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Effect of Relative Humidity on Respiration and Metabolism in the Moss Physcomitrella Patens

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EFFECT OF RELATIVE HUMIDITY ON RESPIRATION AND METABOLISM IN THE MOSS *PHYSCOMITRELLA PATENS*

by

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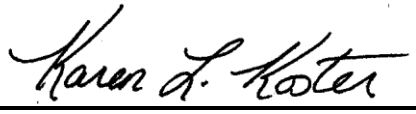

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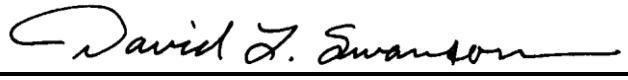

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ABSTRACT

Effect of Relative Humidity on Respiration and Metabolism in the Moss *Physcomitrella Patens*

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The goal of this research was to test the effect of relative humidity on respiration and metabolism in the moss species *Physcomitrella patens*. Although this moss does not usually survive rapid desiccation, tolerance of extreme water loss can be induced by very slow drying and acclimation for 6-10 days at high relative humidity (RH). During acclimation, numerous changes in metabolite content occur, but metabolic rates during this process have not been measured. My goal was to determine how respiratory metabolism is affected during acclimation of the moss *P. patens*. I used a Hansatech oxygen electrode to measure O_2 uptake by protonema samples of *P. patens* at full hydration and after varying times, up to 10 days, at 89% RH. The data suggest that respiration rates initially increased for up to 24 hours after the moss was transferred to 89% RH, then declined during the following days of acclimation. However, insufficient data were collected during this initial study to provide conclusive understanding of the effect of relative humidity on respiration in the moss *Physcomitrella patens*.

Keywords: *Physcomitrella patens*, respiration, desiccation, dehydration, relative humidity, oxygen consumption

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Introduction

Physcomitrella patens is a moss species that is being used as a model system to answer genetic, metabolic, and developmental questions. As a bryophyte, it is at an evolutionary position that is in some ways ideal for considering the biological processes of all land plants (Schaefer and Zrÿd 2001). Despite its simple morphology, at the cellular level, *P. patens* has similar responses to environmental stimuli as seen in many other land plants (Schaefer and Zrÿd 2001). It has been a model for plant experiments for many years, as initial reports by Cove (1992) suggested that *P. patens* could become a model system to study developmental genetics. The moss is simple in structure, has a short life cycle, and a dominant haploid generation. It does not require expensive or large facilities to grow. The life cycle of *P. patens* makes it ideal for studies, as it has the typical life cycle of a moss, with alternating haploid gametophyte and diploid sporophyte generations (Cove 1992). The growth pattern allows for quick sexual reproduction, with the whole life cycle complete in 12 weeks in optimal conditions. Within 3 weeks a gametophore can grow out of the protonema. Even as quickly as 7 days into the life cycle, a small 20 cell growth of a small plant can be identified (Prigge and Bezanilla 2010). The simple structure allows for a better analysis of the moss itself. Considering these factors, *Physcomitrella patens* can serve as a model system for further understanding desiccation tolerance in plants, along with other metabolic processes.

Under most conditions *P. patens* behaves as a mesic bryophyte: it grows in moist, temperate regions (Cove 2005) and it is killed by rapid drying to water contents below

approximately 0.3 g H_2O/gDW (Koster et al. 2010). It can, however, become desiccation tolerant during very slow drying conditions lasting several days to weeks (Wang et al. 2009, Xiao et al. 2018), or when the phytohormone abscisic acid ABA is applied to the plant by researchers (Oldenhof et al. 2006, Koster et al. 2010). ABA is seen to trigger at least 11 "stress" genes in *Physcomitrella patens* (Machuka et al. 1999). Studies have shown that this moss can survive mild drought, salt and osmotic stresses (Frank et al. 2005, Decker et al. 2008), making this moss a useful system for functional studies of dehydration stress at a cellular level. Thus, this plant might also serve as a model species when investigating the importance of metabolism in plant stress tolerance. Bewley and colleagues (1978) were among the first to do detailed studies on plant desiccation tolerance. They suggested, at the time, that there were few studies that had considered a focus on plants with a higher degree of drought tolerance, in comparison to those on plants with partial drought tolerance (Bewley et al. 1978). Many studies have considered plant desiccation tolerance since then, supporting Bewley's idea that understanding desiccation tolerance is important, as understanding desiccation tolerance has practical application. Desiccation tolerance has shaped the evolution of land plants and adapted over time to allow plants to survive (Oliver et al. 2005). This ability allows plant cells to survive severe water deficits that have direct effects on agricultural productivity, discussed later.

Despite much research in the field on desiccation tolerance, there is still much to discover and understand about desiccation tolerance and the role of aerobic respiration in helping plants to acclimate to desiccating conditions. Decreased water content can lead to lowered respiration rates, leading to lower overall metabolism (Flexas et al. 2005). This,

overall, might negatively influence the plant if metabolic changes are needed to tolerate the stress.

The relevance of this idea can then be placed in a larger scale, such as other plants. In a world that is faced with frequent, widespread, and reoccurring droughts, the effects of water deficit on plants demand attention. The environment places constraints due to drought and salinity in high magnitudes. This has a large impact on the world (Bartels and Sunkar 2007). Understanding desiccation tolerance, as a stress response, would allow for agricultural yields to be increased. If researchers could understand mechanisms of desiccation tolerance, then it may be possible to breed crop plants that have improved tolerance of drought. This could, hopefully, help to improve agricultural productivity in drought-prone regions.

