2016

Effect of Endophytes in Physcomitrella patens on Cellular Respiration During Abiotic Stress

Michael Danh
Butler University, mdanh1@butler.edu

BUTLER UNIVERSITY HONORS PROGRAM

Honors Thesis Certification

Please type all information in this section:

Applicant Michael Danh
(Name as it is to appear on diploma)

Thesis title Effect of Endophytes in *Physcomitrella patens* on Cellular Respiration During Abiotic Stress

Intended date of commencement May 7, 2016

Read, approved, and signed by:

Thesis adviser(s) _____ May 5, 2016
Date

_____
Date

Reader(s) _____ May 5, 2016
Date

_____
Date

Certified by Rusty Jones
Director, Honors Program
5/5/16
Date

Effect of Endophytes in *Physcomitrella patens* on Cellular Respiration During Abiotic Stress

A Thesis

Presented to the Department of Biological Sciences

College of Liberal Arts and Science

And

The Honors Program

Of

Butler University

Michael Danh

March 13, 2015

Dedication

This thesis is dedicated to my all my family, friends, and to anyone who has ever believed in me.

Acknowledgements

I would like to show gratitude to Drs. Philip Villani and Sean Berthrong for their resourcefulness and generosity that made this thesis possible.

Table of Contents

Abstract

Endophytes are bacteria or fungi that ubiquitously reside in plant tissue and do not cause apparent disease. With the mutually symbiotic relationships between plant and endophyte being scientifically evident, it is supported that endophytes provide an augmentative method to absorb nutrients and additional tolerance under abiotic stress for the plant while benefiting from the host plant's reduced carbon sources. The moss *Physcomitrella patens* is expected to share the same type of mutualism with endophytes, being able to treat endophytes as endosymbionts and having a reduced carbon reservoir. However, it is unclear if these endophytic relationships alter under abiotic stress. In this study, the carbon dioxide levels in *Physcomitrella patens* and fungal endophyte will be monitored under the abiotic stress simulations of darkness, drought and nutrient deprivation for any trends and conclusions regarding cellular respiration and endophyte presence. Endophytic presence was inconclusive under the simulation of abiotic stress. Further studies with endophytes are required to make more definitive statements about endophytic symbioses in plants.

Introduction

Endophytes and Symbiosis with Plants

Endophytes are bacteria or fungi that ubiquitously reside in plant tissue and do not cause apparent disease (Carrol 1988). By nature, endophytes are highly variable due to the magnitude of diversity seen in the Monera, the bacteria and Fungi Kingdoms. Plants and endophytes share a mutual symbiotic relationship (Carrol 1988). A mutual symbiotic relationship occurs when two different species interact and provide positive effects for each other.

The plant benefits in the mutual symbiosis by having increased access to macro nutrients, particularly nitrogen (Faeth 2002). In particular, fungal endophytes use their mycelia, which grow in a radial pattern, to search and absorb nutrients. By inhabiting plant tissue, endophytes augment the mobilization of nutrients as the plant and endophyte work synergistically. Specifically, nitrogen is a component in chlorophyll, which correlates to the photosynthetic activity and green coloration of the plant. A lack of nitrogen would cause chlorosis. Chlorosis is the yellowing of plant tissue. Since nitrogen is mobile in the plant, chlorosis usually occurs in the older plant tissues and any remaining nitrogen will be used in the new tissues.

A few primary macronutrients for plants include nitrogen (N), phosphorus (P) and potassium (K). The secondary macronutrients include calcium (Ca), sulfur (S) and magnesium (Mg) (Mauseth 2014). Supplementing the macronutrients are the micronutrients. Often called trace minerals, they include boron (B), zinc (Zn), manganese (Mn), iron (Fe), copper (Cu), molybdenum (Mo) and chlorine (Cl) (Mauseth 2014).

For the microbial symbionts, the endophytes, they benefit from having a reduced carbon source from having the plant nearby. This is because oxidized forms of carbon are harder for the endophytes to metabolize (Faeth 2009).This alleviates some of the scavenging that the heterotrophic endophytes must do for energy (Carrol 1988). Heterotrophic organisms are ones that cannot produce their own food sources and must feed off of other organisms.

Although it is evident that there is a mutual symbiosis between plants and endophytes, it is uncertain if these endophytic relationships operate differently under certain abiotic stressors and conditions (Levitt 1982).

Plant Abiotic Stress

Plants require many certain abiotic conditions to be met in order to grow properly. Traditionally, plants require an ample amount of sunlight, fertile soil, water, and carbon dioxide-rich air. Sunlight is used as an energy source for photosynthesis, which results in food for the photoautotrophic organism. Fertile soil is essential as it contains micro- and macronutrients for the plant. Furthermore, soil can act as a substrate for the anchorage of many plants. Water is important in plants for many reasons. Firstly, water can be used to store and mobilize nutrients as it travels through the thallus, stems and leaves. Secondly, water is one of the two reactants in photosynthesis. The other reactant, carbon dioxide, is acquired from the atmosphere. All of these components work synergistically to provide the means for proper growth and development.

