**Photosynthesis and Transpiration Rate as Indicator of Carbon Sequestration and the Effect of Three Hormones: Jasmonic Acid, Ethylene and Cytokinin on Leaf Senescence of an Evergreen *Thuja plicata* (Western Red Cedar)**

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**Abstract**

The objective of this study was to measure photosynthesis and transpiration rate of *Thuja plicata* as indicators of carbon sequestration through environmental and seasonal changes. Photosynthesis and transpiration rates were measured automatically by CI-340 Handheld photosynthesis machine from November 10, 2017 to March 28, 2018. Our hypothesis matched our results that both photosynthesis and transpiration rates decrease during winter compared to fall and spring. Photosynthesis and transpiration rates were highest in the beginning of November and they both dropped by about 20% in winter, reaching lowest in middle of January. Another objective of this study was to observe whether the presence of exogenous hormones ethylene (CEPA), cytokinin (BAP) and jasmonic acid (JA) promotes leaf senescence in the evergreen *Thuja plicata*. 5mL of 1uM, 10uM, and 100uM concentration stock solutions of each hormone and 0.7-0.8 grams of leaflets were added to a total of 40 petri dishes; samples were taken from each plate once a week for up to 6 weeks. We hypothesized that JA and CEPA promote leaf senescence and BAP prevents leaf senescence. It was observed that JA was the first hormone to cause leaf senescence and CEPA was the second hormone that caused leaf senescence, and in both cases chlorophyll and proteins were degraded as indication of leaf senescence; BAP prevented leaf senescence in the first 3 weeks but at the end of week 6, 100uM BAP induced leaf senescence.

**Introduction**

Atmospheric Carbon dioxide ($CO_2$) has been increasing in the past decade due to burning fossil fuels, deforestation and over population, which is causing big issues and negatively effecting the environment. Global warming can affect the growth of tissue composition, photosynthesis, and respiration of plants (Hughes, 2000). Species might have to migrate to a different location due to the change in the climate temperature and the environmental changes (Hughes, 2000). Also, the phenological relationship of species that lived once together is affected (Hughes, 2000). $CO_2$ and other greenhouse gases trap heat and cause climate changes; therefore, increase in $CO_2$ will result in an increase in global temperature (Hansen et al., 2006). The mean global temperature has increased by 0.3-0.6 $C^O$ over the last century (Houghton et al., 1992). Atmospheric $CO_2$ can be returned to the carbon pool through photosynthesis and it can be stored in plants biomass (Lorenz & Lal, 2009). It is important to keep in mind that carbon sequestration cannot affect the atmospheric $CO_2$ increase that is a result of anthropogenic activities; but growing trees

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can strengthen and enhance carbon fluxes into forest carbon pools, reducing the risks of abrupt climate change (Lorenz & Lal, 2009).

Photosynthesis is the conversion of $CO_2$ using light and water into organic compounds, such as carbohydrates (Najafpour et al., 2017). These carbohydrates can be used as nutrient and energy for the plant. Photosynthesis and transpiration are closely related because carbon assimilation involves plants losing water (Krober and Bruelheide, 2014). Transpiration is the movement of water through the plant and its evaporation. Plants use transpiration to get the water needed for survival and to perform reactions such as photosynthesis.

In this study, the environmental regulation and seasonal changes of western red cedar (*Thuja plicata*), a conifer native to North America, was studied. Western red cedar is native to cool habitats, it is adapted to the low temperature environment and it can photosynthesize efficiently. Plants can control $CO_2$ uptake by controlling stomata and the various partial photosynthetic processes in the chloroplast (Öquist, 1983). Stomata are controlled by environmental variables such as light, temperature, $CO_2$, and humidity (Öquist, 1983). Temperature can affect the metabolism of guard cells through effecting plant water balances and causing differences in water vapor pressure between intercellular space of the leaf and the ambient air (Öquist, 1983). The rate of photosynthesis decreases in winter but with the increase in air temperature only for a few days, the rate of photosynthesis is recovered (Tanja et al., 2003). Low temperature increases the amount of intercellular $CO_2$ concentration due to reduction of photosynthesis, and stomata tend to close when the concentration of $CO_2$ is high (Öquist,

1983). Therefore, it can be said that reduction in temperature results in stomatal closure and a reduction in transpiration and photosynthesis rate.

Reducing photosynthesis rate is also part of the survival mode that the plant goes through. Adaptation of evergreens to annual temperature changes between summer and winter is by developing physiological changes that allows them to modify their growth and dormant periods based on environmental changes (Öquist & Huner, 2003). During poor conditions, evergreens can maximize their carbon gain and nutrient efficiency (Öquist & Huner, 2003). During harsh conditions such as winters, evergreens build thick cell walls to protect themselves from winter damage and they will repair any metabolic disturbances that winter might have caused for them (Öquist & Huner, 2003). Over-excitation of photosystems can cause permanent damage to the photosynthetic apparatus; therefore during winter when photoperiods and temperature are reduced, evergreens down regulate photosynthesis and will undergo cold hardening to prevent photo-damage (Wong & Gamon, 2015).  As spring approaches, the temperature increases and photoperiods get longer, evergreens go through de-hardening and so photosynthesis is recovered (Wong & Gamon, 2015).

In order to adapt to the new cold temperature, biochemical differentiations in lipids, carbohydrates, proteins, amino acids and expression of genes are made (Öquist & Huner, 2003). During winter, evergreens change their lipid composition, membrane structure and sugar concentrations due to cold hardening; these changes result in stomatal closure, inhibition of gas exchange and inactivation of Calvin cycle enzymes (Tanaka, 2007). Chlorophylls harvest light energy but this energy cannot be used in photosynthetic reaction and so it can form lethal reactive oxygen species (Adams et al., 2004).