Understanding the *Physcomitrella patens* Life Cycle

P. patens is a monoecious moss. This means that both sex organs are presented on the same individual. In total, this means that simple growth conditions are needed in order for *P. patens* to complete its life cycle (Schaefer and Zrÿd 2001). The life cycle can be broken down into two phases: a haploid gametophyte generation and a diploid sporophyte generation. *P. patens* can be used as model system, based on the simple developmental pattern of the haploid gametophyte that controls the life cycle (Schaefer and Zrÿd 2001), and the genetic approaches that arise from this dominance of the gametophyte (Cove et al. 1993). The heterotrophic sporophyte is simple and supported by the photoautotrophic gametophyte (Schaefer and Zrÿd 2001). The sporophyte is simple in comparison to the haploid gametophyte, as the gametophyte is broken into two distinct growing periods. The first stage consists of protonemata, which grow after the haploid spore germinates.

Protonema is a filamentous network of chloronemal and caulonemal cells. These develop by apical growth or cell division of apical and subapical cells (Schaefer and Zrÿd 2001). The second haploid stage is the gametophore. This is the leafy shoot. It differentiates from the protonemata by growth from a simple apical meristem. This gametophore contains photosynthetic, non-vascularized stem and leaf-like structures called phyllids. At the tip of the gametophore the reproductive organs, archegonia and antheridia, form and produce eggs and sperm, respectively. At the base of the gametophore stem filamentous rhizoids grow to anchor and absorb nutrients from the substrate (Reski 1998). Somatic cells in gametophyte tissues allow for extended culture, due to their high regeneration capacity. This life cycle can be completed in roughly three months. In addition to sexual reproduction, nearly all the cells, regardless of being gametophytic or sporophytic, are able to regenerate and produce new protonemata following mechanical damage to the tissue (Cove 1992). The protonemal tissue generated later gives rise to gametophores. The ability to regenerate new moss plants following mechanical damage enables vegetative propagation *in vitro* and also enables moss plants to regenerate from a few cells that may survive environmental damage in the field.

Defining Desiccation Tolerance:

Tolerance to desiccation can be considered in some moss species as a protective mechanism from periods of drought in the environment, but not all moss species are tolerant of extensive desiccation (Proctor et al. 2007b). Some define complete desiccation tolerance as drying to a water content of 10%, on a dry weight basis (g H_2O/ gDW), or less, then being able to resume normal growth upon rehydration (Cui et al. 2010). Desiccation tolerance, in this study, can be defined as a moss that can suspend its

metabolism in a low humidity state (<50% RH) and return functionality to itself when humidity increases (Alpert 2000). The plant must be able to sustain its structures during this lack of water content and be able to repair these structures to a sustainable level upon rehydration (Alpert 2000). Genes (Cuming et al. 2007), metabolites (Rathnayake et al. 2017), and proteins (Wang et al. 2009) change based on the extent of desiccation. Recent studies using *P. patens* have found that desiccation tolerance can be induced by acclimation at 89% relative humidity (RH) for 6-10 days (Rathnayake et al. 2017). The dehydration rate correlates to the relative humidity of the surrounding atmosphere, so at this high RH, water loss is slow. During this acclimation period, the moss loses 90% or more of its water, but continues to undergo some metabolism (Rathnayake et al. 2017). Data from Rathnayake et al. (2017) shows hundreds of changes in metabolites that occur during acclimation at 89% RH. Many of these metabolic changes are thought to protect the moss cells from desiccation damage, although not all mechanisms for desiccation protection are understood. However, the structural changes that occur during desiccation must be repairable or tolerable for the plant to be considered desiccation tolerant, so these metabolic changes may help the moss to survive desiccation. Desiccation can be harmful to plants in many ways, including cytoskeletal damage that occurs due to cell volume change (Oliver et al. 1998). This is one example of structural change that could occur that must be repairable upon rehydration for the moss to be considered desiccation tolerant.

Precisely how metabolic changes take place as cells dehydrate is under investigation. Oliver et al. (2007) suggests that cells in a drier state do not have enough water for most aqueous metabolic reactions to occur, which can increase cellular damage, if reactions are

not regulated correctly. Water loss creates a cell that has increased viscosity. As dehydration advances, the cellular metabolites that could react with one another increase in concentration. However, the increased viscosity slows down reactions, as the metabolites are less likely to be able to diffuse.

The moss *Tortula ruralis* (*Syntrichia ruralis*) tolerates almost complete loss of protoplasmic water (Wood and Oliver 2004). Key factors to this tolerance are an abundance of sucrose and the accumulation of a dehydrin-like protein; dehydrins are highly hydrophilic and water-soluble proteins that lack extensive secondary structure (Wood and Oliver 2004). These molecules are thought to limit damage in the presence of stressful factors. These are key factors of the same desiccation tolerance system in vascular plants (Wood and Oliver 2004). In *T. ruralis*, the dehydrin-like protein was abundant during rehydration of the moss, while high concentrations of the sugars were found both before and after desiccation. Wood and Oliver (2004) suggested a constitutive cellular protection was working in conjunction with a repair mechanism during rehydration. However, this hypothesis faces scrutiny. Walters et al. (2002) suggest that the critical moisture level that a moss can survive is determined by treatment duration, the metabolite concentration, and any physical barriers that separate metabolites.

