

**The Effects of Soil Compaction on Photosynthesis,  
Transpiration and Chlorophyll Levels in Japanese  
Katsura (*Cercidiphyllum japonicum*)**

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Directed Studies in Biology (BIOL 448)

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**BIOL 448 – DIRECTED STUDIES IN BIOLOGY**

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**January 4, 2017  
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## **Abstract**

This study focused on the effects of soil compaction on photosynthesis, transpiration and chlorophyll levels in *Cercidiphyllum japonicum* trees. The photosynthesis and transpiration rates were measured using the CI-340 Handheld Photosynthesis Machine and chlorophyll levels were measured using the CL-01 Chlorophyll Content System. The transpiration rates were relatively similar between the control and soil compaction treatment in the beginning of this study. In the latter part of the study, the transpiration rates were higher in the soil compaction treatment. The photosynthesis rates and chlorophyll levels were generally lower in the trees experiencing soil compaction. By tracking the differences in trees grown in normal soil and trees grown in compact soil, there is a deeper understanding of the relationship between plants and their environment. Furthermore, this understanding can be applied towards using plants as a solution to the increasing carbon dioxide levels in the atmosphere.

## **Introduction**

The earth has experienced alternating warming and cooling periods throughout its 4.5-billion-year life span. Now, earth's global temperature is continuously increasing (Hansen et. al, 2006). This is in part due to the rising atmospheric concentrations of carbon dioxide, a greenhouse gas that traps heat in the earth's atmosphere (Solomon et. al, 2008; Kammann et. al, 2011). Today's CO<sub>2</sub> levels are already showing disastrous consequences. Higher sea levels, acidification of the ocean, loss of glaciers and increased intensity of hurricanes are just a few examples (Solomon et. al, 2008). Of the many proposed solutions, plants have been thoughtfully considered. They can capture carbon dioxide in the atmosphere and convert it into sugars to use as an energy source. However, the ability to assimilate carbon dioxide depends on the physiology of the plant and the environment in which it grows.

Soil is a non-renewable resource in a plant's environment that stores and delivers essential nutrients (Beylich et. al, 2010). It contains air, water and ions that plants require for proper growth and development. The porosity of soil naturally varies from one environment to another. But, in situations of extraneous pressure, soil

compaction can occur. This is the process by which soil particles are pushed closer together and the space between them is reduced (Kozlowski, 1999). Heavy machinery, pedestrian traffic and urban development can cause severe compaction of soil (Kozlowski, 1999).

Soil compaction can lead to startling disturbances in plant physiology. It decreases the rate of water infiltration and soil water retention while increasing water runoff and soil erosion (Kozlowski, 1999; Gregory et. al, 2006). This leads to decreased absorption of vital nutrients that are contained in soil water (Kozlowski, 1999). Plants grown in compacted soil experience reductions in leaf size, shoot biomass and root growth (Komatsu et. al, 2007; Tubeileh et. al, 2003).

Compacted soil also affects a process called transpiration. This is the movement of water upwards in a plant and it functions to transport water, nutrients and sugars. It is initiated by the opening of stomata, which are microscopic pores on leaves (Taiz et. al, 2015). Stomatal opening causes the evaporation of water and this indirectly pulls water from the roots toward the leaves (Taiz et. al, 2015). To prevent excessive water loss, plants do not open their stomata during hot and/or dry days (Taiz et. al, 2015). Plants in compacted soil have less available soil water and thus, transpiration rates are lowered (Komatsu et. al, 2007).

Photosynthesis, like transpiration, is another essential plant process that is affected by soil compaction. This is the process by which sunlight, carbon dioxide and water are converted into sugars that are then used for energy (Taiz et. al, 2015). Plants can support higher rates of photosynthesis when they have increased nitrogen content in their leaves (Eamus, 1999). However, soil compaction leads to a decreased uptake of

nitrogen because nitrate and ammonium cannot easily diffuse into the roots (Douglas and Crawford, 1993). Also, the capture of carbon dioxide can only occur when the stomata are open. Reduced transpiration rates mean that stomata are not open as often and therefore, carbon dioxide is not being captured as frequently from the atmosphere. Thus, photosynthesis is reduced in plants that grow in soil compaction conditions.

Due to the unbelievably important role of soil, it is no surprise then that the overall health of a plant experiencing soil compaction is severely affected.

The focus of this study is on the effects of soil compaction on *Cercidiphyllum japonicum* (Japanese Katsura). This deciduous tree is native to China and Japan and is often grown for its physical appeal. The objectives of this experiment include: to determine net photosynthesis and transpiration rates, to examine differences in chlorophyll levels and to track morphological changes between *C.japonicum* trees that are grown in compact soil conditions and those that are not. I hypothesize that *C.japonicum* trees experiencing soil compaction will have lower chlorophyll levels as well as lower net photosynthesis and transpiration rates than the control trees.

## **Materials and Methods**

### **Samples**

The *C.japonicum* trees used in this study are located on the Vancouver campus of the University of British Columbia (UBC), specifically on the perimeter of the bus loop on University Boulevard. A total of three trees under soil compaction conditions were used. These trees were in areas where the ground was covered with cement bricks and surrounded by sidewalks and roads. Each treatment tree had an opposing control tree, which were in areas with more exposed soil and other vegetation.