Evergreens can survive the lethal reactive oxygen species because their photosystem II light harvesting system can dissipate the excess absorbed light energy (Horton and Ruban, 2004).

One important protein in the photosynthesis process is the Light Harvesting Complex, which transfers light energy to chlorophyll, which is located at the reaction center of the photosystem (Horton and Ruban, 2004). Another important protein that participate in the photosynthesis process is the enzyme ribulose-1,5- bisphosphate carboxylase/oxygenase (RubisCO). RubisCO catalyzes carboxylation of ribulose-P2 to create two P-glycerate, in order to be used in Calvin cycle for making glucose in the plant (Roy & Andrews, 2000). Another important protein is the D1 protein; this protein, which is part of the reaction core of Photosystem II (PSII), receives electrons from the Light Harvesting Complex (LHC IIb), and converts $CO_2$ to sugar using those electrons (Kawamori et al., 2005).  Therefore, protein and chlorophyll analysis can help observe environmental and seasonal changes in western red cedar.

In this study, the effect of jasmonic acid, cytokinin and ethylene on leaf senescence was also studied. Leaf senescence is a deterioration process in which the cell structure, gene expression and metabolism of the cells change and lead to death (Lim et al., 2007). Leaf senescence does not only happen with aging; other factors such as stress or leaf detaching can cause leaf senescence (Jiang et al., 2014). One of the primary steps in leaf senescence is the breaking down of chloroplast that contains 70% of the leaf protein (Lim et al., 2007). During leaf senescence photosynthesis is replaced by catabolism of protein, membrane lipids, chlorophyll and RNA (Lim et al., 2007). The

second objective of this study is to observe whether exogenous hormones, JA, BAP and CEPA, will result in early leaf senescence in the evergreen *Thuja plicata*.

Plant hormones can affect leaf senescence (Liu et al., 2016). Ueda et al (1981)., was the first to report that leaf senescence is accelerated by methyl jasmonate. Jasmonic acid (JA) or methyl jasmonate (MeJA) regulates expression of various senescence-associated genes and causes senescence phenotypes, such as degradation of chlorophyll and reduction in the level of RubisCO (Jiang et al., 2014). Methyl jasmonate increases the transcription of senescence developing genes such as SEN4, ERD1 and the senescence-associated gene SAG21 (Liu et al., 2016). Jasmonic acid and the transcription of genes that are involved in jasmonic acid synthesis increases during leaf senescence, suggesting that JA promotes leaf senescence (Liu et al., 2016). Similarly, ethylene accelerates leaf senescence but in order for ethylene to cause leaf senescence, it has to be coupled with age-dependent factors, such as OLD genes (Lim et al., 2007). Gan and Amasino (1995) suggested that Cytokinin prevents senescence. During leaf senescence, cytokinin biosynthesis is down regulated and its degradation is up regulated (Liu et al., 2016).

During leaf senescence, chlorophyll is degraded, which results in the loss of the greenness of the plant (Liu et al., 2016). Lipid peroxidation during leaf senescence results in the deterioration of the membrane, increasing membrane permeability, resulting in more deterioration (Liu et al., 2016). Photosynthesis is reduced during leaf senescence because translation and transcription of CAB2 gene and RPS protein decrease and expression of senescence associated genes (SAGs) increase (Liu et al., 2016).

The purpose of this study was to measure the photosynthesis and transpiration rate as indicators of seasonal and environmental regulations and to observe the effect of jasmonic acid, cytokinin (BAP) and ethylene (CEPA) in the *T. plicata* leaf senescence. We hypothesized that the photosynthesis and transpiration rates would decrease during winter compared to fall and spring; and that JA and ethylene would result in early leaf senescence compared to the cytokinin treatments.

**Materials and Methods**

**Environmental and seasonal changes experiment:**

**Sample collection and experimental setup**

Samples from two trees of *T. plicata* species located on UBC campus were taken. The tree closer to main mall was designated as tree number two and the other one was designated as tree number one. Each week, 2 branches of leaves were cut from each tree and located in water. To measure photosynthesis and transpiration rate, CI-340 Handheld photosynthesis system was placed on an open bench space, with a water filled glass container 16 cm in front of it and then a light box 16cm in front of the water container. An 11-cm2 chamber was placed for the CI-340 Handheld photosynthesis system. Each leaf was placed in the chamber while submerged in water and 5 measurements were taken for each sample, totaling 20 measurements per week. The machine automatically calculates the photosynthesis and transpiration rate by measuring water vapor and $CO_2$. Samples were frozen with liquid nitrogen stored in the freezer for later chlorophyll and protein measurement.

**Chlorophyll analysis**

2mL of DMF was added to 0.02 gram of western red cedar leaf and placed on the shaker covered by aluminum foil overnight. In order to completely extract all the chlorophylls from the tissues, the samples were grinded. Using the Spectronic 20D spectrometer, the absorbance rate of the extracts was measured at 647 and 665nm and the chlorophyll content were measured using a formula according to the laboratory manual (Singh, 2016).

**Protein analysis**

Samples from different weeks were run through a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the laboratory manual (Singh, 2016). In order to observe the presence and quantity of RubisCO in those dates, a western blot procedure was performed according to the laboratory manual (Singh, 2016).

➢ **The leaf senescence experiment:**

**Sample collection and experimental setup**

Leaves from both of the *T.plicata* trees were collected. Thanks to Mr. Jarnail Chandi, for $10^{-2}$M BAP, CEPA and JA stock solutions, solutions were made in 1uM, 10uM and 100uM concentrations. 2 drops of Tween 10 with the concentration of 1:10 were added to each 25ml of solution. 40 medium sized petri dishes were used. Two layers of filter paper were located in each petri dish. 5ml of each solution were added to each petri dish. 10 petri dishes were made for each week, totalling 40 dishes: 1uM BAP, 10uM BAP, 100uM BAP, 1uM CEPA, 10uM CEPA, 100uM CEPA, 1uM JA, 10uM JA, 100uM JA. Leaves were washed with distilled water and 0.8-0.9 grams of leaflets were added to each petri dish plus 5mL of the solutions. The control contained 5mL of distilled water. The plates

were sealed using parafilm. The petri dishes were stored in the dark growth chamber at $24^O$ C covered by an aluminum foil. Every week, one sample was taken from each treatment, frozen with liquid nitrogen and stored for further protein and chlorophyll analysis. Chlorophyll analysis and protein analysis were conducted similar to previous experiment.