The balance of these components can be interrupted under abiotic stress. Abiotic stress is a detrimental influence of non-living factors on living organisms within an environment (Jenks & Hasegawa 2008). Examples of abiotic stressors can include, but

are not limited to, extreme temperatures, high winds, droughts, floods, ionizing radiation, and poor edaphic, or soil related, conditions. Although abiotic stressors are naturally present in every ecosystem, the organisms, particularly plants, within the ecosystems must find a way to acclimate and adapt to the stressors (Levitt 1982).

Vascular Plants

Evolutionarily, vascular plants are the most recent deviation of plants. The vascular tissues are characteristic of vascular plants. The vascular tissues are responsible for distributing water and nutrients throughout the plant. Specifically, the xylem is used to carry water throughout the plant. The second transport tissue is the phloem. The phloem carries the sugar product produced by photosynthesis to the heterotrophic regions of the organism. In short, the vascular tissues of the xylem and phloem work together and allow vascular plants to grow to become relatively larger than their non-vascular ancestors.

Non-vascular Plants

The non-vascular plants, the bryophytes, consist of the mosses, hornworts and liverworts. In this study, moss and liverwort were used because they are structurally less complex as compared to vascular plants.

Non-vascular plants lack the aforementioned vascular tissues of the xylem and phloem, and are considered to be the lower plants since they were the earliest to evolve. These plants do not have true leaves nor roots, but instead have rhizoids, which are root-like structures that aid in the anchorage of the plant, and a single layer of cells for photosynthesis. Non-vascular plants do not rely on soils to obtain nutrients. Thus,

they often grow on top of rocks, trees and other plants. Additionally, since non-vascular plants do not use soils for nutrients, soil fertility can be eliminated as a confounding factor for plant growth.

Physcomitrella patens is a model organism for studies involving moss. Besides the fact that *P. patens* has its entire genome sequenced for more accessible genetic studies, it was used in this experiment for its simple growth conditions and manipulation in the lab (Schaefer & Zrÿd 2001).

Mosses have different life cycles as compared to vascular plants. Mosses start as a haploid spore that produces protonemata, a filamentous chain of cells that will grow into a gametophore. Gametophores are dioecious. Being dioecy means that organisms of the population are either distinctly male or female. In mosses, haploid females have egg storing archegonia. Haploid males have antheridia that are filled with sperm. Once a zygote is formed through fusion of the egg and sperm, or fertilization, the zygote will grow in the archegonium of the female gametophyte. Eventually, a stalk will come to rise from the archegonium and the sporangium will be placed at the top. Being now diploid, the sporangium produces haploid spores and more haploid gametophytes are formed to repeat the cycle (Campbell et al. 2008).

Marchantia polymorpha, the common liverwort (Figure 1), was used in this experiment as a reservoir for endophytes for two reasons. Firstly, *M. polymorpha* and *P. patens* are both classified as non-vascular bryophyte land plants. This similarity could render an increased likelihood of compatible endophytes because their tissues are both non-vascular. This is important considering that endophytes may be specialists when it comes to host selection (Davis et al. 2003). Secondly, *M. polymorpha* was chosen due

to its flat structure. Surface sterilizing flat plant tissue would make the treatment more thorough, allowing for an easier and more confident sterilization of tissue. *P. patens* does not provide that same luxury.

Figure 1. *Marchantia polymorpha.*

Photosynthesis

A hallmark of plants and the resource for primary productivity lies in photosynthesis. Photosynthesis is a process universally used by plants and other photoautotrophic organisms to convert light energy into chemical bond energy. Being photoautotrophic means that the organism can provide its own food source via light. In plants and algae, photosynthesis occurs in the chloroplasts of the plant cells. Generally, each plant cell should contain about 10 to 100 chloroplasts. At a molecular level,

atmospheric carbon dioxide and water yield the products of oxygen gas and the carbohydrate, glucose, after being energized by light. Glucose acts as a storage of chemical bond energy because carbon is reduced as it goes from carbon dioxide to carbohydrate. This chemical bond energy in glucose could then be used later when in need of energy. Furthermore, the amount of energy from the primary producers in the ecosystem can then be elevated to other trophic levels as organisms feed off of plants and one another.

Equation 1. *Photosynthesis:* $6CO_2 + 6H_2O \xrightarrow{light} C_6H_{12}O_6 + 6O_2$

Cellular Respiration

Complementing the process of photosynthesis is cellular respiration. In contrast to photosynthesis, cellular respiration focuses on releasing chemical energy, rather than storing it. Cellular respiration consists of catabolic reactions that break down large molecules into smaller molecules.