In comparison, *P. patens* is not able to perform desiccation tolerance to this degree. The gametophores tend to survive desiccated situations better than the protonema tissue (Cui et al. 2010). However, tolerances among tissues need to be further explored (Cui et al. 2010). This demands understanding of the changes in metabolites, organelles, or cells that contribute to desiccation tolerant responses. There are 130 genes that are induced in

response to dehydration in protonemal tissue when compared to the 19 genes that respond in gametophores, based on microarray analysis (Frank et al. 2005, Cuming et al. 2007, Decker et al. 2008). Similar gene responses in vascular plants are associated with abiotic stress. In *P. patens*, this reinforces the idea that their desiccation response is similar to that of angiosperms. They can mobilize protection mechanisms, and repair damage after rehydration. There is some research towards understanding induced proteins and their responsive genes and their functionality, but much left to be learned. This leaves the mechanisms of desiccation tolerance in *P. patens* uncertain (Frank et al. 2005, Cuming et al. 2007). Bryophytes can survive desiccation through many complex mechanisms, not just one, leaving much to be discovered (Oliver 1996, 2007, Oliver et al. 2005).

Understanding Respiration in Moss

Respiration plays a vital role in the development of desiccation tolerance. The majority of plants use aerobic respiration. This is the normal respiratory process. This occurs through the oxidation of carbohydrates and fats. They are broken down into carbon dioxide and water, as oxygen is used from the atmosphere to effect the oxidation process (Raich and Schlesinger 1992). A decline in water content during desiccation can be linked to an accompanied disability to respire and photosynthesize. This is also linked with studies that show gas exchange does not occur in a desiccated state of these plants, but is reactivated once rehydration occurs (Bewley 1979). For example, oxygen use is impaired during desiccation, but it improved upon rehydration (Bewley et al. 1978). There is a significant "respiratory burst" in plants, such as moss, when recovering from a dehydrated state (Bewley et al. 1978). These basal plants can even, in many cases, be seen to have enhanced respiration abilities upon rehydration (Stiles 1960). To some

extent, there has been little evidence, besides the works of Bewley and colleagues, to measure respiratory function after differing rates of desiccation. There is also a lack of knowledge in this subject, relative to the moss *Physcomitrella patens.* However, there is good evidence from Bewley's work that the recovery of respiration allows these moss samples to be able to return to a normal function after a desiccated state. Bewley et al. (1978) compared effects of slow and rapid desiccation of *Tortula ruralis*, a desiccation tolerant moss, and *Cratoneuron filicinum*, a moss that does not tolerate desiccation. Despite three different drying rates, most moss samples seemed to resume normal activities in oxygen exchange, including respiration. Bewley et al. (1978) further demonstrated that the timing of desiccation affects the rate of oxygen consumption during rehydration. When moss was rapidly dried in the initial growth stage of the moss, consumption was only slightly elevated. When rapidly dried in the later stage of growth, the increase in oxygen was pronounced upon rehydration. However, the most consistent increase was when the moss was completely dried at the rapid speed (Bewley et al. 1978). Recovery of respiration, or these "respiration bursts" is correlated with the speed of dehydration. If dehydrated quickly, the rate of respiration burst is seen to be quicker upon rehydration. This poses the question of whether this is to help the plant recover more quickly using energy and products of respiration (Bewley et al. 1978). Respiration bursts have been shown to be relevant in other bryophytes, as well (Proctor and Dilks 1974). The dependence of O_2 consumption could be relevant to the dehydration process, as slower desiccation may lead to more controlled adjustments to be made throughout organelles or enzymes in the plant system. Overall, Bewley's and others' discoveries in these moss samples suggest that similar factors could be affecting desiccation tolerance in

Physcomitrella patens. A follow-up study by Bewley et al. (1987) looked more closely at the ATP contents of desiccation sensitive mosses in relation to O_2 uptake during desiccation. ATP content of the mosses did not seem to be correlated with this O_2 consumption. It seemed to be more affected by the rate at which water loss was occurring in the moss. With rapid water loss, there was prevalent ATP found. Overall, the observations on desiccation sensitive moss after desiccation and rehydration seemed to point to the idea that these phenomena could be related to damage in mitochondria or cellular damage caused from differing speeds of dehydration. However, it was noted that the rate of water loss mattered more than the levels of ATP in recovery. The moss tended to show no correlation of O_2 uptake to drying rate, while ATP available after slow and rapid drying differed significantly. ATP production was relatively similar, despite different drying rates. (Bewley et al. 1987).

Desiccation damage tends to happen based on the proximity of the cellular entities. The byproducts of respiration and chloroplast activity contribute to this damage, due to proximity in a desiccated species (Oliver et al. 1993). *Tortula ruralis*, which is desiccation tolerant, is able to repair severe damage caused by rapid desiccation. *Crateroneruon filicinum*, which does not tolerate desiccation, was unable to do so. O_2 uptake may be related to the amount of damage that moss is subjected to during studies. In this study (Oliver et al. 1993), it was seen that the metabolism of *T. ruralis* was affected more by prior rapid desiccation than by slower rates. In the moss *C. filicinum*, there was greater correlation between ATP collected, O_2 consumed, and the damage detected. There was a low level of O_2 consumption with large damage, and low levels of ATP were detected (Krochko et al. 1979). Although it is clear that the rate of desiccation

impacts respiration, the effects of prolonged exposure to decreased water contents on respiration are unknown.