**Results**

**Part I: Environmental and seasonal changes**

Photosynthesis and Transpiration rates

According to Figure 1.1, net photosynthesis rate of western red cedar was highest in November $22_{nd}$ at around 4.3 umols/$m^2$ and lowest in January 17 at around 0.8. The mean temperature is also highest in November $22^{nd}$ at $14^O$ C and lowest in March $2^{nd}$ at $2C^O$. It is noticeable that when temperature drops from 14 to 5 degrees Celsius in November $22^{nd}$ to $29^{th}$, the average net photosynthesis rate also drops from 4.3 to 3 umols/$m^2$ (Figure 1.1). Both mean temperature and average net photosynthesis rate stay low during winter and only show minor fluctuations (Figure 1.1). The temperature in February $5^{th}$ is 1.5 times more than in January $31^{st}$ and it is seen that the net photosynthesis rate in February $5^{th}$ is 1.47 times more than in January $31^{st}$ (Figure 1.1). From February $5^{th}$ to $16^{th}$ the mean temperature dropped by $1.2^o$ C and the average net photosynthesis rate also dropped by 1.2umols/$m^2$/s It is also observed that at the end of March both mean temperature and net photosynthesis rate are increasing (Figure 1.1). The temperature starts increasing again from March $2^{nd}$, while net photosynthesis rate starts increasing from February $16^{th}$. Throughout November until March, the average photosynthesis rates were above zero (Figure 1.1).

The average transpiration rate was highest at 0.7 in November $10^{th}$, and then it gradually started decreasing and reached its lowest 0.14 in January $17^{th}$ (Figure 1.2). From January $17_{th}$ to February $5_{th}$, the transpiration rate increases gradually similar to photosynthesis rate (Figure 1.2). From February $5^{th}$ and $16^{th}$, the transpiration rate drops by 0.7 $mmol/m^2/s$, similar to net photosynthesis rate and the mean temperature (Figure 1.2). After February $16^{th}$ transpiration rate starts increasing similar to net photosynthesis rate until March $28^{th}$ (Figure 1.2).

Protein analysis

Results from SDS-PAGE shows that all bands from November $10^{th}$ to February $16^{th}$ show proteins approximately weighting at 50, 30 and 25 KDa (Figure 1.3). Due to the presence of high quantity of proteins, the bands are dark blue and the intensity of proteins in each sample does not differ significantly among samples (Figure 1.3). Results from Western Blot shows that the protein RubisCO is present in the samples of December $14_{th}$ onwards but absent in samples from November $10^{th}$, $22^{nd}$, $29^{th}$ and December $7^{th}$ (Figure 1.4). Figure 1.4 suggests that the intensity of the protein RubisCO increased in January $31^{st}$, February $5^{th}$ and $16^{th}$ because the bands were bolder compared to other bands (Figure 1.4).

Chlorophyll analysis

Results from chlorophyll analysis showed no significant difference between the total amounts of chlorophyll in the tissues of the samples from November $10^{th}$ to March $2^{nd}$ (Figure 1.5). The chlorophyll content in the tissues fluctuated between 1.8 and 1.2 mg/ g tissue (Figure 1.5). Also, the leaves taken from November $10^{th}$ to March $28^{th}$ look very much similar (Table 1.2).

**Part II: Hormones and leaf senescence**

BAP treatments

According to Table 2.1, BAP did not cause leaf senescence in the samples until week 4. In week 4, BAP 10uM and 100uM treatments, minor leaf senescence is observed, as the middle part of the leaflets had turned brown. In week 6 samples, there are darker brown sections showing more senescence compared to previous week. During the chlorophyll analysis of week 3, BAP did not show any chlorophyll degradation compared to the control (Figure 2.1). In week 6 chlorophyll analysis, BAP in 10uM and 1uM concentrations showed more chlorophyll content than control of week 6 but less chlorophyll content than week3 control (Figure 2.2). According to Figure 2.3, BAP did not show any protein degradation or showed minor protein degradation compared to week3 control. In week6, major protein degradation in BAP treated samples is observed in SDS-PAGE results, especially in 1uM and 100uM concentrations (Figure 2.4).

CEPA treatments

CEPA did not show major leaf senescence until week 4; during week 4 some browning was observed on the leaflets. In week 3, CEPA did not show chlorophyll degradation because the chlorophyll content was similar to week3 control (Figure 2.1). In week 6, CEPA showed chlorophyll degradation in 10uM and 100uM concentrations but not in 1uM concentration (Figure 2.2). The protein content in week 3 CEPA samples did not defer from the control (Figure 2.3). Week 6 CEPA samples showed slightly less

protein content than week 6 control but the protein degradation was less than JA and BAP (Figure 2.4).

