Examining the influence of calyptra size on sporophyte morphology in Funariaceae mosses

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ABSTRACT

Maternal care is a critical component of offspring survival, development, and fitness in many organisms. In bryophytes (mosses, liverworts, and hornworts), offspring sporophytes are physically attached to and nutritionally dependent on the maternal gametophyte throughout their lifespan. Mosses have a structure called the calyptra, which is a maternal cap of gametophyte tissue that covers the sporophyte apex during critical developmental stages. The calyptra has shown to positively impact sporophyte survival and fitness. However, the influence of calyptra size on sporophyte development, survival, and fitness have not been tested. We hypothesize that sporophytes with larger calyptra experimentally placed on their apex will be taller and produce more spores. To test our predictions, we conducted a removal replacement experiment. We found that larger calyptra influenced sporophyte growth and produced longer capsules, but sporophyte fitness was not impacted. Maternal investment in larger calyptra and its positive effect on sporophyte height may also directly impact spore dispersal and in relation to species distributions and fitness.

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SECTION 1: INTRODUCTION

Bryophytes include three morphologically distinct clades: Anthocerotophyta (hornworts), Bryophyta (mosses), Marchantiophyta (liverworts; Renzaglia et al., 2000, 2018; Puttick et al., 2018). All land plants have life cycles that undergo an alternation of phases between haploid gametophytes and diploid sporophytes. Bryophytes are the only terrestrial plants that are gametophyte dominant. The gametophyte is the main photosynthetic phase that is observed on the landscape, while the bryophyte sporophytes are smaller, physically attached to, and nutritionally dependent on the gametophyte throughout their lifespan (Vanderpoorten and Goffinet, 2009). The sporophytes arise within the female sex organ, the archegonium, and develops to produce the sporangia (capsule) in which meiosis occurs and spores are produced. The sporophytes and sporangia differ morphologically across the three lineages. Liverwort sporangia are elevated above the substrate by an ephemeral seta that generally increases in length through cell elongation rather than cell division. The hornwort sporangium is highly elongated and the sporophyte and lacks a seta (Vanderpoorten and Goffinet, 2009). In mosses, terminal sporangium is elevated by a seta that persists until long after spore dispersal and increases in length via both cell division and elongation (Figure 1 in Appendix).

Mosses have a maternal gametophytic structure, known as the calyptra. The calyptra is covered in relatively thick and layered cuticle (Budke et al., 2011), which impacts the overall survival and fitness of the sporophyte; it protects the developing sporophyte from desiccation and increases sporophyte survival and reproductive output (Budke et al., 2013). The calyptra is formed from tissues of the archegonium and the gametophyte stem below and surrounds the sporophyte during early development (Budke, 2019). As the sporophyte develops and increases in height, the calyptra detaches from the rest of the maternal gametophyte and remains on the sporophyte apex. During development, the sporophyte apex moves beyond the still boundary layer of the substrate and is exposed to increasing dehydration stress (Rice and Schneider, 2004). This makes the calyptra especially critical for taller sporophytes.

It is important to study structural maternal investments, which could be interpreted as maternal care, comparatively in order to understand on how different moss species have evolved to protect their offspring. As previous observations and studies have pointed out, the calyptra plays an important role in sporophyte development (Paolillo, 1968; French and Paolillo, 1975*a*, 1975*b*; Haig, 2013; Budke et al., 2011, 2012, 2013; Budke and Goffinet, 2016). However, the influence of maternal calyptra size on sporophyte survival, development, and fitness have not been explored.

To examine the impact of calyptra size on sporophyte survival, development, and fitness, a removal-replacement experiment was conducted. *Funaria hygrometrica* Hedw. and *Physcomitrium pyriforme* (Hedw.) Hampe, two species with contrasting sizes of calyptrae, were used. Both species are readily cultured in the laboratory and have been used for similar removal-replacement experiments; French and Paolillo, 1975*a* examined the role of the calyptra in capsule formation while Budke et al. 2013 investigated calyptra cuticles. Using species with different sizes of calyptrae will enable us to test the impact of calyptra size on sporophyte survival, development, and fitness. We hypothesize that large calyptra will provide more protection than small calyptra, and thus we predict that sporophytes with larger calyptra on their apex during early development will be taller and produce heavier capsules (proxy for spore count; Budke et al., 2013) compared to sporophytes with smaller calyptra.