Photosynthesis and respiration can recover rapidly from desiccation in tolerant species. Slowly-dried, desiccation tolerant bryophytes that are rehydrated have been shown to have no lasting damage to cellular structure (Proctor et al. 2007a). *Polytrichum formosum* slowly dehydrated with ambient air at 50% RH showed cell structure that was not damaged by desiccation. Respiration did recover with initial respiration rates being rapid upon rehydration and seeming to be independent of protein synthesis (Proctor et al. 2007a). Full recovery of respiration occurred within hours, while the cell cycle recovered in 24 hours or more (Proctor et al. 2007a).

Summary and Rationale:

In a world faced with widespread drought and warming climates, understanding desiccation tolerance in a model organism, such as *Physcomitrella patens*, allows for rapid research that can be applicable to larger plant models. Identifying variations in metabolism and respiration caused by changes in RH and cellular water content allows greater understanding of mechanisms of desiccation tolerance. The purpose of this study is to measure aerobic respiration in *P. patens* during the acquisition of desiccation tolerance. As noted earlier, desiccation tolerance in *P. patens* is an inducible feature (Koster et al. 2010) and Rathnayake et al. (2017) developed a protocol to induce tolerance by a slow acclimation at 89% RH. During acclimation, hundreds of metabolites change, including those in cellular respiratory pathways (Rathnayake et al. 2017). The goal of this study was to measure aerobic respiration in the dehydrating moss plants to see how respiration rates are affected by extended dehydration. By calculating aerobic

respiration rates, based on oxygen uptake as measured by an oxygen electrode, the effect of lowered RH can be compared between the gametophores and protonemata as they acclimate and acquire desiccation tolerance. Although there is knowledge of the many molecular features of desiccation tolerance, the mechanisms that come together to confer the phenotype are not yet well understood (Frank et al. 2005, Cuming et al. 2007, Cui et al. 2012). The goal of this study was to measure aerobic respiration to determine whether, and how long, respiration can occur in partially dried moss and provide substrates and energy for the metabolic changes detected by Rathnayake et al. (2017) during acclimation of the moss leading to desiccation tolerance.

Materials and Methods

Moss Culture and Acclimation Process

P. patens (Hedw.) Bruch and Schimp., 'Gransden' ecotype was donated by Dr. Daniel Lynch (Williams College). Ranthnayake grew the moss in axenic culture on 9 cm diameter cellophane discs. These discs were placed on top of solid BCDA medium, described by Cove et al. (2009). The moss was cultured and maintained for 3 to 5 weeks in a Percival 136-LLX growth cabinet prior to use at 89% RH. The temperature was maintained at 24 °C with a 16:8 light: dark cycle at 55-60 μmol-m^{-2}/s^{-1} photosynthetic photon flux density. At 3-weeks, the moss was dominated by protonemata, while at 5-weeks, the moss had numerous gametophores.

Research by Rathnayake demonstrated that *P. patens* can develop desiccation tolerance during prolonged acclimation at 89% RH and 25 °C. Protonemata become tolerant after 8 days of acclimation, while gametophores require only 6 days of acclimation to become tolerant (Rathnayake et al. 2017). Since the purpose of my research was to investigate

respiration rates during acclimation, similar growth and acclimation conditions to those used by Rathnayake were used.

Controlled relative humidity chambers were made to allow samples of *P. patens* to dehydrate and acclimate to acquire desiccation tolerance. Each chamber contained a saturated salt slurry made of aqueous $MgSO_4$. Aqueous solutions are a relatively inexpensive and simple method to maintain relative humidity. The solute usually is non-volatile. At any temperature that is constant, a saturated solution can be fixed at a certain concentration, meaning the solute does not need to be determined. The excess solute allows for the solution to remain saturated (Greenspan 1976). One given salt solution can produce one relative humidity, at one given temperature. A variety of selected saturated salt solutions can help to produce a range relative humidities, in conditions researchers create (Greenspan 1976).

Samples for assay were prepared by cutting sections of the moss culture on the cellophane into pieces measuring approximately 2 cm in diameter. Samples were placed into aluminum weighing dishes and suspended on plastic mesh above the $MgSO_4$ slurry. The chambers were kept in the same growth cabinet as before, at 24 °C and with a 16:8 photoperiod. The chamber RH was measured through constant Bluetooth monitoring, using Sensirion SHT31 RH probes. These apparatuses were attached to the lid of the chamber and measure at a ± 2% RH accuracy. If the chamber had inconsistent humidity, the water content in the saturated salt solution was adjusted.

The 2 cm pieces of moss remained in the RH chamber for varying times to match the time course for acclimation determined by Rathnayake et al. (2017). Samples were removed for non-destructive measurement of O_2 uptake and then returned to the 89% RH

chamber. Measurements were taken after 12, 24, and 72-hour periods and then after 5, 7, and 10 days

Measuring Oxygen Uptake using a Hansatech ™ Oxygen Electrode

Measurements of oxygen uptake or release by tissues are easily done using a closed analysis system (Walker 1987). Oxygen exchange from moss tissue was measured using a Hansatech (King's Lynn, England) measuring system with an LD2/3 gas chamber connected to a CB1-D controller. The CB1-D controller was interfaced to a Windows-based computer to permit continuous electronic acquisition of voltage data from the oxygen electrode. Data were processed in Microsoft Excel® software. The Hansatech system uses a closed chamber in which the sample is completely sealed inside the apparatus; changes in oxygen concentration in the chamber atmosphere are measured using a Clark-type electrode beneath the base on the sample chamber. This is called the leaf disc well. The O_2 sensing electrode disc is under this chamber. The electrode disc consists of a dome-shaped platinum (Pt) cathode that is exposed to the air in the sample chamber and a silver (Ag) anode in a groove around the periphery of the disc. A saturated KCl solution forms a salt bridge between the cathode and anode. For use, the electrode is covered by a drop of the KCl solution, a small piece of cigarette paper, and a thin cellophane membrane that is permeable to oxygen. The paper serves to maintain a uniform KCl solution between anode and cathode. These are all held in place by an O-ring that seals around the dome of the cathode and fits into the groove holding the anode. The moss sample will be pushed slightly against the temperature controlled roof of the chamber by foam discs for accurate measurements. After the moss is placed in the leaf disc well, clips draw the middle and top sections together to form an air-tight seal.