Among the reactions of cellular respiration is aerobic respiration. Aerobic respiration occurs in the mitochondria of cells, specifically in the mitochondrial matrix. Thus, all eukaryotic cells can exhibit this reaction since they have mitochondria. The spontaneous reaction of aerobic respiration results in heat, carbon dioxide gas and water, after glucose is catabolized with oxygen gas.

Equation 2. *Aerobic Respiration:* $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP + heat$

Thesis Research and Hypothesis

Endophytes play a supportive role in plant growth (Carroll 1988). As mentioned earlier, it is unclear whether the mechanisms for the endophytic symbiosis becomes altered under certain abiotic stress (Levitt 1982). However, when considering the biological success and resilience within the Monera and Fungi Kingdoms, it would be logical to predict adaptations to drastic environments (Clay & Schardl 2002).

Therefore, I hypothesize that endophyte presence in plant tissues will allow for metabolic processes, like photosynthesis and cellular respiration to function under abiotic stressors.

In this experiment, I use endophytes extracted from *M. polymorpha* and grew them in *P. patens* under abiotic stresses of light deprivation, water deprivation and nutrient deprivation. I then measured the levels of atmospheric carbon dioxide concentrations in sealed growth conditions and compared them relative to dry plant mass. This served as a proxy for calculating metabolism, since carbon dioxide is a reactant of photosynthesis and product of aerobic respiration. Thus, a lower ratio of carbon dioxide to plant dry mass would suggest more photosynthesis occurring.

Methods

Plant and Endophyte Growth Conditions

P. patens was used in the endophytic symbiosis experiment because it grows easily on media and can be transferred onto new media very quickly and sterilely. Axenic *P. patens* was grown in Dr. Philip Villani's laboratory (Figure 2). *P. patens* was grown on sterilized BCD agar with alternating 12 hours of fluorescent lighting at 27.5 ℃ and 12 hours of dark. Standard BCD stock was prepared in 500 mL increments with 5

mL of stock B containing 25 g $MgSO_4 \cdot 7\ H_2O$ filled to 1 L with H_2O, 5 mL of stock C containing 15 g KH_2PO_4 filled to 1 L with H_2O and adjusted to pH 6.5, 5 mL of stock D containing 101 g KNO_3 1.25 g $FeSO_4 \cdot 7\ H_2O$ filled to 1 L with H_2O, and 460 mg diammonium tartrate, then brought to a volume of 500 mL with deionized H_2O and adjusted to pH 6.5 before the addition of 2 g Agargel™ (Sigma Aldrich). The solution was then microwaved for 3.5 minutes and swirled to fully dissolve the agar, before being autoclaved at 121 °C at 15 psi for 25 minutes. Once the sterilization was complete, 0.5 mL of 1 M $CaCl_2$ was added and the solution was poured into sterile petri dishes to solidify before use (Ashton et al. 1979).

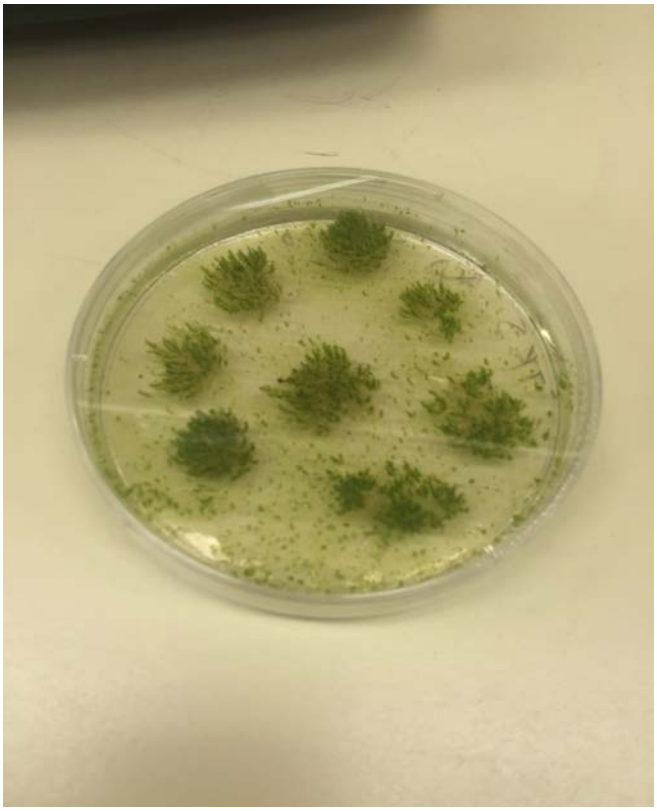


Figure 2. Axenic *Physcomitrella patens.*

In preparation for the endophytic fungi propagation, 1/8th strength potato dextrose agar plates were prepared. PDA plates were prepared in 500 mL increments,

composing of 6.563 g BD Difco agar and 2.438 g PDA stock, containing 15 g/L agar, 20 g/L dextrose, and 4 g/L potato extract, and brought to 500 mL with deionized H_2O (Fawcett 2014).

