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**UGR 294: Mini Research Paper**

**Abstract**

Ion Chromatography Research is being conducted by Undergraduate Students of North Seattle College. Our team was formed by students of the UGR program who are interested in using the LICOR 6800 Ion Chromatography Device. Our purpose was to investigate the relationship between leaf assimilation, 1st in a natural environment with a control sample, and 2nd, with a known area of leaf removed. The intention of the experiment is to determine what types of change can be seen in our Ion Chromatography data. Data shows that our control sample shows highly efficient trends in assimilation when submitted to specific intensities of light from our Ion Chromatography Device. When submitted to artificial damage in the form of a removal of a 6mm area via 1-hole punch, Ion Chromatography data shows that immediately after damage is inflicted, the measured section of leaf with a hole loses all ability to use light as a stimulant for photosynthesis.

**Our Team**

The LICOR 6800, Ion Chromatography team consists of UGR 294 students who have decided to participate in Ion Chromatography research. We intend to learn more about how scientists are currently studying plants via Ion Chromatography using the LICOR 6800 device. Our group is collecting and analyzing data obtained from the LICOR 6800 to prepare ourselves for further Ion Chromatography experimentation in our scientific careers.

Our team consists of undergraduate students in multiple fields and backgrounds including materials science, mechanical engineering, biology, aerospace, chemistry, etc. The following members of our class choose to join us in our LICOR 6800 Ion Chromatography research.

* Cory Andrew Hofstad
* Michelle Clarissa
* John Krausser
* Sabrina Bell
* Lindsay Owings
* Thiên Kim Trần Hoàng
* Sophia Herrmann

During our research and throughout the process of learning how to use and manipulate the LICOR Ion Chromatography device we are assisted by our instructor Ann Murkowsky. Our Instructor Ann assisted in preparing and programming the LICOR device during the process of our readings of plants within our campus watershed.

**Introduction**

The plants in the wetland areas located on the north-east end of the North Seattle College Campus produce oxygen which can be measured by using Ion Chromatography. The assimilation of CO2 into oxygen can be measured for a variety of light levels, CO2 levels and humidity levels. We can then learn how individual plants react under various conditions and how assimilation is affected by these conditions.

The purpose of this experiment is to learn a system of understanding, interpreting and comparing data collected from Ion Chromatography using a LICOR-6800. Our Experiment involves learning the basic functionality of our LICOR-6800 device, then taking readings to collect plant data, which we can then use to study the effects of leaf damage by herbivore threat or removal of a known area of sample readings.

Control readings are taken from a sample leaf without any previous leaf damage to determine a baseline of assimilation rates from a LICOR testing series which uses various light ranges to determine assimilation rates of a sample plant under normal conditions. The LICOR testing series is repeated on damaged leaf samples. Data from control readings and data from post damage readings can then be compared. Our team is then able to look for relations and/or patterns in Ion Chromatography data which provides readings on plant data including assimilation readings.

The experience which our group is learning through this experiment is critical in any future plant sciences which we are involved in, and the technology we are learning to use “makes the LI-6800 the most technologically advanced photosynthesis system in the world.” Data observed in our readings gives our group a better understanding of how our LICOR 6800 - Ion Chromatography device functions and how we will collect data for future experiments. Taking readings from a controlled environment as well as an experimental environment gives our team comparable data which we can use to build skills in Ion Chromatography.

**Methods**

Our group’s main objective was to become familiar with the LICOR equipment and less in doing critical research. We wanted to choose a location that reflected these values A location for our experiment was chosen from the wetland area of North Seattle College at GPS coordinates: ( , ), within 5 meters of the north, eastern end of the campus parking lot.

An attempt to take readings was made on Thursday October 19th, 2017 between 14:00-16:00, but testing was scrubbed after multiple equipment shutdowns due to excess condensation from heavy rain and weather conditions. Successful Ion Chromatography readings were taken on Tuesday October 24th, 2017 @ 14:43. These readings were taken during dry conditions which allowed for LICOR readings of plant data without equipment shutdowns during the 10+ minute readings intervals.