JA treatments

JA showed small leaf senescence and browning in some parts of the leaflets in week 1 at 100uM concentration but not in other concentrations. In week 2, leaf senescence was observed in small areas in 10uM concentration and fully in 100uM concentration. In week 3, JA 100uM concentration showed brown liquids in the dish, which are secreted sugars from the tissues. In week 4, sugar secretion was observed in both 10 and 100uM concentrations and so the dishes were changed, and in week 6 the leaves had turned dark brown in these 2 treatments. JA 1uM concentration showed very minor leaf senescence in week 6. In week 3, JA in 10 and 100uM concentrations showed 40% less chlorophyll content than week 3 control and JA 1uM had similar chlorophyll content to the control of that week (Figure 2.1). JA 10uM and 100uM treatments showcased more chlorophyll degradation than other treatments in week 3 (Figure 2.1). In week 6, JA 10uM and 100uM had less chlorophyll content than week 3 and it was less than week6 control but to a lesser degree and JA 1uM chlorophyll content was similar to the control of week 6 (Figure 2.2). No difference in the protein content of week 3 was observed in JA treatments in all concentrations compared to the control of week 3 (Figure 2.3). Major protein degradation was seen in JA 10uM and 100uM concentrations because the SDS-PAGE bands were much lighter than the control of week 6 (Figure 2.4).

Comparing controls from week 0 to week 6, the leaves started to appear lighter after week 3 onwards but no browning was observed. Chlorophyll contents decreased in the tissues when comparing week 0, 3, and 6; week 3 chlorophyll content was 0.3 mg/g tissue less than week 0 and week 6 chlorophyll content was 0.2 mg/g tissue less than week 6 (Figure 2.2 and 2.3). Protein content in the tissues did not show noticeable degradation in week 3, but in week 6 the SDS-PAGE was brighter than week 0 control (Figure 2.3 and 2.4).

**Conclusion**

The measured transpiration and photosynthesis rate of western red cedar throughout different seasons were expected to be lower in winter compared to fall and spring (Figure 1.1 & 1.2). According to Hawkins et al (1995), and Öquist (1983), transpiration and photosynthesis rate reduces during winter as the temperature drops. The reason for the reduction in photosynthesis and transpiration rate during winter is the effect of temperature on stomata. During cold temperature, signals cause stomatal closure and as a result, transfer of water vapor and $CO_2$ is reduced (Öquist, 1983). Since the gas exchange and transpiration are dependent on stomatal opening and closure, then it can be concluded that stomatal closure will reduce atmospheric intake, reducing photosynthesis and transpiration rates.

The SDS-PAGE results showed that the proteins with molecular weights around 50KDa, 30KDa and 25KDa are present in all the samples from November to March (Figure 1.3). RubisCO consists of a large 56KDa and a small 14KDa subunit (Ma, 2009). According to Efimov et al (1994), D1 protein is 33.5 KDa. The light-harvesting complex

has a molecular weight of 26 KDa (Sigrist and Staehelin, 1994). Therefore, bands observed in Figure 1.3 suggest that RubisCO, D1 and light harvesting complex are present from November to March. The presence of photosynthetic proteins in the samples, support our other results that western red cedar photosynthesizes even during winter. The average photosynthesis and transpiration rate data indicate that western red cedar photosynthesizes throughout different seasons (Figure 1.1, 1.2). Our western blot results were different as expected (Figure 1.4). We had expected to see RubisCO in the samples of all dates but RubisCO seems not to be present in November and December 7th (Figure 1.4). We suggested that this might be due to the degradation of RubisCO from our initial samples that proteins were extracted from.

Results showed that there was no chlorophyll degradation throughout different dates from November to March (Figure 1.5). According to Maslova et al (2008), gymnosperm plants show no significant chlorophyll degradation during winter. Similar to our studies result, Hawkins et al (1995), did not find significant difference in the chlorophyll content of western red cedar through different freezing temperatures. Results showed no difference in the chlorophyll content of the samples from different dates, which is as expected because western red cedar are evergreen trees and have chlorophyll content all year round.

In the hormonal and leaf senescence experiment, cytokinin (BAP) showed minor leaf senescence in week 4 and small parts of browning in week 6. We had hypothesized that no leaf senescence would be observed in the BAP treatments. According to Gupta et al (2012), BAP is a senescence retardant, which also reduces chlorophyll loss and chlorophyllase activity. There was no chlorophyll degradation in week 3 and all three

concentrations showed similar or more chlorophyll content than the control (Figure 2.1, 2.2). Wojtania and Wegrzynowicz (2012) indicated that BAP can cause slow senescing in species that is not susceptible to leaf yellowing and first symptoms of leaf senescence can be observed. Therefore, it may be suggested that 100uM concentration of BAP can cause leaf senescence but lower concentrations are senescence retardant. Gan and Amansino (1996) proposed that the application of exogenous cytokinin, delays the degradation of chlorophyll and photosynthetic proteins. Our results indicated that BAP degraded proteins in week 6 but it did not affect proteins in week 3 (Figure 2.2); these results may purpose that 6 weeks is enough time to observe the slow leaf senescing and protein degradation by BAP in species such as western red cedar that are not susceptible to leaf yellowing.

Ethylene, CEPA, showed leaf senescence after JA, in week 4. Ueda and Kusaba (2015), suggested that ethylene plays an important role in causing leaf senescence because in dark incubation ethylene synthesis was promoted and in light ethylene treatments induced senescence. Strigolactone biosynthesis and strigolactone mutants delayed senescence, suggesting that they are hyposensitive to ethylene. Strigolactone enhances the action of ethylene in promoting leaf senescence (Ueda & Kusaba, 2015). In week 3, CEPA did not show degradation of chlorophyll but in week 3, major chlorophyll degradation was seen in week 6 (Figure 2.1, 2.2). Ueda and Kusaba (2015) mentioned how ethylene resulted in the senescence syndrome, which refers to degradation of chlorophyll, chloroplast structure, concomitant lipids, and photosynthetic protein. We did not see as much protein degradation in CEPA treatments as we did in JA and BAP treatments, but the bands are still lighter than the control (Figure 2.4). Our results were

as hypothesized and Gupta et al. (2012), also proposed that ethylene promotes chlorophyllase activity, promoting chlorophyll degradation and leaf senescence.