### **Collection of Samples**

The petiole of a leaf from *C.japonicum* was cut, immediately placed in a water-filled beaker and then cut again. The leaf was a representative sample (constituting more than 50% of the leaves on the tree) and was used in the chlorophyll, photosynthesis and transpiration analyses.

## Experimental Setup

The experimental setup is shown in Figure 1. The CI-340 Handheld Photosynthesis Machine was set up on a clear, open bench space. A water-filled glass container and a light box were placed directly in front of the leaf chamber. A beaker of water was also placed under the chamber. The light was calibrated to  $250 \mu\text{mol}/\text{m}^2/\text{s}$  with a LI-COR Photometer.

## Chlorophyll Measurements

The CL-01 Chlorophyll Content System was used to measure the relative chlorophyll of the leaves of *C.japonicum*. Each leaf was measured three times at various different locations on the leaf blade.

## Analysis of Photosynthesis, Transpiration

The leaf sample was placed into the chamber of the CI-340 Handheld Photosynthesis Machine. Ensuring that it was submerged in the water-filled beaker, the petiole was cut again. The sample was allowed to adjust to the light for two minutes before starting the analysis. Then, the CI-340 Handheld Photosynthesis Machine automatically calculated photosynthesis and transpiration rates by comparing the levels of carbon dioxide and water vapor (respectively) inside the chamber and the atmosphere. Each analysis was conducted for five counts, which roughly equates to ten minutes.

## Results

The average chlorophyll levels in *C.japonicum* are shown in Figure 3, where the green bars represent the control and the yellow bars represent the soil compaction treatment. On August 31<sup>st</sup>, the control had a reading of +14.8 relative chlorophyll units (rcu) while the treatment had a much lower reading of +6.1 rcu. On September 8 and 16, the control showed values of +15.5 and +12.8 rcu while the treatment showed only +6.8 and +5.0 rcu respectively. However, in the next two weeks, the chlorophyll levels between the control and the treatment were relatively similar. The control had values of +13.0 rcu for both weeks while the treatment had values of +12.0 and +11.6 rcu on September 26 and 30 respectively. On October 17<sup>th</sup>, the chlorophyll level was higher in the treatment than the control by a total of +1.4 rcu. Following this week, there was a drop in the chlorophyll levels for both the control and the treatment. On October 28<sup>th</sup>, the control showed +3.4 rcu and the treatment dropped to +1.8 rcu. The last week of this

study (November 4<sup>th</sup>) showed similar chlorophyll levels between the control and treatment with values of +1.3 and +1.2 rcu respectively.

The average transpiration rates for *C.japonicum* are shown in Figure 4, where the dashed line represents the control and the solid line represents the soil compaction treatment. The first five weeks gave readings of +0.6, +0.6, +0.9, +0.9 and +0.8 mmol/m<sup>2</sup>/s for the control and +0.5, +0.8, +0.6, +0.9 and +0.8 mmol/m<sup>2</sup>/s for the treatment. In these weeks, neither the control nor the treatment showed consistently higher transpiration rates than the other. On October 28<sup>th</sup>, the control reached a peak of +1.1 mmol/m<sup>2</sup>/s before decreasing to +0.5 mmol/m<sup>2</sup>/s on November 4<sup>th</sup>. The soil compaction treatment reached a relatively higher peak on October 28<sup>th</sup> with a value of +1.5 mmol/m<sup>2</sup>/s. Similar to the control, the transpiration rates in the treatment decreased to +0.6 mmol/m<sup>2</sup>/s on November 4<sup>th</sup>.

The average photosynthesis rates for *C.japonicum* are shown in Figure 5. The dashed line represents the control and the solid line represents the soil compaction treatment. The first four weeks of this study showed values of +2.3, +3.1, +2.9 and +4.4 μmol/m<sup>2</sup>/s in the control trees. Following the week of September 26<sup>th</sup>, average photosynthesis rates started to decline in the control. The rates fell from +4.4 μmol/m<sup>2</sup>/s on September 26<sup>th</sup> to +2.5 μmol/m<sup>2</sup>/s on October 17<sup>th</sup> before dropping to +0.1 μmol/m<sup>2</sup>/s on November 4<sup>th</sup>. On the other hand, the treatment trees showed relatively lower photosynthesis rates of +0.3, +0.9, +2.2 and +2.8 μmol/m<sup>2</sup>/s for the first four weeks. Unlike the control, the treatment trees did not show a steady decline in photosynthesis rates. Instead, the average rate dropped to +1.2 μmol/m<sup>2</sup>/s on September 30<sup>th</sup> and then,



it increased to  $+2.4 \mu\text{mol}/\text{m}^2/\text{s}$  on October 28<sup>th</sup>. The final photosynthesis reading for the treatment was  $-0.2 \mu\text{mol}/\text{m}^2/\text{s}$  on November 4<sup>th</sup>.

## **Discussion**

The Japanese Katsura, *C.japonicum*, showed relatively similar transpiration rates in both the control and soil compaction conditions. However, the soil compaction treatment had relatively lower photosynthesis rates and chlorophyll levels than the control. The photosynthesis and chlorophyll results supported part of my hypothesis but the transpiration results directly contradicted the remainder of my hypothesis.