A group of plants was selected from a grouping of ground level plants with 10 – 20 leaves and average heights of 20cm - 45cm. A control reading was taken of a leaf with minimal pre-existing damage to collect data that is used to compare with data from our damaged samples to look for any changes in assimilation trends with and without damage. A leaf lowest to the ground was selected for Ion Chromatography readings because of its proximity to a resting area for our LICOR reading sensor.

Two Mealworms which were prepared for one week without food to promote herbivories damage to the leaves. Leaves were subjected to mealworm herbivores for a time period between 15 and 20 minutes in which they did not consume any of the sample leaves. During the time the leaves were subjected to herbivore stress, further research into mealworms was conducted in which we found that mealworms do not consume leaves, and instead consume a variety of other stock suck as carrots, wheat, oats, etc. Herbivore damage tests were concluded without success and the second method of using a standard 1-hole punch was used to artificially simulate herbivore stress on our plant samples.

A 1-hole punch with a diameter 6±0.5 mm was used to wound the leaf of a sample plant in order to gather data on leaf assimilation rates after damage from herbivores. A located of soft leaf material was removed from the leaf using the hole punch approximately 2/5 of the way from the stem to the tip of the leaf and 3/8 the distance from the center of the leaf to the outer edge. Major leaf veins were avoided to mimic damage found on other leaves of mostly soft tissue areas without heavy vein structure. The leaf was given one minute to adjust to the damage and allow the plant to exhibit any symptoms of trauma or shock which could be detected using LICOR data.

Ion Chromatography readings were then taken from the section of leaf sample damaged by removal of a 6mm leaf section by method of one-hole punch. The LICOR device was programmed to account for the missing area of leaf. Our original leaf sample was damaged and accidentally severed from the plant after artificial damaged was induced by one-hole punch method. The weight of our LICOR device broke the leaf from the stem of the plant. Our group selected a leaf similar in size, ground height, lack of damage and plant size to carry out damage tests using the hole punch method to compare to our control data.

**Results**

**Assimilation**

LICOR Ion Chromatography data shows two different trends in assimilation between our Control and Punch Experiments. Control data shows a highly variable range of assimilation rates over the light intensities which our LICOR device submitted our sample to. Our punch data show a trendline with a constant negative slope of assimilation rate per increase in light intensity, lower overall efficiency and a much lower range of variance from the trend line of assimilation values for the domain of light intensities in which the plant was subjected to.

Our control readings show the ability for our sample plant to produce a local maximum assimilation rate of **.2 (µmol m⁻² s⁻¹)** , when exposed to a **200 (µmol m⁻² s⁻¹)** light intensity. The data recorded from the LICOR device shows an immediate loss in the plant’s ability utilize light in the process of assimilation through photosynthesis. Time of leaf puncture was at x = -60(s), the time our readings were started was at x = 0(s), Our test was concluded at x = 1500(s).

* **The equation of the line for our Ion Chromatography readings for Assimilation rates vs Light Intensity on our control sample was f(x) = (-3.98 e-5)(x) +( 0.106), our r2 value was = 0.071.**
* **The equation of the line for our Ion Chromatography readings for assimilation rates vs light intensity on our punch sample was f(x) = (-5.38 e-5)(x)+(0.0775), our r2 value was = 0.807**

Our data shows that the ability to photosensitize in our sample leaf was non-existent immediately after hole punch damage has occurred. To further back our analysis of post damage data, readings could be taken of an area of the damaged leaf without the missing diameter. This would allow us to determine whether a missing circular diameter within our reading head contributed to our null assimilation rate readings.

While our data from the damaged leaf shows a lack of an ability to photosynthesize, our control data shows a rich variance in assimilation rates along the intensities of light in which our sample was submitted to. When data output from the LICOR 6800 device was used to create a graph of our control sample, it showed a maximum efficiency within specific light intensity ranges. This data suggests that plant assimilation can be artificially tuned via exposing a plant species to a specific intensity of light.