Propagation of Endophyte

Due to prior experimental success of others, surface sterilization was used in order to isolate viable endophyte fungi for propagation (Carroll 1988). Surface sterilization is when raw plant tissue is treated in order to eliminate, or at least, dramatically reduce the microorganisms on the plant's epidermis. Wanting only the endophytes, the plant tissue would be cultured on standard BCD medium in order to then propagate the endophyte growing from within the plant tissue onto $1/8^{th}$ strength PDA medium.

M. polymorpha thalli was cut and torn into squares of approximately 0.5 cm in length. With about thirty tissue pieces, the squares were equally divided to form three packets of cheesecloth with the ends of the cheesecloth concealed by the coiling of plastic twist ties rather than the traditional twisting technique. The wrapped coils of the plastic twist ties allow for a simpler opening of the packets since it is difficult to untwist the plastic twist ties with sterile tweezers, and using hands could desterilize and defeat the purpose of the procedure. The cheesecloth packets were soaked in sterile deionized water for the 30 minutes.

In a separate beaker, a 7% bleach solution was prepared using 14 mL of commercial bleach, 186 mL of deionized water, and a few drops of surfactant to lower the surface tension between the solution and tissue. As evidenced from a prior study, a

7% commercial bleach concentration was found to be optimal for surface sterilizing *M. polymorpha* (Vujičić et al. 2010).

Using a magnetic stir rod, the 7% bleach solution was stirred and concealed with foil. The cheesecloth packets were then individually placed in the bleach solution for 5 minutes. Afterward, the packets were rinsed three times in sterile deionized water for 1 minute per rinse using sterile utensils.

The thalli were then removed and handled with utensils that were flame sterilized with 95% ethanol. Liverwort thalli were cut along the perimeter if there was any discoloration or sign of apparent dead tissue. The remaining liverwort tissue was then transferred to sterilized BCD agar plates with about nine to ten pieces per plate and incubated for 24 hours of fluorescent lighting at 27.5 °C.

After the 24 hour period, liverwort samples that had visually asymptomatic fungi growing endophytically were transferred to new petri dishes for further propagation. Once the mycelia of the endophytic fungi expanded on the fresh BCD plate, a small plug (0.5 cm x 1.0 cm) from the outer radius of the fungi was transferred onto a 1/8th strength PDA plate. The outer ring of the fungi was of interest due to it being the location of the active growth of the fungi, likely to lead to successful propagation. The PDA medium provides the fungi with a scarce carbon source, allowing it to propagate without the reliance of the carbon sources, like glucose, from host plant tissues. Samples were grown at 25 [illegible] in the shade in Dr. Villani's

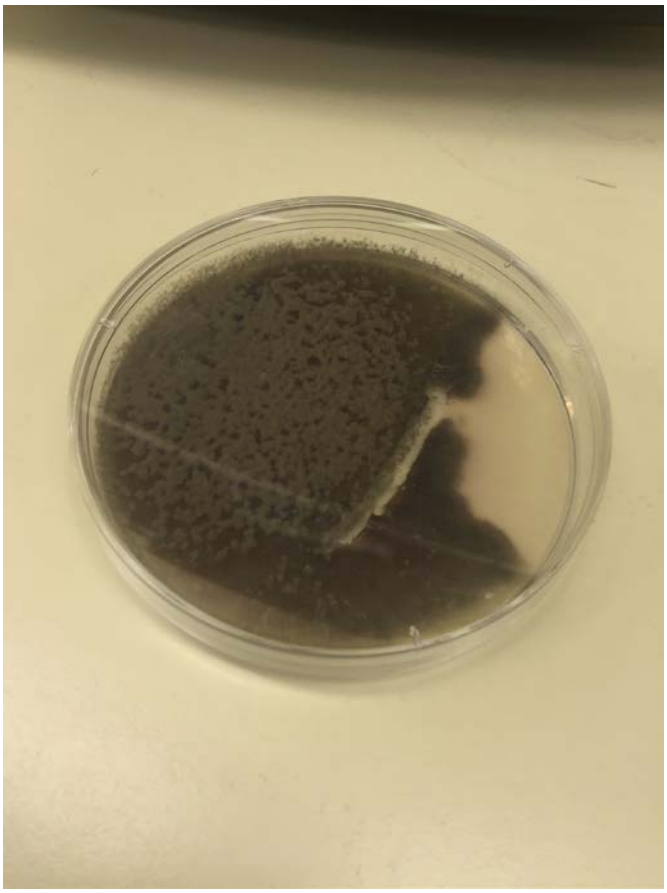


Figure 3. Extracted endophytes from *M. polymorpha* on 1/8th strength PDA plate.