Transpiration rates are usually high when the temperature is high and when the relative humidity is low (Taiz et. al, 2015) Thus, the highest rates should have been seen on September 26<sup>th</sup> and October 28<sup>th</sup> according to Figure 2. This idea only holds true for October 28<sup>th</sup> where both the control and the treatment showed the highest transpiration rates of the study. On September 26<sup>th</sup>, the transpiration rates were relatively low and were similar to previous weeks. This means that the stomata did not open as they should have, suggesting an irregularity within the *C.japonicum* trees.

Furthermore, the difference in transpiration rates between the control and the soil compaction treatment was minimal for most of this study as shown by Figure 4. These results contrast much of scientific literature. First, trees grown in compacted soil have reduced leaf sizes, which means there are fewer stomata and thus, these trees should show less transpiration in similar conditions to the control (Kozlowski, 1999; Tubeileh et. al, 2003). Secondly, roots in compacted soil produce a stress hormone called abscisic acid (ABA) that is transported to the leaves (Tubeileh et. al, 2003). Transpiration rates

decrease because ABA stops the opening of stomata under water stress conditions (Tubeileh et. al, 2003). This is what was seen in a study by Grzesiak that compared transpiration rates under soil compaction conditions in *Zea mays* (2009). The soil compaction treatments had transpiration rates that were approximately +0.54 mmol/m<sup>2</sup>/s less than the controls (Grzesiak, 2009). Conversely, the largest differences in my study were on October 17<sup>th</sup> and October 26<sup>th</sup> where the soil compaction treatment had rates that were +0.39 and +0.36 mmol/m<sup>2</sup>/s higher than the control. A possible reason for the higher rates in the treatment could be that water was evaporating through weakened areas of the leaves and not in a controlled, systematic way through stomatal opening. The CI-340 Handheld Photosynthesis Machine is not able to isolate only the water vapor from transpiration when calculating the rate. So, the transpiration rate presented may not be truly indicative of the real rate. However, it is important to note that the 95% confidence intervals of the control and the treatment overlap, suggesting that there may be no real difference between the two groups. A study by Alameda and Villar showed that soil compaction does not necessarily affect transpiration in the deciduous tree *Fraxinus angustifolia* Vahl (2012).

Now, while the study by Alameda and Villar went on to conclude that photosynthesis is also unaffected, my results show that there is in fact a difference in the photosynthesis rates between the control and soil compaction treatment (2012). According to Figure 5, the treatment showed relatively lower photosynthesis rates than the control. In a study on *Z.mays*, the photosynthesis rates of seedlings grown in compacted soil ranged from +7.0 to +12.8 μmol/m<sup>2</sup>/s while seedlings grown in the control soil had rates of +10.0 to +13.5 μmol/m<sup>2</sup>/s (Tubeileh et. al, 2003). *Z.mays*

showed instances where the seedlings in compacted soil performed better than the control (Tubeileh et. al, 2003). This is similar to the photosynthesis rates on October 28<sup>th</sup> in my study where the soil compaction treatment also had higher photosynthesis rates. Another study by Tu and Tan on *Phaseolus vulgaris* showed similar results (1988). The control treatment showed photosynthesis rates of +7.3 to +9.1  $\mu\text{mol}/\text{m}^2/\text{s}$  while the soil compaction treatment showed considerably lesser values of +4.1 to +5.7  $\mu\text{mol}/\text{m}^2/\text{s}$ . Ultimately, my photosynthesis results were similar to results of previous scientific literature.

Decreased photosynthesis rates in soil compaction are usually due to a drop in stomatal conductance, which is essentially how much water is being evaporated during transpiration (Tubeileh et. al, 2003). However, this explanation falls short in this study because of the similarity of transpiration rates in the control and soil compaction treatments of *C.japonicum*. A possible reason for the lowered photosynthesis rates could be a result of nitrogen deficiency. The photosynthesis machinery in leaf cells require high levels of nitrogen (Fritschi and Ray, 2007). Because of the decreased nitrogen uptake by plants in compacted soil, there is less nitrogen available to create the proteins that make up enzymes (Eamus, 1999; Douglas and Crawford, 1993). RubisCO is an example of an important enzyme needed for photosynthesis. It captures the carbon dioxide in the air and uses it to carboxylate ribulose-1,5-bisphosphate into a form that the plant cell can use to make sugars (Ono et. al, 2013). Under soil compaction conditions, however, decreased nitrogen levels may lead to a decreased amount of RubisCO. This would be able to explain the similar levels of transpiration (and stomatal opening) but the differing levels of photosynthesis. The decreased

amount of RubisCO in the soil compaction treatment would not be able to fix as much carbon as the control. However, this is mere speculation and further experimentation is required to support this idea.

Furthermore, a study by Fritschi and Ray showed a positive correlation between nitrogen content and chlorophyll levels in leaves (2007). Chlorophyll is a type of pigment present in the leaves that captures sunlight and uses it as an energy source to drive the conversion of carbon dioxide into sugars. It is also responsible for the green colour of leaves. Moreover, a deficiency in nitrogen can lead to decreased chlorophyll levels (Douglas and Crawford, 1993; Fritschi and Ray, 2007). In this study, the chlorophyll levels in the soil compaction treatment were generally lower than the levels in the control as shown in Figure 3. The leaves of *C.japonicum* are also shown in Table 1, where the soil compaction treatment has less green leaves than the control. This further supports the idea that the trees may have been deficient in nitrogen. Because chlorophyll plays an important role in photosynthesis, it would seem reasonable to expect a decreased capacity for photosynthesis when chlorophyll levels are low. This would also help to explain the results of photosynthesis seen in *C.japonicum*. To the best of my knowledge, there are very limited studies that have considered the relationship between chlorophyll and soil compaction. This could be an area for future experimentation.