Jasmonic acid was the first hormone to show leaf senescence during week 2. JA induces Chlorophyll breakdown and plastid protein turnover, which promote leaf senescence; during this time, more JA is produced in the plant (Reinbothe et al., 2009). JA induces the expression of a senescence regulator (ESR/ESP), which is involved in senescence and pathogen defense. WRKY53 and ESR/ESP cause leaf senescence by sensing the ratio of JA and salicylic acid (Reinbothe et al., 2009). Another transcription factor family that controls leaf senescence is the TCPs (Reinbothe et al., 2009). Class 1 TCPs are positive regulators, while class 2 are negative regulators (Reinbothe et al., 2009). Both classes bind to promoter motifs of genes to express PCNA, a cell-cycle regulator (Reinbothe et al., 2009). TCP also controls expression of AtLOX2, which is a key enzyme involving JA biosynthesis (Reinbothe et al., 2009). JA breaks down chlorophyll by destabilizing chlorophyll a and b protein binding complexes that are associated with photosystems II and I with the enzyme, chlorophyllase (Reinbothe et al., 2009). In Figure 2.1 and 2.2, it can be seen that JA 100uM and 10uM degraded chlorophylls drastically. Over expression of stay green protein (SGR), causes senescence by controlling the release of chlorophyll and stability of light harvesting complex (Reinbothe et al., 2009). 100uM concentration of JA was first to show senescence and 10uM was next, while 1uM was the last concentration. Therefore, it can be suggested that 100uM and 10uM were high enough concentrations to increase the JA/SA ratio, inducing ESR/ESP expression, which then results in leaf senescence. Also, in Figures 2.1 and 2.2,

JA showed to degrade proteins and Reinbothe et al., suggest that during terminal stages preceding cell death, stress, and defense, protein synthesis is shut down (2009).

Overall, we conclude that transpiration and photosynthesis rates in *Thuja plicata* reduce during winter compared to spring and fall, but *Thuja plicata* photosynthesizes throughout the year. Also, we concluded that JA is the most effective hormone in promoting leaf senescence followed by CEPA. BAP usually does not promote leaf senescence, unless it is at a very high concentration and the treatment takes at least 6 weeks. In future studies, for the first experiment, it is suggested to measure transpiration and photosynthesis rates during summer in order to observe their rates in a yearly cycle and for the second experiment, it is suggested to run a western blot observing the presence/absence and intensity of a photosynthetic protein to be able to more completely decide which hormone promotes or retards leaf senescence.

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**Appendices**
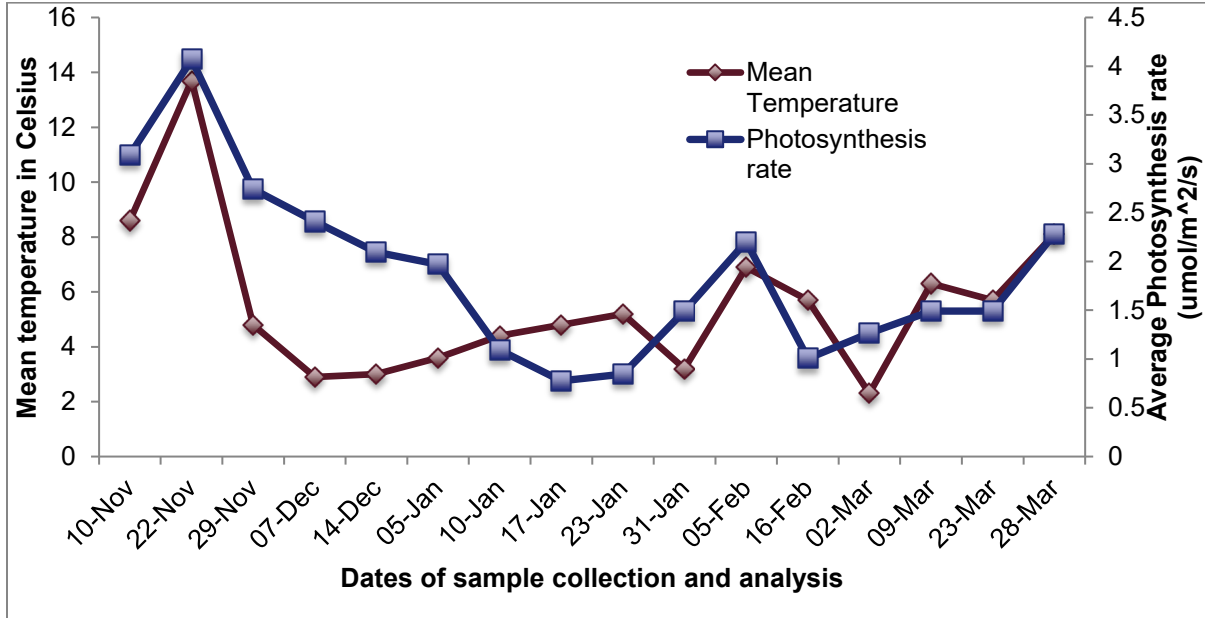