Experimental Growth Conditions to Simulate Abiotic Stressors

A total of four different treatments, including one control, were prepared to simulate the abiotic stressors of light, water, and nutrient deprivation. For each treatment, four types of samples were prepared: samples containing only medium, only endophyte, only *P. patens*, and both the endophyte and *P. patens*. For samples containing endophyte, a 0.5 cm x 1.0 cm plug was inserted into the Vacutainer tube. The samples with plant tissue contain three pieces of leafy gametophyte of *P. patens*. All samples were run in quintuplicate.

Samples with nothing but medium serve to monitor the carbon dioxide levels, which are expected to be stable for this type of sample. Samples containing endophytes and medium were used to monitor the stability of the endophyte metabolism. Samples of only plant tissue and medium served to provide data on axenic plant metabolic rates for comparison with those with endophytes.

The controlled treatment was meant to simulate an environment under neutral conditions, without any major abiotic stressors present and having sufficient light, water and nutrients. Therefore, the controlled treatment was prepared using the aforementioned standard BCD medium and light exposure cycling.

For simulation of darkness, aluminum foil was wrapped around the samples to prevent light exposure. Water deprivation was simulated by altering the water to agar ratio in the standard BCD medium to where there would only be half the amount of water as instructed by Ashton et al. (1979). Finally, nutrient deprivation was simulated by removing the diammonium tartrate supplement in the standard BCD medium. Diammonium tartrate was removed because it is a nitrogen source, and endophytes are known to mobilize nitrogen sources for the plant (Ashton et al. 1979; Carrol 1988).

Carbon dioxide Assessment and Apparatus

In order to approximate the level of carbon dioxide in the presence of organism(s) after a certain period of time, the organism(s) must be contained in a closed system to prevent the escape of gases. This closed system must still be penetrable in order to have a CO_2 reading using a PP Systems EGM-4 Environmental Gas Analyzer and rubber tubing. Thus, sterile and empty 15 mL BD Vacutainer tubes were used to encapsulate the medium and organisms involved in the experiment. The

rubber stoppers sealing the Vacutainer tubes were each then penetrated by a pair of 18 gauge needles of 38.1 mm in length that were extended by 41.45 cm of rubber tubing with an inner diameter of 3.18 mm. The ends of the rubber tubing were tightly sealed using binder clips to prevent the escape of gas.

Before the initial CO_2 level readings (t_o), all samples were opened and aerated with running air in attempts to standardize and equilibrate gases. CO_2 readings were recorded in parts per million. After the t_0 recording, the rubber tubes were sealed with the binder clips and put under fluorescent lighting for 72 hours, repeatedly alternating 12 hours of fluorescent lighting at 27.5 °C and 12 hours of d
chosen due to being evidently substantial for growth of *P. patens* under a similar medium (Hohe et al., 2001) while also not being too excessive to interfere with oxygen supply (Scragg, 1995). After the 72 hour growth period, the CO_2 level was recorded again and the respective plant tissues were dry massed. Dry massing is when biological tissue is heated and incubated for the removal of moisture. This was done as a way of standardizing plant tissue data since residual water and medium could obscure mass readings.

Figure 4. Experimental apparatus of PP Systems EGM-4 Environmental Gas Analyzer and tubing with sample.

Data Analysis

After recording values of carbon dioxide concentrations in parts per million, a ratio of micrograms of carbon to milligrams of dry plant mass was calculated. A carbon dioxide value in parts per million equals the number of moles of carbon dioxide per million moles of air. Thus, by using the Ideal Gas Law and the volumes of air in the vacutainer (0.005 L) and in the rubber tubing (0.003285 L), the moles of carbon dioxide could then be calculated with stoichiometry as the moles of air (n) was solved. Moles of carbon was then converted to micrograms of carbon using stoichiometry.

Equation 3. *Ideal Gas Law* $PV=nRT; n=PV/RT$

The micrograms of carbon in the samples containing plant samples were then used to provide a rate in which carbon is fixed over the 72 hour period. The samples that did not contain plant samples, the control and endophyte only samples, only had the carbon dioxide parts per million concentrations monitored for any fluctuations.

The average ratio of the micrograms of carbon to their corresponding dry plant masses were then compared between the plant only and plant endophyte samples for significant differences. A two-tailed t-test was used to observe any significant differences among the sample types. This procedure was repeated for all treatments for overarching conclusions for endophytic presence under abiotic stress.