SECTION 2: MATERIALS AND METHODS

Species

Two moss species were used for the following experiments. *Funaria hygrometrica* Hedw. and *Physcomitrium pyriforme* (Hedw.) Hampe grow on wet soil and in disturbed environments. *Funaria hygrometrica* sporophytes range from 20 – 45 mm tall with capsules from 2 - 3.5 mm long and has an average calyptra length of 3.2 mm (preliminary data), and develop during the spring (eFloras, 2008), whereas *P. pyriforme* sporophytes range from 6 – 15 mm in height, capsule length reach between 1 – 3 mm with calyptra length averages 1.3 mm (preliminary data), and capsule develops in spring (eFloras, 2008).

Laboratory populations

Laboratory populations were started originally from the following field collected populations originally collected in Connecticut (*Funaria hygrometrica* CONN Budke #142 and #144; *P. pyriforme* CONN Goffinet #9276). The protocol outlined below is modified from Budke et al., 2011. Spores were surface sterilized, germinated on media, and then transferred to a soil mix. Plants were grown on a rich sandy-loam mix in PlantCon containers (Thermo Scientific, Waltham, MA). Mosses in these containers were kept on light carts (50—70 µmol m^{-2} s^{-1}) in the laboratory at room temperature (20—25°C) for three months with 14—16 h of light. After three months, plants were placed in a cold chamber (10°C) and received 8 hours of light (65 µmol m^{-2} s^{-1}). Plants were inspected weekly for antheridia and archegonia development. When the presence of both antheridia and archegonia was observed, plants were flooded with deionized water for 24 hours; then drained and remained in the cold chamber for one week. Thereafter, they were returned to room temperature conditions on the light carts as described above to facilitate sporophyte development.

Experiment

Sporophytes at an early stage of development were used to examine the influence of calyptra size on sporophyte development and capsule reproductive output. Since conditions during this immature stage may have repercussions for later stages, sporophytes at developmental stages three and four (spear-shaped with unexpanded capsule; Figure 2C and 2D in Appendix) were used for this experiment. Sporophytes grown in the laboratory were 8.0 - 23.0 mm tall for *F. hygrometrica* and 2.0 - 8.9 mm for *P. pyriforme* at developmental stages three and four.

The developing sporophytes were assigned one of four treatments: (1) unmanipulated control, (2) manipulated control, calyptra removed and placed back on the same sporophyte, (3) calyptra from *P. pyriforme* (small calyptra) removed and placed on *F. hygrometrica* sporophyte, or (4) calyptra from *F. hygrometrica* (large calyptra) removed and placed on *P. pyriforme* sporophyte. Individuals of *F. hygrometrica* and *P. pyriforme* were randomly paired for the swapping in treatments 3 and 4. Prior to manipulation, both the sporophyte length (mm) and calyptra length (mm) were measured. Sporophyte length was measured from the top of the vaginula to the sporophyte apex. Calyptra length was measured from the calyptra base to tip of calyptra rostrum. Both sporophyte and calyptra length were measured to the nearest millimeter. Individuals were then randomly assigned a treatment and location on a 90 × 90 mm grid in the experimental pots. Random numbers were generated using R 3.5.1 (R Core Team, 2015). To keep samples hydrated, a drop of water was dispensed on two separate slides and a pair of individuals were immediately placed on the droplets on the slides. Then one of the four treatments described above was applied to the pair during a three-minute time window. Then samples were placed in randomly assigned locations in the experimental pot. Experimental pots were rotated on a weekly basis to a new location on the light cart and were alternated between inside and the outer edge under 50 - 70 μmol m^{-2} s^{-1} of light at room temperature (20 - 25 °C).