A major flaw of this study is the lack of quantification for soil compaction. This poses serious threats to the validity of the control. Perhaps the lack of differences in transpiration was due to all the trees in study experiencing soil compaction. This is

something that would need to be compensated if this study were to be repeated. For example, soil bulk density could be measured like in the study by Tubeileh et. al or soil hardness could be measured like in the study by Komatsu (2003; 1988). Another flaw presented in this study was the lack of replicates. In the future, more than one representative leaf should be analyzed for photosynthesis and transpiration.

## **Conclusion**

For plants to be a serious solution to combat the problem of rising carbon dioxide levels in the atmosphere, it is necessary to understand that plants must be grown in environments that allow them appropriate access to water and nutrients. Plants will not assimilate carbon nearly as efficiently if they are unhealthy.

Furthermore, the Japanese Katsura, *Cercidiphyllum japonicum*, is a beautiful tree known for its characteristic foliage colour change in the fall. However, as shown by the results of this study, the beauty of this tree is severely impacted when grown in heavily paved and cemented areas. It is important then to note that even if the reason for planting trees is superficial, the trees still require proper environments to flourish.

## **Acknowledgements**

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## **Literature Cited**

- Alameda, D. and Villar, R. 2012. Linking root traits to plant physiology and growth in *Fraxinus angustifolia* Vahl. Seedlings under soil compaction conditions. *Environmental and Experimental Botany*. 79: 49-57.
- Beylich, A., Oberholzer, H., Schrader, S., Hoper, H. and Wilke, B. 2010. Evaluation of soil compaction effects on soil biota and soil biological processes in soils. *Soil and Tillage Research*. 109: 133-143.

- Douglas, J.T. and Crawford, C. E. 1993. The responses of a ryegrass sward to wheel traffic and applied nitrogen. *Grass Forage Science*. 48: 91-100.
- Eamus, D. 1999. Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. *Trends in Ecology & Evolution*. 14: 11-16.
- Environment of Canada. 2016. *Historical Data – Climate*. Jan 4, 2017. <https://www.canada.ca/en/services/environment/weather/past.html>.
- Fritschu, F.B. and Ray, J.D. 2007. Soybean leaf nitrogen, chlorophyll content, and chlorophyll *a/b* ratio. *Photosynthetica*. 45: 92-98.
- Gregory, J.H., Dukes, M.D., Jones, P.H. and Miller, G.L. Effect of urban soil compaction on infiltration rate. *Journal of Soil and Water Conservation*. 61: 117-124.
- Grzesiak, M.T. 2009. Impact of soil compaction on root architecture, leaf water status, gas exchange and growth maize and triticale seedlings. *Plant Root*. 5: 10-16.
- Hansen, J., Sato, M., Ruedy, R., Lo, K., Lea, D.W. and Medina-Elizade, M. 2006. Global temperature change. *Proceedings of the National Academy of Sciences of the United States of America*. 103: 14288-14298.
- Kammann, C., Ratering, S., Eckhard, C. and Muller, C. 2011. Diochar and Hydrochar Effects on Greenhouse Gas (Carbon Dioxide, Nitrous Oxide, and Methane) Fluxes from Soils). *Journal of Environmental Quality*. 41: 1052-1066.
- Komatsu, H., Katayama, A., Hirose, S., Kume, A., Higashi, N., Ogawa, S. and Otsuke, K. 2007. Reduction in soil water availability and tree transpiration in a forest with pedestrian trampling. *Agricultural and Forest Meteorology*. 146: 107-114.
- Kozlowski, T.T. 1999. Soil Compaction and Growth of Woody Plants. *Scandinavian Journal of Forest Research*. 14: 596-619.
- Ono, Y., Wada, S., Izumi, M., Makino, A. and Ishida, H. 2013. Evidence for contribution of autophagy to Rubisco degradation during leaf senescence in *Arabidopsis thaliana*. *Plant, Cell & Environment*. 36: 1147-1159.
- Solomon, S., Plattner, G., Knutti, R. and Friedlingstein, P. 2008. Irreversible climate change due to carbon dioxide emissions. *Proceedings of the National Academy of Sciences of the United States of America*. 106: 1704-1709.
- Taiz, L., Zeiger, E., Møller, I. M. and Murphy, A. 2015. *Plant*. Sinauer Associates, Inc., Sunderland. 681-684.