Stopcocks on each side of the leaf disc well allow for air to be added or removed from the system. This allows for calibration using nitrogen gas (N_2), or air saturation of the system.

When moss is enclosed in this system, oxygen concentration in the sealed chamber will decrease as respiration occurs in the dark. If the tissue is exposed to light, oxygen generation could occur via photosynthesis.

Oxygen exchange from the moss in the apparatus was always measured in the same room with a constant temperature of 24 °C, in darkness. After experimentation, the moss was allowed to oven dry for 4 days at 70 °C. This allowed for the dry weight of the moss to be measured and used a constant basis for comparing respiration rates.

Oxygen Measurements

As a voltage is applied to the electrode, the current that first occurs is minimal and the electrode becomes polarized. This is due to adaptation to the externally applied potential (Walker 1987). As potential is increased, close to 600-700 mV, oxygen is reduced at the electrode and the polarity discharges. This is due to electrons being donated to oxygen, which acts as an electron acceptor. The current is electrically generated by this reduction of oxygen that occurs at the cathode and is displayed as voltage by the Hansatech CB1-D control box. When using this system, the output signal was monitored in the 1-volt range. To ensure accurate readings, calibration must occur to adjust the numerical signal so that it reflects a known amount of O_2 reacting at the electrode. For convenience, the electrical zero and oxygen zero were made equivalent by passing 60 mL of nitrogen gas (N_2) through the electrode and adjusting the readout to 0 mV (Fig. 1). Afterwards, the chamber was flushed with 60 mL of air to obtain a mV reading for the concentration of

atmospheric O_2, calculated based on the known O_2 content of air at the elevation of Vermillion, SD (372 m). At the current elevation and 0 °C, 1 mole of gas occupies 23.341 L (Walker 1987). Air is 21% oxygen; 1 mL contains 210 μL oxygen and thus 8.96 μmoles (210 μL /23.431 μL· μmol^{-1}). At any other temperature the following equation is used: $\frac{273}{273+T} * 8.96$ (= 8.21 μmol O_2/mL air at 25 °C).

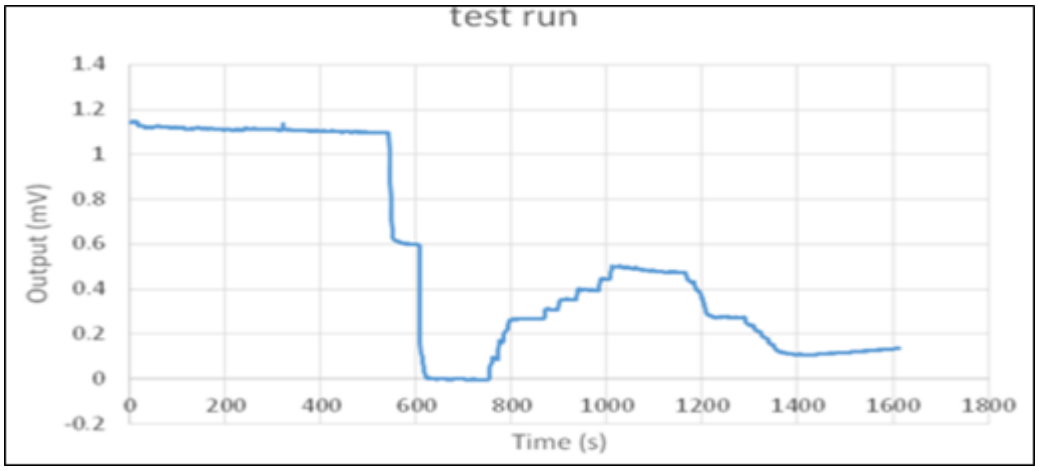
To calculate rates of O_2 change, this number was divided by the initial mV readout, to give the voltage conversion factor in terms of μmol O_2/mL air/mV output. Since the volume of air in the chamber was constant during each trial, the conversion was used as μmol O_2/mV. The slope of the change in O_2 concentration per minute (mV/min) for each 5-minute section of output from the sample was analyzed. This number was multiplied by the voltage conversion factor in μmol O_2/mV. This allowed for the calculation of oxygen consumption in units of μmol O_2/min for each 5-minute interval. These rates for each interval were then compared to see if oxygen consumption changed during the course of the measurement.

Sample Experimentation

Moss samples on cellophane were placed into the oxygen electrode apparatus, which was then sealed. The initial samples were fully hydrated and were placed on dampened filter paper in the chamber to ensure humidity and water levels remained the same throughout the experiment trial. Moss was left for 20 to 30 minutes, with mV readings taken manually every 5 minutes, as well as with the CB1-D controller interfaced to a Windows-based computer. This allowed for continuous electronic input of voltage data from the oxygen electrode.