Results

Control and Endophyte Samples

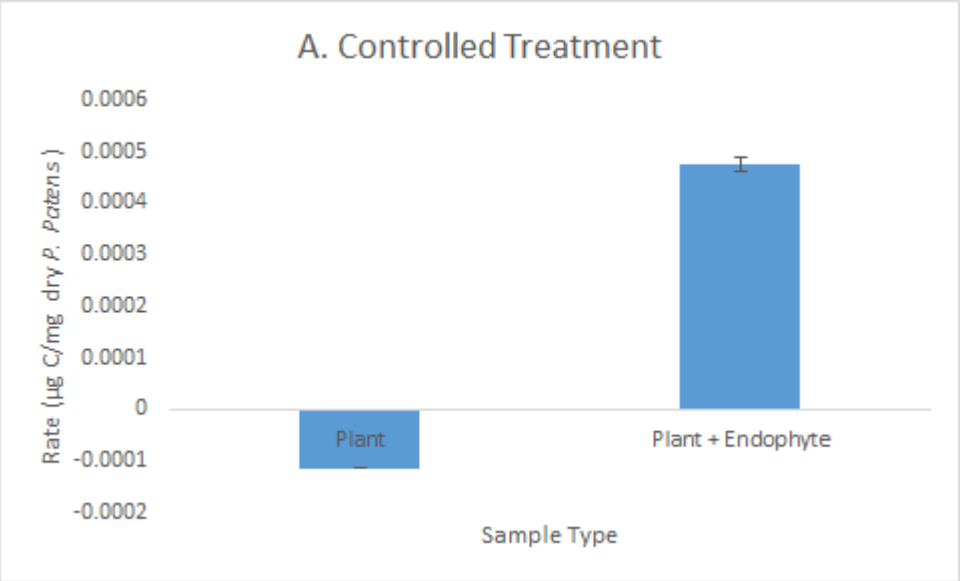
The parts per million of carbon dioxide for both the control and endophyte only samples were generally stable across all treatments (Table 1). The standard deviation of the recordings among these quintuplicate trials were calculated (Table 1).

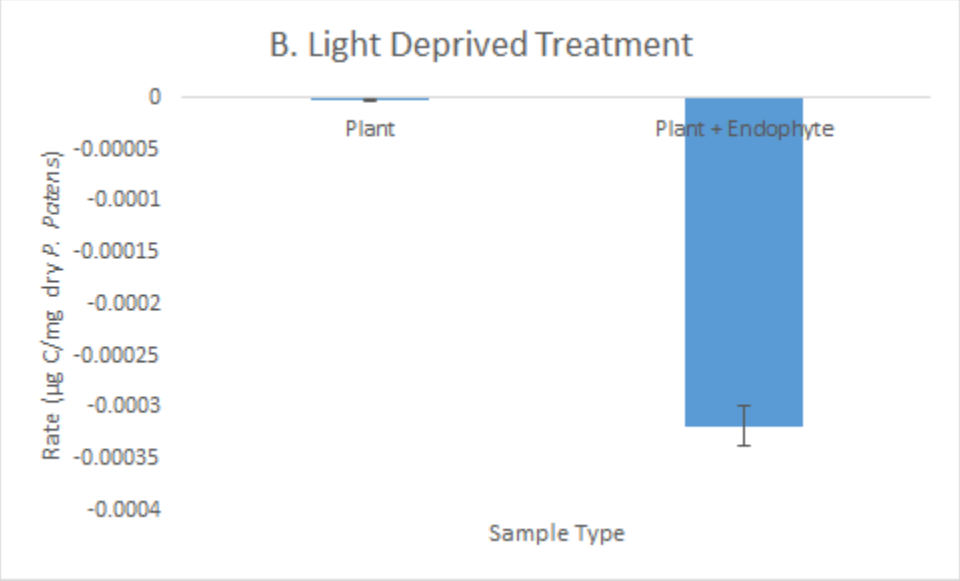
Sample Type	Control	Light Deprivation	Water Deprivation	Nutrient Deprivation
Control	1.36172E-06	1.64327E-05	4.92599E-05	6.18137E-06
Endophyte	1.9639E-05	1.75175E-05	3.26843E-05	8.29329E-05

Table 1. Standard deviation values of the carbon dioxide ppm for the control and endophyte samples (n=5).

Comparison of Carbon Mass to Plant Dry Mass

In the controlled treatment, the carbon fixation rate was higher in the plant samples containing endophytes as compared to the axenic ones (Figure 5A). This trend was also similar in the water deprived treatment (Figure 5C). For the darkness simulation, the mass of carbon to dry mass ratio for the plant and endophyte sample was lower (Figure 5B). Similarly, the nutrient deprivation treatment had the same trend of a lower relative carbon mass to dry mass ratio (Figure 5D).