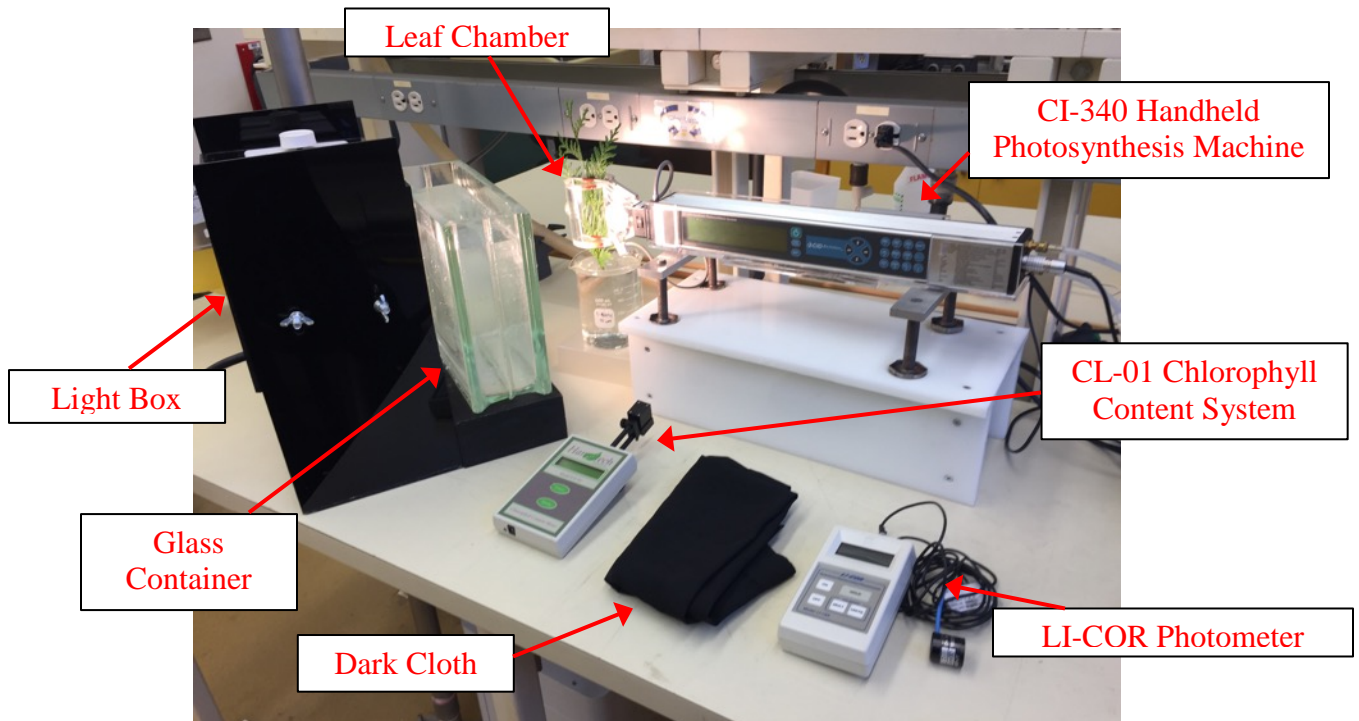
Tu, J.C. and Tan, S.C. 1988. Soil Compaction Effect on Photosynthesis, Root Rot Severity, and Growth of White Beans. *Canadian Journal of Soil Science*. 68: 455-459.

Tubeileh, A., Groleau-Renaud, V., Plantureux, S. and Guckert, A. 2003. Effect of soil compaction on photosynthesis and carbon partitioning within a maize-soil system. *Soil and Tillage Research*. 71: 151-161.

## Appendices

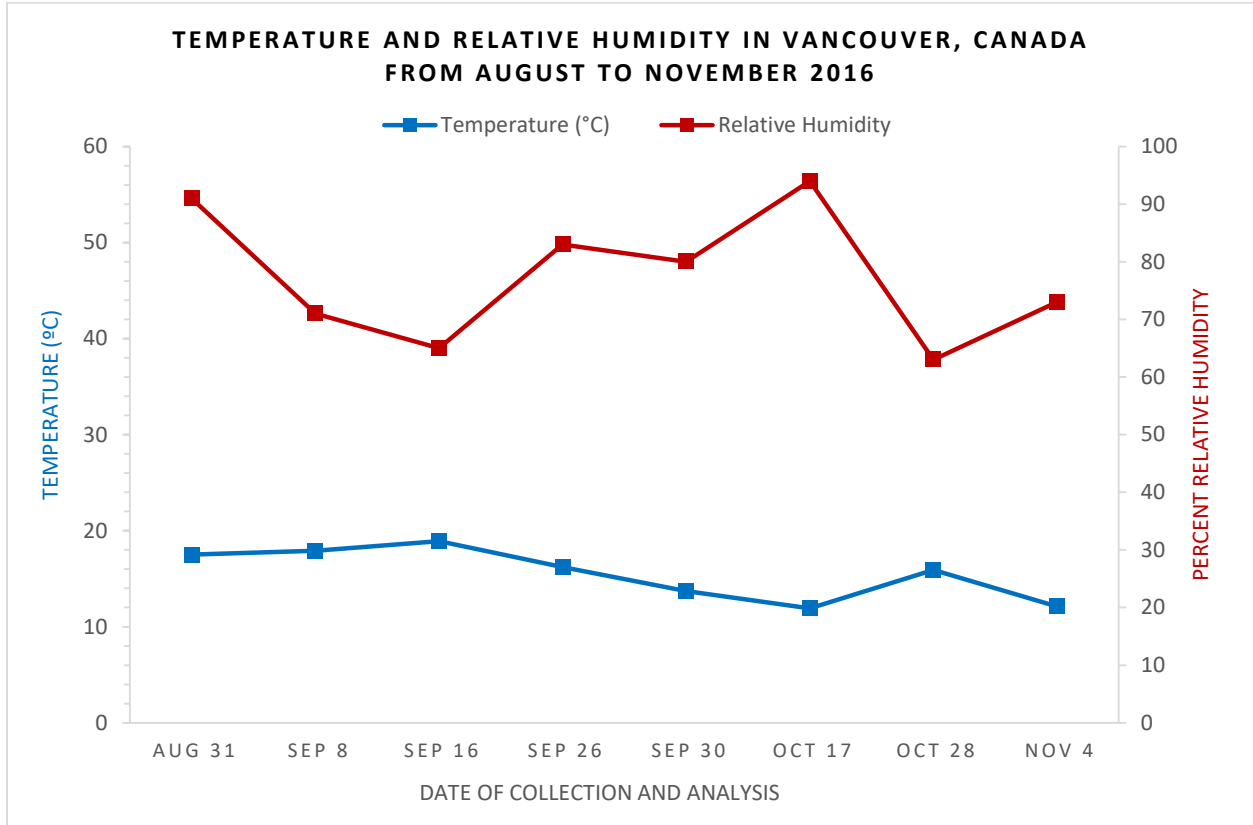
The figures and tables in this study have been split into the following: Experimental Setup, Temperature and Relatively Humidity, Chlorophyll Data, Transpiration and Photosynthesis Data and Morphological Data.