Moss samples measured during acclimation at 89% RH were placed into the leaf disc chamber on cellophane, without addition of damp filter papers. A Luer-lock syringe was then used to flush 89% RH air from a controlled humidity chamber into the leaf chamber to ensure that the O_2 exchange by the moss would be measured under the same RH as the acclimation conditions. The initial mV reading of the CB1-D control box was recorded and oxygen consumption was measured for 20 min as voltage changed from this initial value. Millivolt output data were recorded at 5-minute intervals. Following this, the moss was immediately returned to the 89% RH chamber for a designated period, before the next measurement of oxygen consumption. The temperature of the lab was recorded and was 24.5 – 25.8 °C throughout the course of the trials. The trials were done during the same time of day, approximately beginning at 7 p.m. Between each sample, the electrode was flushed with N_2 gas then calibrated with air to ensure more precise readings (Walker 1987).

Figure 1: Example of O_2 electrode calibration prior to use. The machine was allowed, after initial setup, to settle for a period of about 500 s. The machine was then subjected to N_2 insertion, at 20 mL segments, until 60 mL was reached. After "electrical zero" was reached the system was then subjected to 1 mL intervals of atmospheric air. This measured the effective calibration, as well as the system's reaction to oxygen measurements.



Results

Oxygen exchange by *P. patens* was measured sequentially for pairs of samples at each date of sampling before and during acclimation. Figures 2-5 show oxygen uptake traces from moss protonemata prior to drying and after 5 and 10 days of acclimation at 89% RH. Sample numbers remained the same throughout, so that, for example, "sample 1" in each figure from shows data from the same section of protonema, measured at different times. Hence, this is one acclimation experiment with two replicates.

Figure 1 shows oxygen uptake by the fully hydrated moss before it was introduced to 89% RH chambers. This provides an oxygen uptake curve for reference. Focusing on the first five minutes allows for the greatest changes in respiration to be seen. This is because it may be possible that the moss was respiring at high rates, when initially in the chamber. After consumption of O_2, slower rates may occur since there was less O_2 in the chamber. This makes the initial rate, or first five-minute segment, most accurate for measurements. The slopes in the following are given as $\frac{mV}{min}$.

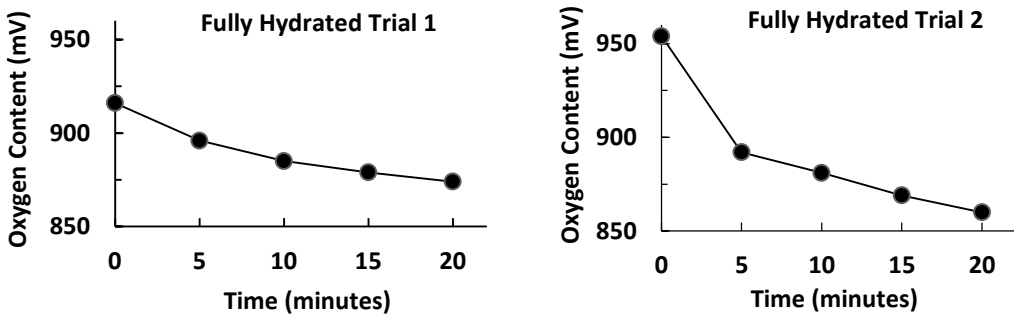


Figure 2: Oxygen uptake by fully hydrated protonemata, prior to acclimation. The temperature in the lab was 24.7 °C.

The data here were analyzed as described above. The slope of the first five-minute segment was -4 mV/min in trial 1. The slope of the moss in trial 2 was -12 mV/min. Some variation exists between these two values, indicating the need for additional trials. The values of this slope mean the moss had high levels of O_2 consumption, indicating that the moss may be respiring relatively quickly in the first five-minute section. The data in Fig. 2 show that the level of O_2 uptake slowed somewhat after that initial segment.

The next curves (Fig. 3) show oxygen consumption from moss left in an 89% RH chamber for 5 days. The slope for trial 1 was -3.5 mV/min for the first five-minute period. The slope for trial 2 was -2.2 mV/min for the first five-minute period. This slope shows that the moss was still consuming O_2 in the first five-minute segment, but uptake was slower than in the fully hydrated trial. This suggests that the moss was not respiring at the same rate it initially was. It could indicate that the water content loss, from the exposure to 89% RH, had decreased respiration rates.

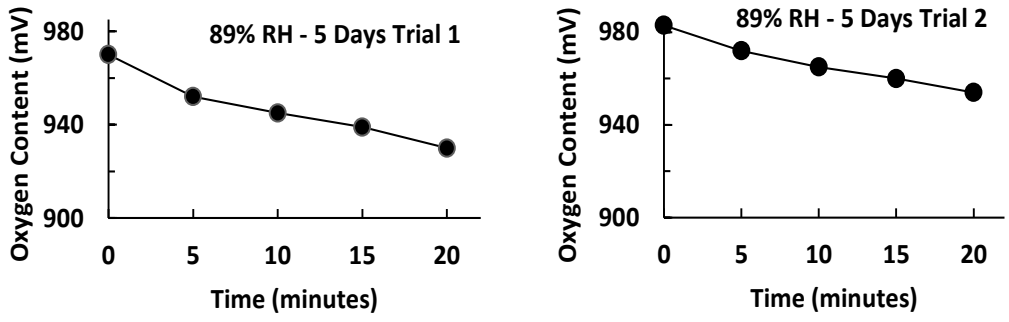


Figure 3: Oxygen uptake from protonemata acclimated at 89% RH for 5 days. The temperature in the lab was 24.8 °C

The last data shown (Fig. 4) shows oxygen consumption by samples left in the 89% RH chamber for 10 days. The slope for trial 1 was -0.4 mV/min for the first five-minute period. The slope for trial 2 was -0.6 mV/min for the first five-minute period. The slope had decreased from the fully hydrated trials and was also lower than after 5 days at 89% RH (Fig. 3).