**Figure 1.1.** Average net photosynthesis rate and mean temperatures of *Thuja plicata* from November 10th 2017 to March 28th 2018. According to the graph, higher photosynthesis rate is observed in November compared to January.
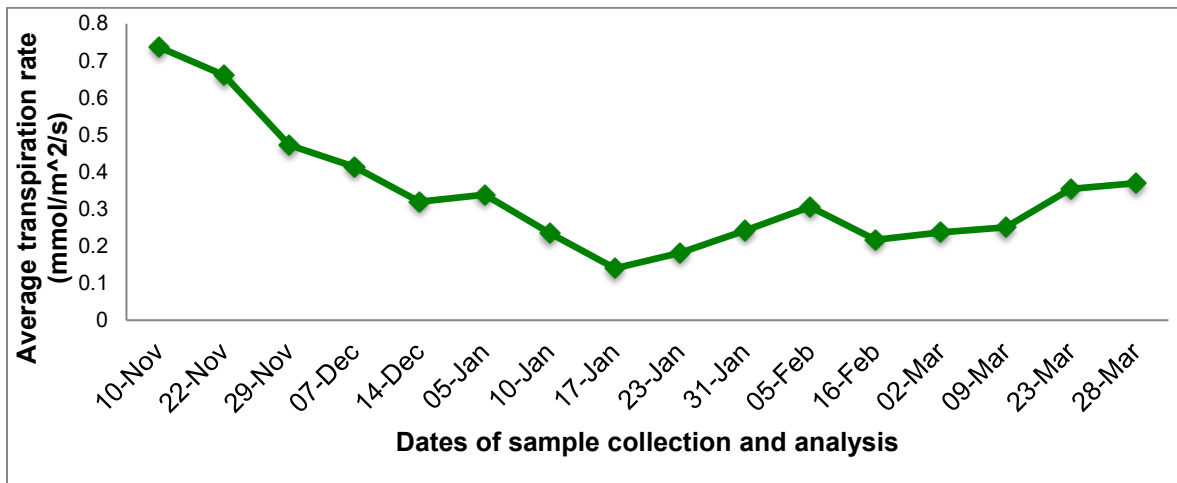


**Figure 1.2.** Average transpiration rate of *Thuja plicata* from November 10th, 2017 to March 28th, 2018. According to the graph, higher transpiration rate is observed in November compared to January.
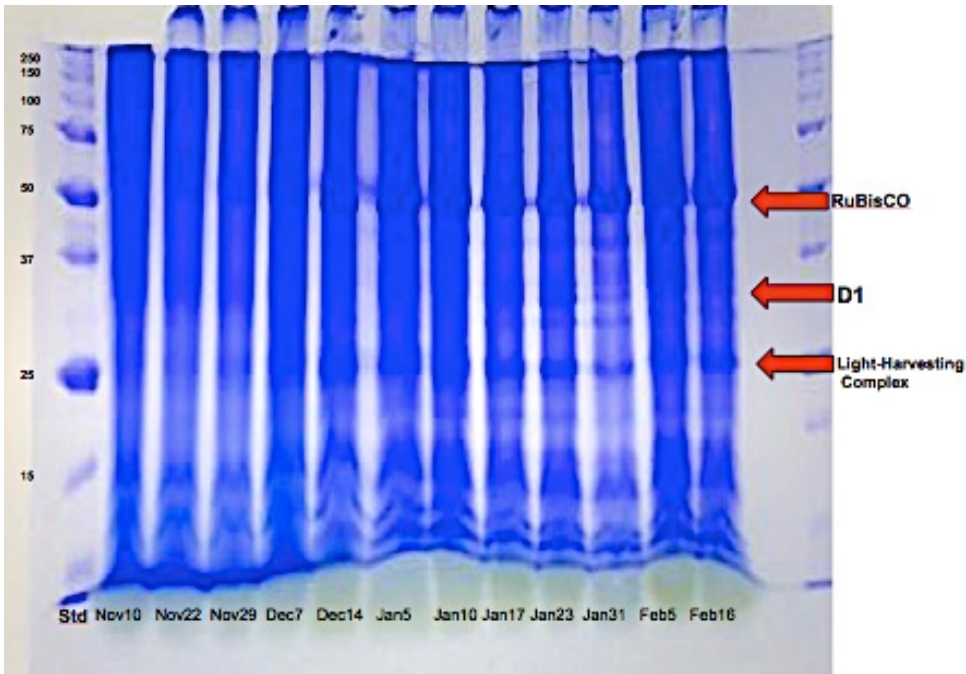
**Figure 1.3**. SDS-PAGE results for protein analysis of western red cedar samples. First lane contains the protein standard. Following lanes are from November 10th to February 16th of 2018. 3 bands weighting at around 50KDa, 30KDa, and 25KDa are observed, indicating that they are RubisCO, D1, and light harvesting complex proteins.
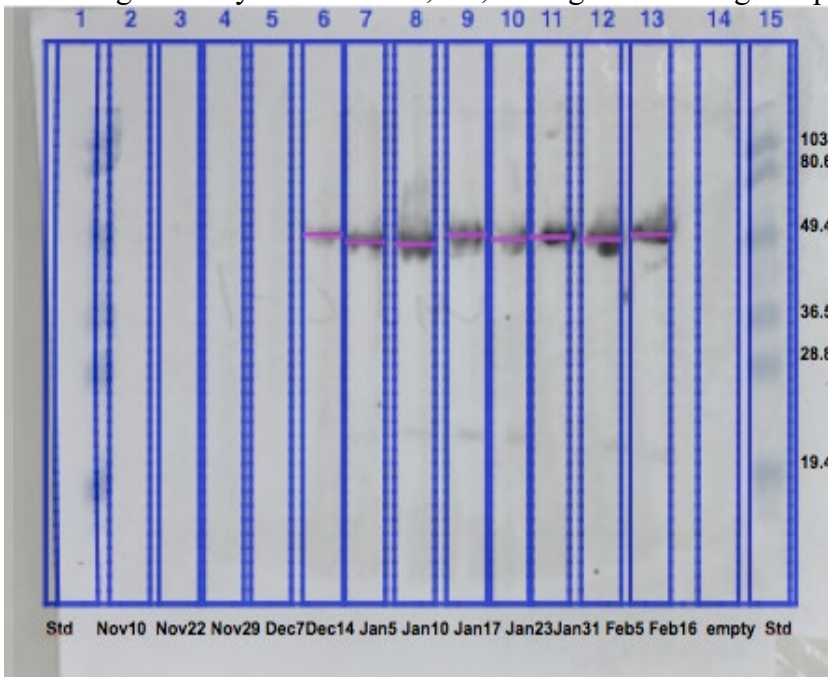