### Appendix 1: Experimental Setup



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

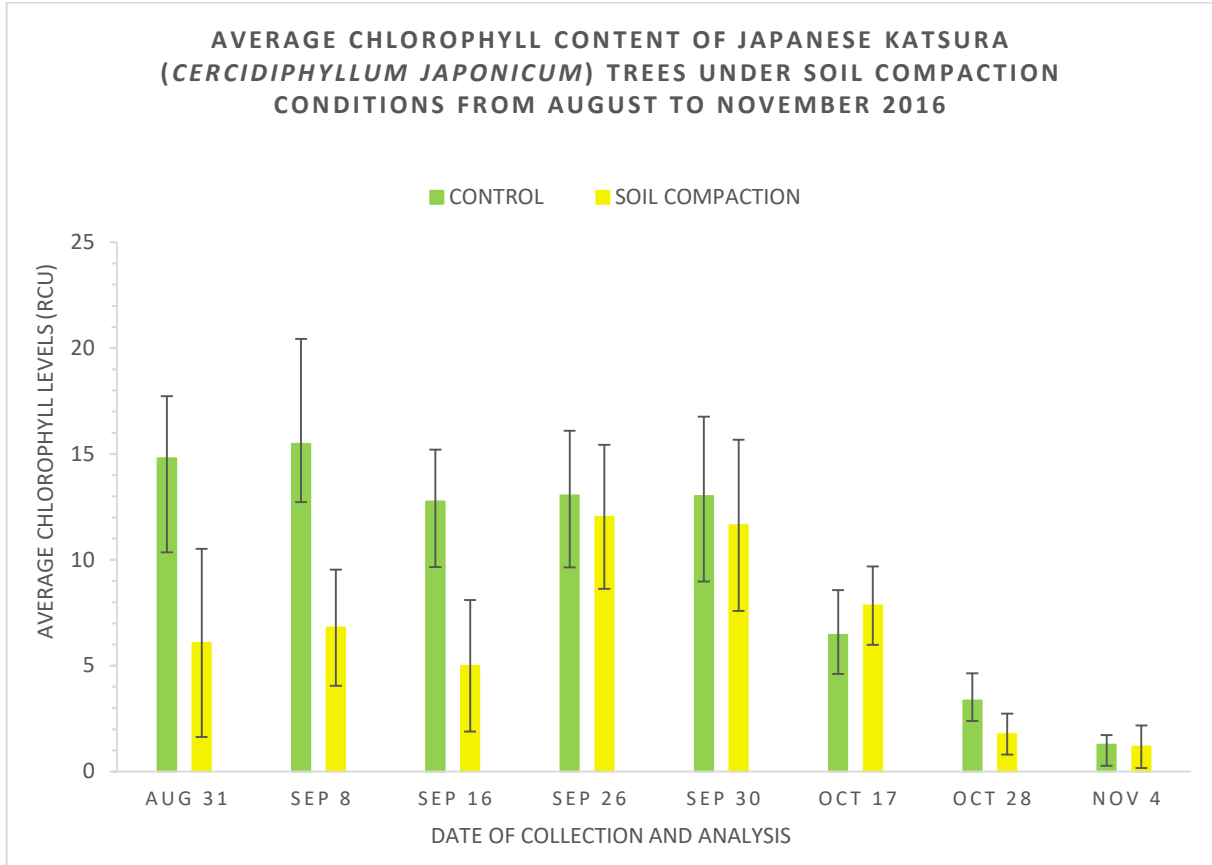
## Appendix 2: Temperature and Relative Humidity



**Figure 2.** The average temperature (blue line) and relative humidity (red line) on each day of collection and analysis in this study are shown above. The location was the campus of the University of British Columbia, Vancouver. Samples were collected and analyzed between 12:00am and 1:00pm (Environment Canada, 2016)