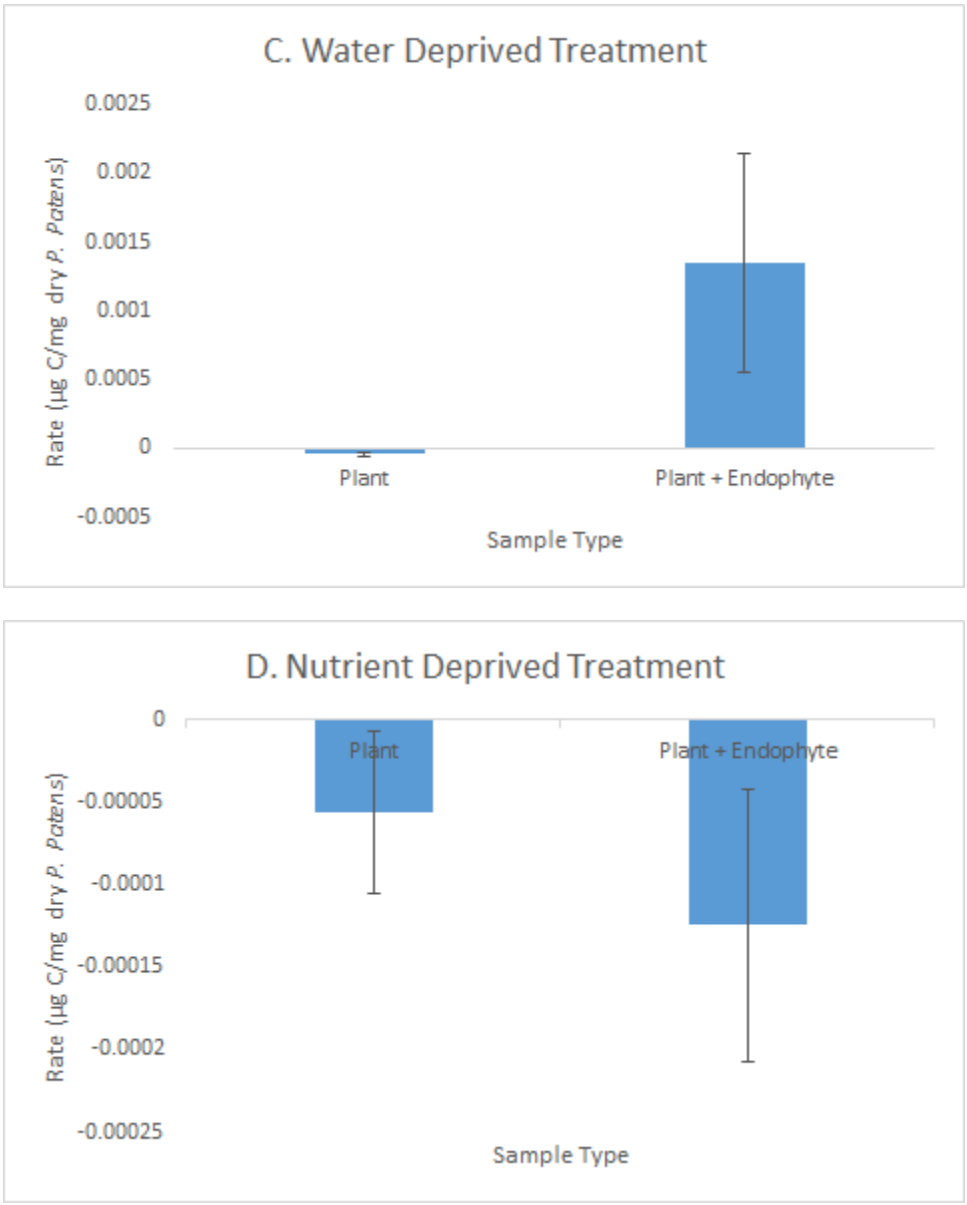


Figure 5. Average carbon respiration rates of the Control (A), Light Deprived (B), Water Deprived (C) and Nutrient Deprived (D) Plant and Plant + Endophyte samples in µg C/mg dry *P. patens*. Standard deviation is expressed using error bars and two-tailed t-tests were conducted for statistical analysis (n = 5; (A) P = 1.13E-07, (B) P = 0.10E-04, (C) P = 0.47E-02, (D) P = 0.154).

Discussion

Endophytic Effect Evaluation

Endophyte presence in a stress-free environment does not cause an apparent increase in photosynthesis (Figure 5A). Considering that this type of result does not match any previous study, it may be possible that certain conditions must be met in order to trigger endophytic symbiosis. Furthermore, endophytic presence in the light deprived treatment allowed for more photosynthesis to occur, based off of the proxy (Figure 5B). This result is rather strange because photosynthesis because the reaction requires stimulation from sunlight to properly occur (Eq. 1).