**Figure 1.4.** Western blot results for protein analysis of western red cedar samples treated with RubisCO antibodies. First and last lanes contain the protein standard. Following lanes are samples from November 10th, 2017 to February 16th, 2018. RubisCO was present in samples from December 14th, to February 16th.
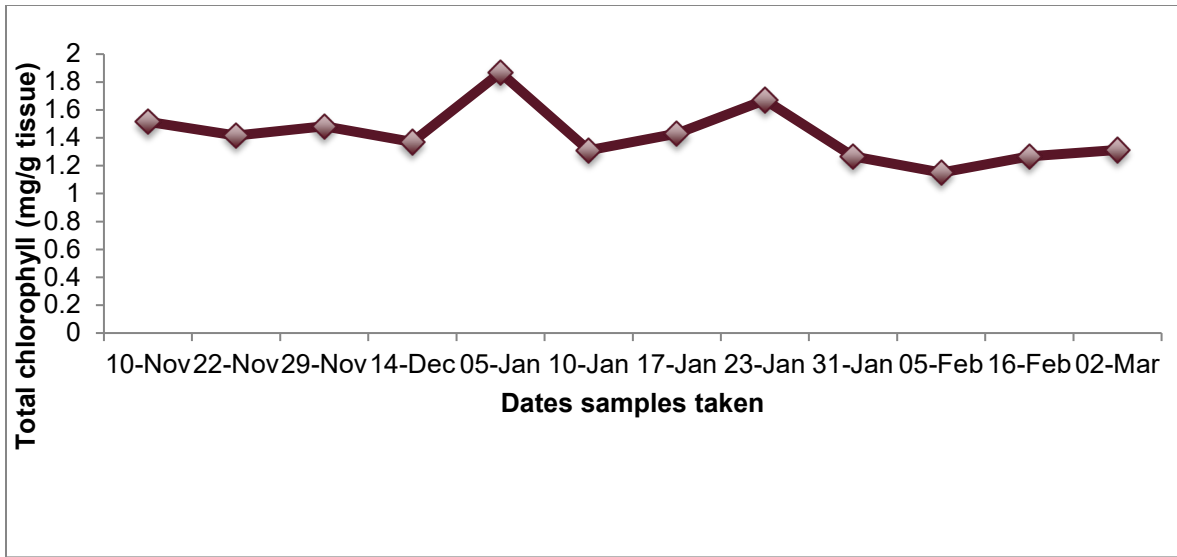
**Figure 1.5.** Chlorophyll analysis of western red cedar samples from November 10th, 2017 to March 28th, 2018. No significance difference was observed between the chlorophyll content among these dates.
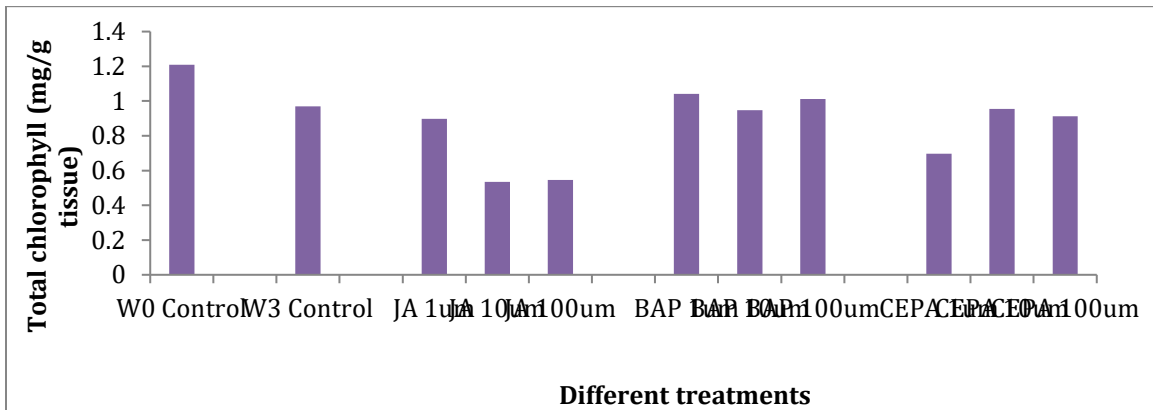


