steady transpiration rate trend, yet the Lawson Cypress had sharper increase or decrease during this period. The environmental aspects of this time period was very similar - temperature around 6 °C, not much rain fall, but relatively humid air. The more dramatic changes observed in Lawson Cypress might be that its transpiration mechanism was more sensitive to environmental changes.

The transpiration rate from in this study was compared with the previous data in Figure 13. The transpiration rate of Western Red Cedar in springtime was relatively moderate comparing to other seasons', but still much higher than that of the deciduous tree species. Therefore, in comparison, despite the Western Red Cedar had a more efficient transpiration mechanism, lower transpiration rate, among the three evergreen species in this study, the evergreen tree species still had an overall higher transpiration rate than the deciduous species.

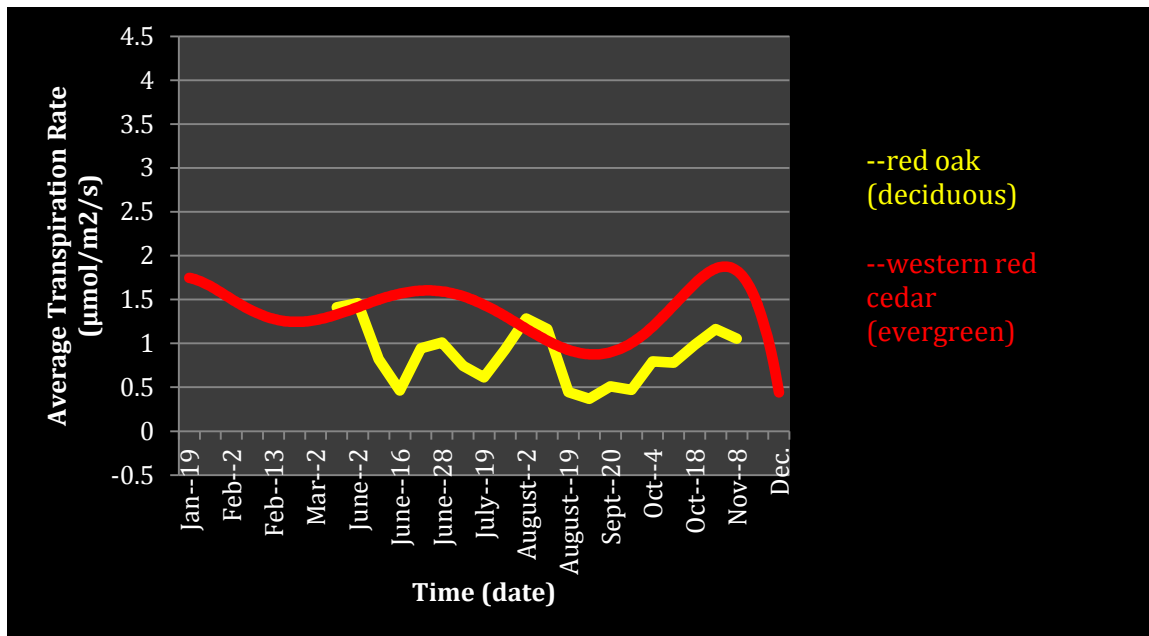


Figure 13. Transpiration rate of Red Oak and Western Red Cedar on a one-year base. June 2 to December data collected from previous studies (Singh *et al.*, 2012).

### Protein Expression Level

The results from the SDS-PAGE gel were consistent with the photosynthesis rate results. The Western Red Cedar had more consistent and intense rubisco and LHC-IIb throughout the spring season than the other two evergreen species. This provided more support to the observation that the Western Red Cedar had a better photosynthetic efficiency than that of the other two evergreens in this study. The Lawson Cypress and Juniper both had higher protein expression around the late January to February period, which was consistent with the increase in photosynthesis rate during that time period.

### 5.0 Conclusion

During the springtime, the evergreen tree species had definite advantage on photosynthesis than the deciduous tree species because they did not have leaf abscission as the deciduous trees do. Of the three evergreen species in this study, the Western Red Cedar showed

an overall better efficiency on carbon sequestration during the springtime. Nevertheless, deciduous tree species should not be excluded from tree forest planning just because the evergreen had a better overall efficiency at reducing atmospheric carbon dioxide. The reason was that, in a community with higher biodiversity, the species could compliment each other better, and deciduous trees could also provide other benefits such as fertilizing soil with their fallen leaves than photosynthesis.

### **6.0 Possible Errors and Future Study**

The sample petioles were not cut under water in earlier experiment, which might cause air bubble in the petiole blocking the water transportation. In the Li-Cor chamber, the leaf sample should fill up half of the chamber, this was difficult to achieve because of the evergreen species' needle-shape leaves, so there was some overlapping and gapping between the leaves. The background stain intensities for the gel products were different, so the interpretation of the band intensity was subjective to the interpreter. Future experiments involving more variety of tree species could be done to continue on finding better sustainable candidates for reducing atmospheric carbon dioxide. Larger scale experiments such as observing the correlational relationship between carbon sequestration efficiency of trees and global average temperature and atmospheric carbon dioxide level would also give insightful knowledge for further experiments as this might provide more understanding on the tree species' impact on global warming.

### **7.0 Acknowledgement**

Special thank you to Mr. Jarnail Mehroke and Dr. Santokh Singh for their guidance and immense help throughout this study. Thanks to Brenda Sawada from SEEDS program for insights to the program. Thanks to Teale and Harleen for their great advice and their data from previous studies. Finally, thanks to all the volunteers that had helped with the data collection.

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