The results suggest that endophytes do not enhance photosynthesis when under water deprived conditions (Figure 5C). However, it may be possible that a higher concentration of agar in the medium may introduce more carbon in the sealed air. This is because agar is composed primarily of organic material from algae and the polysaccharide, agarose (Hohe et al. 2002). In terms of the nutrient deprived treatment, the error bars are far too big and overlap one another. This makes any observed differences due to chance (Figure 5D).

Future Directions

To find more promising results, modifications to the experiment will be needed. Firstly, I would suggest adding additional replicates to the samples as well as more temporal recordings to trace the levels of carbon dioxide over time. Another important consideration would be of protein misfolding. The protein RuBisCO plays a major role in carbon fixation, but may misfold and operate differently due to a suboptimal pH. If there is an excess of carbon dioxide gas, the pH of the system could change and disrupt

conventional carbon fixation (Mauseth 2014). If the study were to be repeated, an easier way of calculating photosynthesis would be ideal. Perhaps, invest in a photosynthesis meter. For this study, financial limitations prevented access to such an instrument. Additionally, it would be beneficial to identify the endophyte genus or species for connections to other endophytes and their compatibilities. The interspecific interactions with other plants and microbes should be considered in order to simulate actual communities and ecosystems. This way, a more genuine endophytic response can be accounted for, since it may be possible that endophytic performance is dependent on multiple species.

Conclusion

Using *P. patens* and endophytes, this study attempted to find patterns of metabolic performance when varying abiotic conditions. The hypothesis that endophytic presence enhances photosynthetic performance is unsupported in this experiment because many confounding factors and experimental design flaws still exist. Albeit that the initial recordings of carbon dioxide concentrations were fairly consistent for many trials, further examination and analysis of the endophytic symbiosis are required to truly grasp an understanding of endophytic effects during abiotic stress.

References

Aston, N. W., N. H. Grimsley and D.J. Cove. 1979. Analysis of gametophytic development in the moss, *Physcomitrella patens*, using auxin and cytokinin resistant mutants. Planta 144: 427-435.

Campbell, N.A., J.B. Reece, L.A. Urry, M.L. Cain, S.A. Wasserman, P.V. Minorsky, and R.B. Jackson. 2008. Biology: Eighth Edition. Pearson, New York, New York.

Carroll, G. 1988. Fungal Endophytes in Stems and Leaves: From Latent Pathogen to Mutualistic Symbiont. Ecological Society of America 69.1: 2-9.

Clay, K. and C. Schardl. 2002. Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses. The American Naturalist 160: S99-S127.

Faeth, S. H. 2002. Are endophytic fungi defensive plant mutualists? OIKOS 98: 25-36.

Faeth, S. H. 2009. Asexual Fungal Symbionts After Reproductive Allocation and Herbivory over Time in Their Native Perennial Grass Hosts. The American Naturalist 173.5: 554-565.

Fawcett, B. 2014. The Influence of Light on Bryophytes and Their Response to Pathogen Infection: A Story of *Mnium cuspidatum* and *Physcomitrella patens*. Undergraduate Honors Thesis Collection, Butler University, Indianapolis, IN.

Hohe A., E.L. Decker, G. Gorr, G. Schween, and R. Reski. 2002. Tight control of growth and cell differentiation in photoautotrophic growing moss (*Physcomitrella patens*) bioreactor cultures. Plant Cell Reports 20:1135–40.

Jenks M. A. and P. M. Hasegawa. 2008. Plant Abiotic Stress. Pages 14-81 in M. Jenks and P. Hasegawa, editors. John Wiley & Sons, West Lafayette, Indiana.

Levitt, J. 1982. Responses of Plants to Environmental Stresses. The Journal of Ecology 70.2: 696.

Mauseth, J. D. 2014. Botany: an introduction to plant biology. Pages 41-200 in M. Johnson, editor. 5th edition. Jones & Bartlett Publishers, Burlington, Massachusetts.

Saikkonen, K, P. R. Wäli, and M, Helander. 2010. Genetic Compatibility Determines Endophyte-Grass Combinations. PloS ONE 5.6: e11395.

Schaefer, D. G. and J. P. Zrÿd. 2001. The moss *Physcomitrella patens*, now and then. Plant Physiology 127.4: 1430-1438.

Scragg A. H. 1995. The problems associated with high biomass levels in plant cell suspensions. Plant Cell Tissue Organ Cult 43:163–17

Vujicic M., T. Cvetic, A. Sabovljevic, and M. Sabovljevic. 2010. Axenically Culturing the Bryophytes: A Case Study of the Liverwort. *Kragujevac J. Sciences* 32: 73-81.