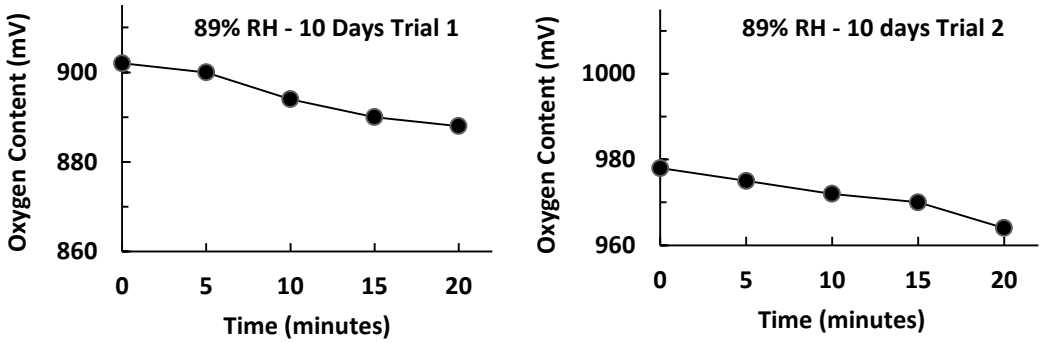


Figure 4: Oxygen uptake from protonemata acclimated at 89% RH for 10 days. The temperature in the lab was 26.1 °C

Table 1 shows how the data from oxygen uptake curves were converted to respiration rates. The columns labeled mV/min show the slopes of the O_2 exchange curve (mV output from O_2 electrode) for different time periods of the trial. From the left, the second column (mV output) shows the initial O_2 measurements from the chamber. The initial O_2 concentration of the atmopshere at this elevation and temperature (8.21 μmol O_2/mL) is divided by this second column. This number was then multiplied by the $\frac{mV}{min}$, or the slope of the trials, as given earlier. This gives the O_2 consumption, in μmol/min, for each time minute segment. This analysis is repeated for each segment with the specific O_2

consumption of that segment multiplied by the $\frac{mV}{min}$. The final rates graphed in Figure 5 were obtained by dividing O_2 consumption by the dry mass of the tissue.

Table 1: Calculations of Oxygen uptake by fully hydrated protonemata, prior to acclimation. The data are shown as an example of how the raw O_2 uptake data were converted to respiration rates. Details of calculations are described in the text.

	Initial O_2 Content and Conversion Factor			**0-5 min**		**5-10 min**		**5-15 min**	
	µmol O_2/ mL air	**mV output**	**µmol O_2/mV**	**slope mV/min**	**RATE µmol O_2/ min**	**slope mV/min**	**RATE µmol O_2/ min**	**slope mV/min**	**RATE µmol O_2/ min**
Trial 1	8.21	916	0.0090	-4	0.0359	-2.2	0.0197	-1.7	0.0152
Trial 2	8.21	954	0.0086	-12.4	0.1067	-2.2	0.0189	-2.3	0.0198

Figure 5 summarizes the data obtained from protonemata throughout the course of acclimation. The initial points, for fully hydrated moss, show the greatest difference among replicates, but subsequent measurements gave more consistent rates. The curve at the beginning of the graph shows the increased O_2 uptake during the initial stages of dehydration at 89% RH, which indicates increased respiration occurring. Respiration rates peaked at 24 hours of dehydration, then began to decrease. By 72 hours, the protonema are responding to their new environment with a depressed respiration rate. By days 7 and 10, the respiration rates are very low, probably because the moss is dehydrated after lengthy exposure to 89% RH. Rathnayake et al. (2017) found that moss under these conditions loses about 90% of its water content. The data indicate that moss initally experiences an increase in oxygen consumption as it begins to dehydrate, follwed by a decline in oxygen consumption around 72 hours, to begin to compensate for

dehydration. Oxygen consumption tapers out by day 7 and is negligible by day 10. Overall, these data indicate that RH and water content do have a large effect on respiration rates in the moss *P. patens*.