**Figure 2.1.** Chlorophyll analysis of treatments containing JA, BAP, and CEPA at 3 different concentrations of 1uM, 10uM and 100uM in week 3. Reduction in chlorophyll content of JA at 10uM and 100uM was observed, while there was no reduction in CEPA and BAP chlorophyll content.
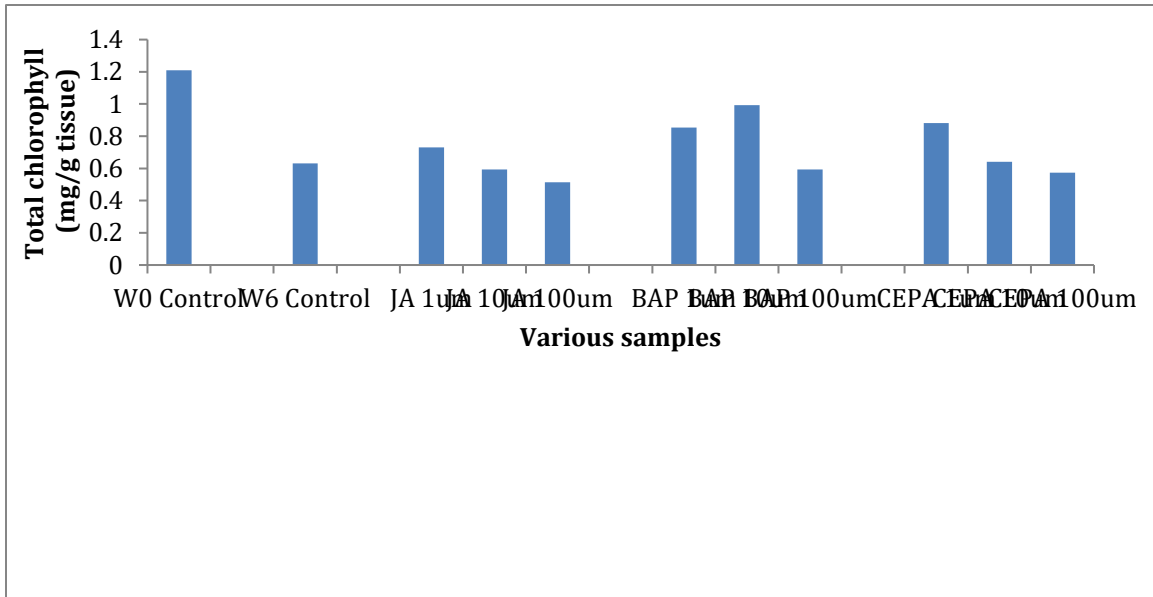
**Figure 2.2.** Chlorophyll analysis of treatments containing JA, BAP, and CEPA at 3 different concentrations of 1uM, 10uM and 100uM in week 6. Reduction in chlorophyll content in JA and CEPA at 10uM and 100uM concentrations was observed, while BAP showed reduction at only 100uM treatment.
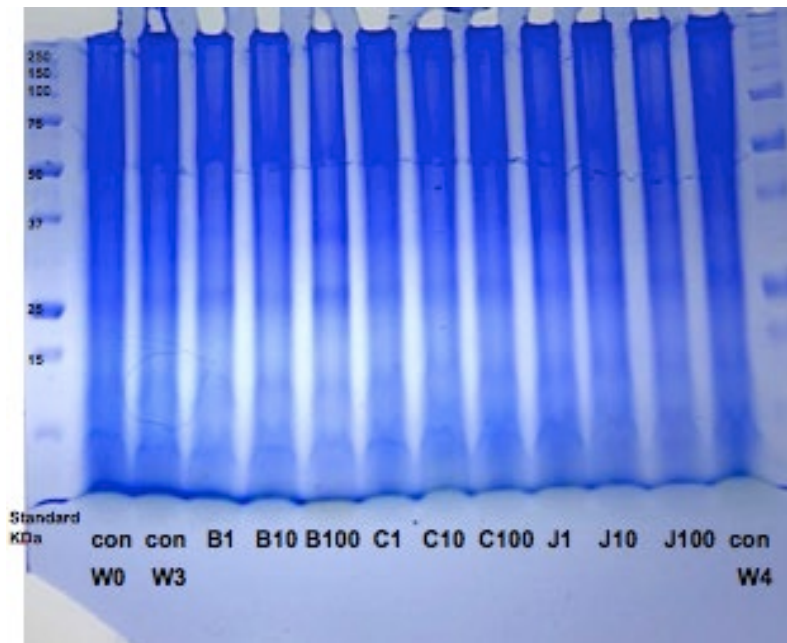


**Figure 2.3.** SDS-PAGE results from week 3 of hormone treated samples. First and last lanes are standard proteins. Following lanes are control week 0, control week 3, BAP 1uM, 10uM, 100uM, CEPA 1uM, 10uM, 100uM, JA 1uM, 10uM, 100uM, and control week 4. Minor protein degradation can be observed comparing treatments to the controls of week 0.
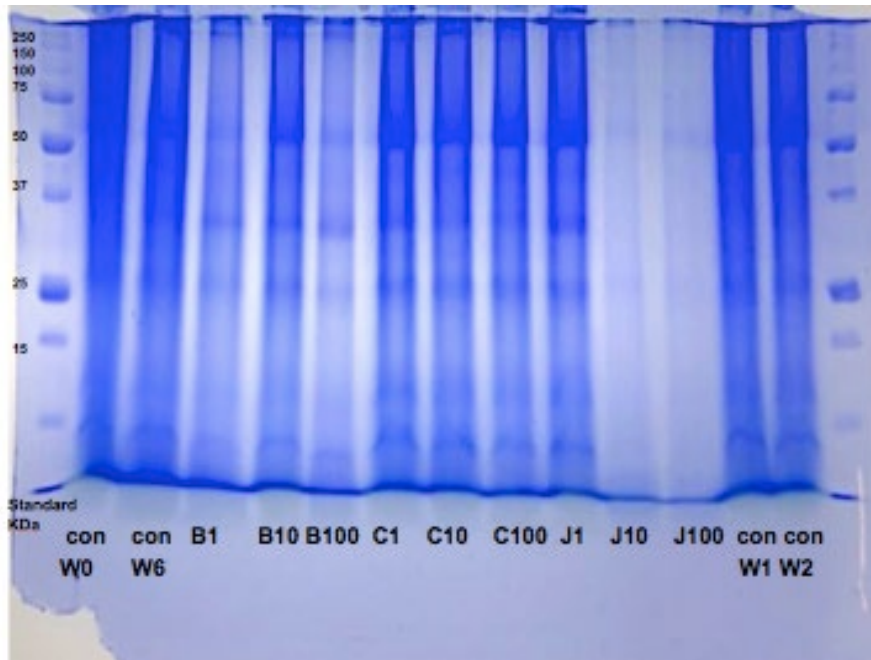
**Figure 2.4.** SDS-PAGE results from week 6 of hormone treated samples. First and last lanes are standard proteins. Following lanes are control week 0, control week 6, BAP 1uM, 10uM, 100uM, CEPA 1uM, 10uM, 100uM, JA 1uM, 10uM, 100uM, control week 1 and control week2. Major protein degradation is observed in JA 10uM, 100uM and minor degradation in other treatments compared to the controls.