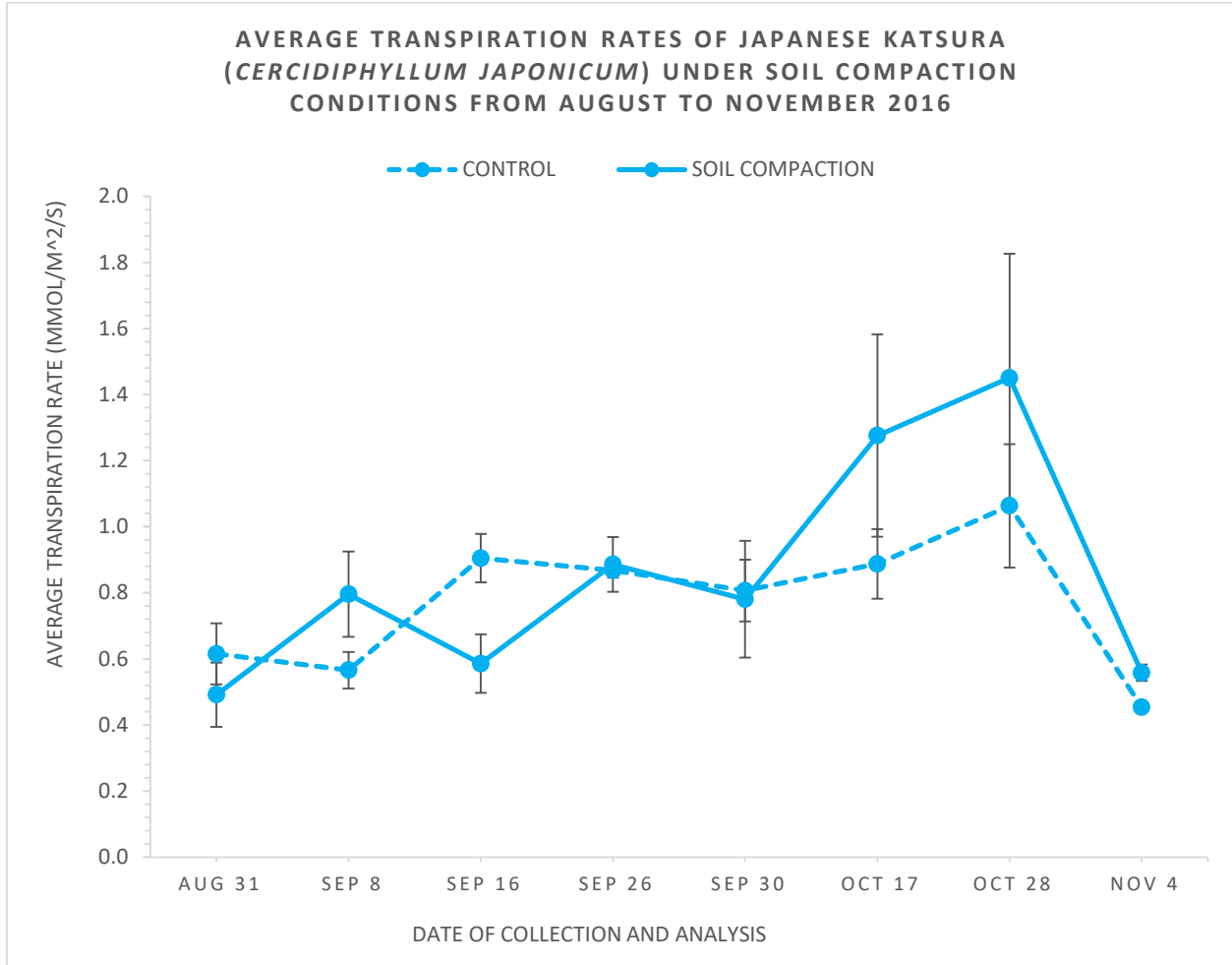


### Appendix 3: Chlorophyll Levels

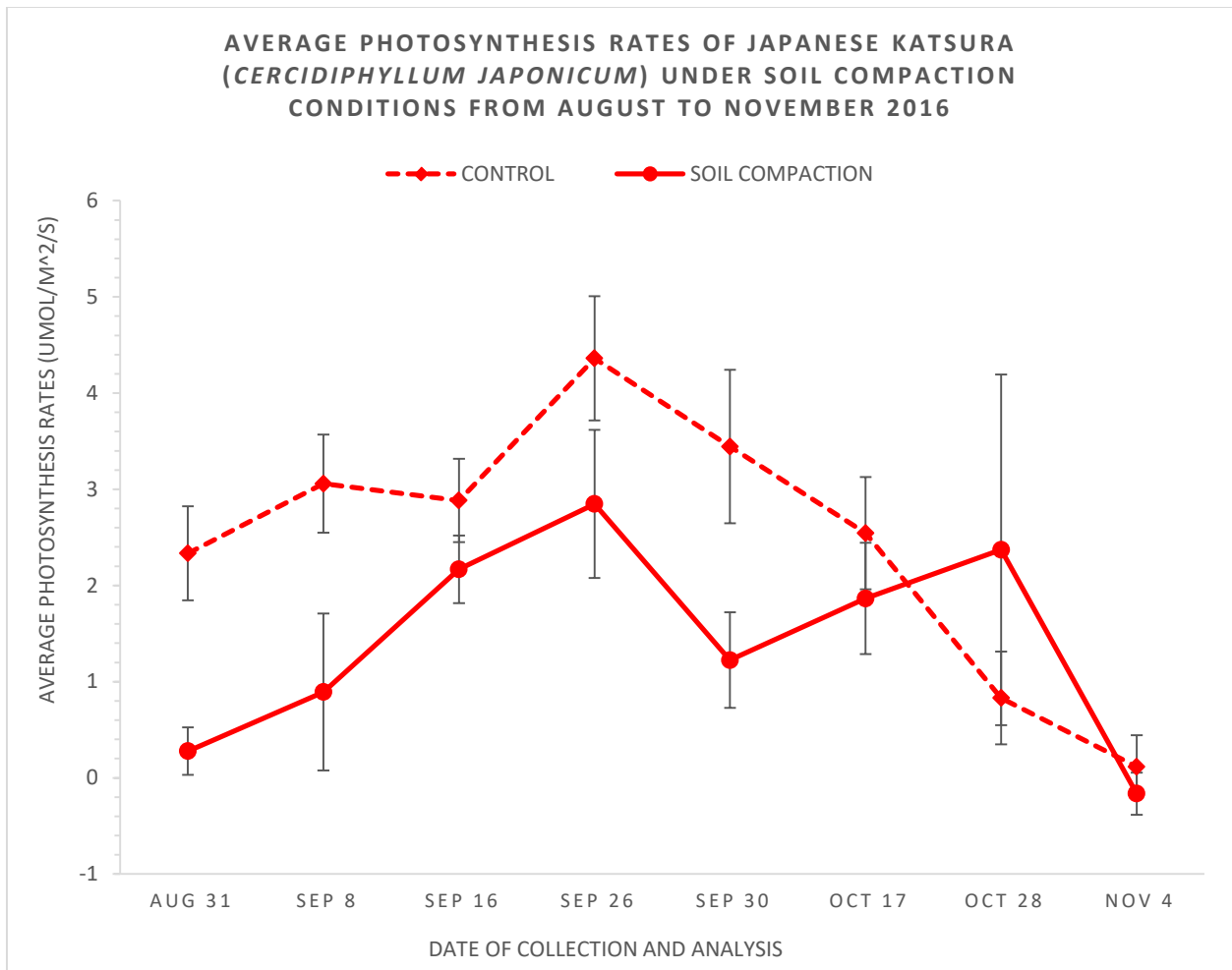


**Figure 3.** Average leaf chlorophyll measurements for Japanese katsura (*Cercidiphyllum japonicum*) under soil compaction conditions are shown above. Each average is a collection of three measurements on one representative leaf of three different trees. Readings were taken with the Hansatech CL-01 Chlorophyll Content System.

#### Appendix 4: Transpiration and Photosynthesis Data








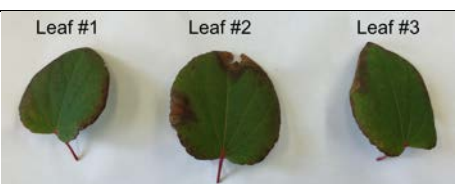




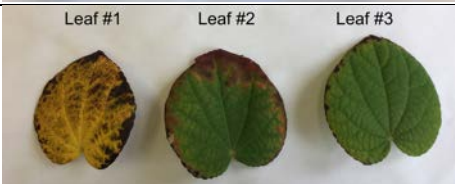




**Figure 4.** The average transpiration rates for the leaves of Japanese Katsura (*Cercidiphyllum japonicum*) are shown above. One representative leaf sample from tree trees was used for each average value. The error bars represent the 95% confidence intervals.










**Figure 5.** The average photosynthesis rates for the leaves of Japanese Katsura (*Cercidiphyllum japonicum*) are shown above. One representative leaf sample from tree trees was used for each average value. The error bars represent the 95% confidence intervals.

**Appendix 5: Morphological Data**

**Table 1.** The leaves of one of the Japanese Katsura (*Cercidiphyllum japonicum*) trees used in this study are shown below. The “X” indicates that there were no longer any leaves left on the tree. Images are not to scale.

	Control	Soil Compaction
Aug 31, 2016		
Sep 8, 2016		
Sep 16, 2016		
Sep 26, 2016		
Sep 30, 2016		
Oct 17, 2016		
Oct 28, 2016		
Nov 4, 2016		<p style="text-align: center; color: red; font-size: 2em;">X</p>

**Table 2.** The trees of Japanese Katsura (*Cercidiphyllum japonicum*) are shown below. Please note that the tree in soil compaction conditions is in the right side of the image while the control tree is on the left side and further in the background. The “X” indicates that there were no longer any leaves left on the trees. Images are not to scale.

<p>Aug 31, 2016</p>		<p>Sep 30, 2016</p>	
<p>Sep 8, 2016</p>		<p>Oct 17, 2016</p>	
<p>Sep 16, 2016</p>		<p>Oct 28, 2016</p>	
<p>Sep 26, 2016</p>		<p>Nov 4, 2016</p>	<p style="text-align: center; color: red; font-size: 2em;">X</p>