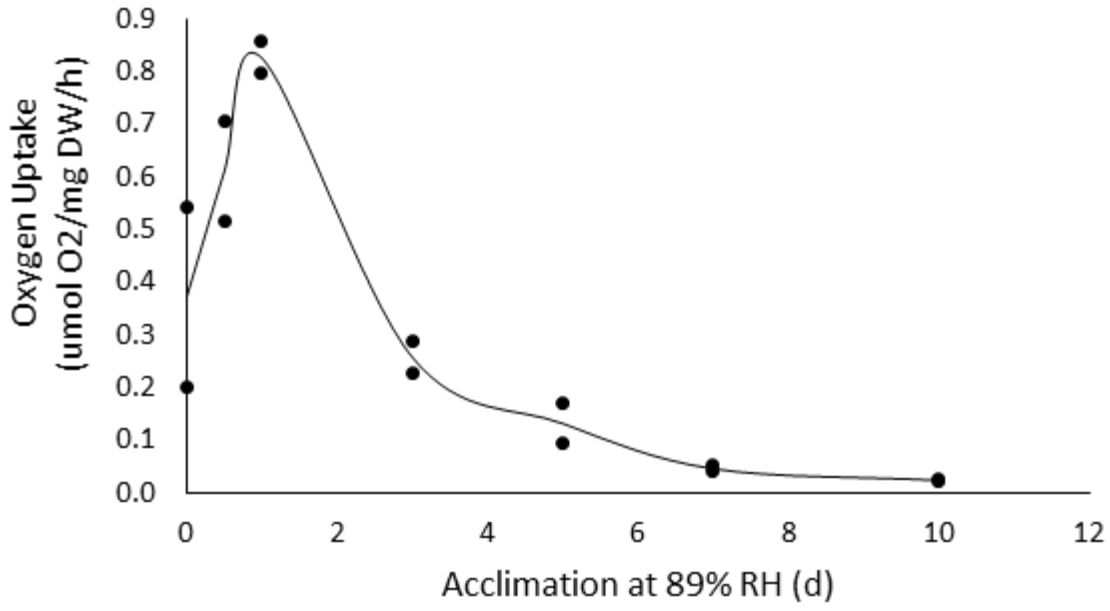


Figure 5: Oxygen uptake from protonemata acclimated at 89% RH over 0-10 days. Each point represents a rate measured from a single moss sample after exposure to 89% RH for the period of time noted on the x-axis. Time 0 samples are from fully hydrated moss, prior to dehydration. Respiration rates were calculated based on the dry mass of the samples, obtained after acclimation. The line connects the means of the two rates from each acclimation time and is intended to aid the eye in following the trend.

Conclusion

Overall, the moss displays signs that there are respiration accommodations during exposure to 89% RH. Figures 2-4 indicate that the moss was not consuming as much O_2 after 3 days of exposure to the dehydrating conditions in the RH chamber. This indicates lower respiration rates. This could be linked to greater loss of water content, leading the moss to experience greater dehydration stress. Figure 5 summarizes these findings and indicates that as the moss faces longer acclimation periods, it is less likely to consume O_2. These decreased respiration rates followed a rise in O_2 consumption rates detected during the first 24 hours of exposure to 89% RH. A similar increase in respiration was reported by Bewley and Thorpe (1974) for the moss *Tortula ruralis* that was partially dehydrated to about 65-76% of its initial weight. In that study, further dehydration led to respiration rates lower than those of the hydrated moss, as was also seen in our samples (Fig. 5).

Figure 5 also shows variability between the respiration rates measured for fully hydrated moss. This could indicate that moss was initially recovering from damage done while obtaining the samples. The initial trauma and stress that follow the snipping of the sample from larger moss cultures could explain the variable respiration rates, as the extent of damage might differ from sample to sample.

The moss changes respiration rates in response to the stress experienced by dehydration during acclimation. Overall, figures 2-5 seem to state that there is a connection between conservation of metabolism s and respiration. Lowered respiration rates could lead to overall lower metabolism due to decreased water content (Flexas et al. 2005). Studies from Bewley et al. (1979) indicate that gas exchange does not occur in a desiccated state

of bryophytes, but is helped with increased water content. However, the preliminary data presented here suggests that gas exchange could occur during the first days of acclimation, as the moss adjusts to the dehydration stress. The longer the moss adjusts to decreased water content at 89% RH, the slower respiration and gas exchange occur, indicating conservation mechanisms for plant survivial.

Bewley et al. (1978) states that "respiratory bursts", or respiration recovery, were needed to allow the moss to sustain normal function upon rehydration. In Bewley's study (1978) with *Tortula ruralis*, a desiccation tolerant moss, and *Cratoneuron filicinum*, a moss that does not tolerate desiccation, it was shown that oxygen consumption was impaired following desiccation, but was short lived following rehydration, in *T ruralis*. The data presented above suggest that the reason for this small recovery time is that the moss continues to respire at higher rates for 24 hours, before slowing respiration.

Similar findings were reported by Proctor and colleagues (2007a). In Proctor's study (2007a), *Polytrichum formosum* was slowly dehydrated with ambient air at 50% RH. Proctor measured an initial respiration for a control, then measured respiration after desiccation and rehydration. Respiration rates recovered to the initial respiration rate with rapid rehydration and seem to be independent of protein synthesis (Proctor et al. 2007a). Bewley and Proctor and their colleagues concluded that respiration resumes quickly upon rehydration. The present study, combined with those of Proctor and Bewley, indicate that oxygen exchange and desiccation are strongly correlated. Decreasing RH leads to decreasing water content of the moss and, thereby, causes changes in the respiration rate to occur over time, as seen in Figure 5. The moss shows signs of slow respiration as both water content and nutrient availability decrease.

Overall, it is suggested that lowered RH contributes to decreased respiration rates by not allowing metabolites to diffuse. This is due to the increasee in viscosity in the cell with the loss of water at lower RH.

The extent of the connection between relative humidity, dehydration , and respiration cannot be determined based on this study. Unfortunately, there are insufficient data to make a credible claim, indicating a need for additional studies.

Continuation

As this thesis and research comes to an end, I would like to continue with this study. The thoughts and research presented are preliminary and beg to be further explored in Dr. Koster's lab. Understanding how metabolites are affected by dehydration at different relative humidity levels through its influence on respiration, will allow for desiccation tolerance in the moss *Physcomitrella patens* to be better understood. I would like to verify my findings with more data and connect my results with the work done by Rathnayake in the Koster and Wone labs to see how differing aerobic respiration rates contribute to acclimation and the acquisition of desiccation tolerance in *Physcomitrella patens.*

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