**CO2 Absorption**

LICOR Ion Chromatography data shows that CO2 levels decrease with increased light intensity. This inverse reaction to light shows that plants absorb CO2 and convert it into oxygen more efficiently with an increase of light energy. Absorption data shows a decrease in the slope of CO2 absorption in the samples damaged by hole punch. Our team is under the assumption that the lower initial values for CO2 are a result of calibration errors. These errors give our team an opportunity to learn more about operating and calibrating the LICOR for changes that are made to our samples. The testing done on our leaves with one hole punched of a known area is a good control for learning the proper LICOR 6800 operation procedures for future experimentation in environments in which all variables are unknown and we must collect data using only our LICOR Device with no calibration factors besides what is provided in the unit by LICOR.

**Discussion**

Our control data shows that our plant samples show trends in efficiency within certain ranges of light intensity that would allow scientists to maximize the efficiency of plant assimilation in artificial conditions. Our Ion Chromatography data contained maximum assimilation rates at specific light intensities, that could be charted for different plant species in order to improve selection methods for producing larger volumes of oxygen for artificial environments and terraforming research.

* Plants used to produce oxygen in space flight would produce higher volumes of O2 under specific intensities of light which could be determined for specific plant species via Ion Chromatography
* If light intensity of specific areas of a planet are known, specific plants could be looked at for their efficiency in producing oxygen under those specific conditions. This data is valuable for future terraforming projects.

Our data shows that immediately after being damaged by the 1-hole punch method of removing a diameter of area from a plant leaf, the test leaf loses all ability to use any intensity of light to efficiently assimilate through photosynthesis. As we see many cases in nature where damaged leaves are still part of the plant structure long after being damaged, we are faced with the question of whether or not plants are able to recover the ability to use damaged leaves to assimilate through photosynthesis. Further readings of the damaged leaf should be taken to determine if the leaf has regained the ability to assimilate.

If readings of the same damaged leaf are positive for efficient assimilation data, we could then analyses data related to long term changes in efficiency of single leaves damaged by physical removal of a known diameter. We could then compare data from single leaves to data collected on average damage of plants in our area to determine at what efficiency these plants are able to photosynthesize in their natural habitat.

* If we find that plants are able to recover the ability to photosynthesize with damaged leaves, further data on could be collected to determine average recovery times in plant leaves by size and other variables.
* New readings of a damaged leaf sample could be taken in decreasing time intervals until a consistent recovery time could be determined.
* Multiple readings of different sized leaves could be taken to find recovery time per gram of plant, which could be used to determine recovery rate per mol of plant substance or per volume.

As we further research into plant recovery, we could attempt to look at how the plant recovery process works.

* Do plants re-route resources around damaged leaf sections?
* Do plants cauterize their wounds and work around damaged tissue?
* Do plants perform compensation for damaged leaf sections to perform at maximum efficiency?
* Can plants learn to compensate for new leaf damage after initial trauma is recovered from?
* What steps are involved in plant recovery?

Also, separate readings should be recorded of a section of the damaged leaf without the hole on it. It would bring confidence to the analysis of the data to know if the recording of data is affected by the hole punch being within the recording area.

**Conclusion**

Initial readings of our control experiment show high efficiency areas in our data that produce higher assimilation rates at specific values of light intensity. In Ion chromatography tests conducted on our 1-hole punch experiments, immediately after a section is removed from a leaf, it loses the ability to efficiently use the light of our LICOR device for photosynthesis. These two data tables show that immediately after damage has been inflicted on our test leaf, it losses the ability to use any intensity of light for photosynthesis. The fact that our leaf showed decreasing assimilation rates with increasing light intensity shows that for photosynthesis the leaf is at this point non-functional.

Data from our control specimen shows that our plant sample reacted to a specific intensity of light to produce high levels of assimilation. Further research in local minimums and maximums of assimilation vs light intensity would allow scientists to produce higher volumes of oxygen in artificial environments through photosynthesis.

Further research needs to be carried out to discover whether plants have the ability to recover from the punch damage and operate with efficient levels of assimilation instead of providing data that shows a linear downward slope of assimilation vs light intensity. Further research would allow scientists to learn more about plant recovery, including recovery time, recovery methods and post recovery efficiency.

**Bibliography**

Using the LI-6800 Portable Photosynthesis System Instruction Manual. (n.d.). Retrieved November 05, 2017, from https://www.licor.com/documents/6afbbpwybdanht6xrbgwicur4yohpx1n