Individuals were inspected 24 hours after the experiment to assess sporophyte apex damage, which was indicated by an apex color change from green to dark red. These damaged individuals were removed from the experiment. Sporophytes were evaluated on days 3, 6, 9, 15, 24, and 34 post-manipulation for developmental stage (ordered categorical variable; Figure 2) and survival (binary). The capsules of individuals that reached developmental stage 9, which is characterized by fully expanded capsules, red peristome teeth and annulus, and differentiated spores (Figure 2I) were prepared for post-manipulation measurements. On day 34, sporophyte length and capsule length were measured. Sporophyte length was measured from sporophyte base to tip of capsule while capsule length was measured from capsule base (differentiated from the seta by the apophysis) to tip of the operculum (capsule lid). Following these measurements, the top 0.5 mm of the seta and the entire capsule were detached from the sporophyte and placed in a labeled paper envelope for drying at ambient room temperature and humidity. After drying for approximately two weeks after capsules were placed in envelopes, the capsules were weighed to the nearest microgram (capsule weight) using a microbalance (Mettler Toledo XS3DU, Columbus, OH), fine forceps, and weigh boats.

Statistical analyses

Physically removing the calyptra and replacing it on the sporophyte apex has negatively effects both sporophyte survival and development seen in other manipulation experiments (French and Paolillo, 1975*a*; Budke et al., 2013) and in this experiment. To account for these negative effects and to focus on the influence of calyptra size as applied in treatments 3 and 4, these experimental groups were analyzed in comparison to treatment 2 (manipulated control).

All statistical analyses were conducted with R 3.5.1 (R Core Team, 2015). We performed a series of regression analyses independently for each species to compare the individuals with either larger or smaller to the manipulated controls. To examine the effect of calyptra size on sporophyte development and reproductive output, sporophyte length, capsule length, and capsule weight were used as continuous response variables in mixed effects models. To measure sporophyte growth, the difference between initial sporophyte height (pre-treatment) and sporophyte height at the end of the experiment (on day 34) was calculated. Capsule weight was used to assess sporophyte fitness since the number of spores per capsule is associated with capsule weight (Budke et al., 2013).

Initial sporophyte length (pre-treatment) and treatment (unmanipulated control, manipulated control, and replaced calyptra with different size) were included as covariates while experimental pot, location in the experimental pot, population lab culture date, population number, and experiment date were included as random effects in all preliminary models. The fixed and random variables were assessed by model selection through a stepwise method using maximum likelihood and the Akaike Information Criteria (AIC). Variables were removed or included if the model significantly improved the AIC score. As such, the most appropriate reduced models that corresponded with the lowest AIC score, were used to assess the impact of the treatments on the responses of sporophyte growth, capsule length, and capsule weight.

The mixed effects model for *F. hygrometrica* sporophyte growth (*n*=76) included treatment as a fixed variable and population date (when spores were plated to start the laboratory cultures) nested in population number and experimental date as random effects. For *P. pyriforme* sporophyte growth (*n*=95), treatment and initial sporophyte length were fixed variables included in the selected reduced model, lowest AIC score. The model for capsule length for *F. hygrometrica* (*n*=72) contained treatment as a fixed variable and population date as a random effect, while the model for capsule length for *P. pyriforme* (*n*=107) had treatment and initial sporophyte length as covariates and pot number as a random effect. The linear model for *F. hygrometrica* capsule weight (*n*=70) only included the treatment variable while the linear model for *P. pyriforme* capsule weight (*n*=96) had treatment and initial sporophyte height as covariates.

Regression assumptions were evaluated with Q-Q plots from R package “car.” Response variables were transformed as needed to normalize the residuals. Transformations that were made included sporophyte length (square root transformation), capsule length (log transformation), and capsule weight (log transformation) for *F. hygrometrica* and capsule length (square root transformation) and capsule weight (log transformation) for *P. pyriforme*.

The significance of models from the “lmer” function R package “lmerTest” were calculated using confidence intervals. 95% confidence intervals for the models that did not include 0 are statistically significant. Additionally, the significance of the models were derived from t-tests, and degrees of freedom using Satterthwaite approximations (Kuznetsova et al., 2017).

Survival analyses

To determine the difference of survival across a time distribution of each treatment, a log rank test was conducted to compare survival curves between treatments. Statistically different distributions were indicated by a significant p-value. Multivariate and univariate Cox regressions were used to measure the covariates and their impact on survival. Model selection in the “MuMIn” package compared AIC of different Cox proportional hazard models and interaction terms (Barton, 2009). The Wald statistic was used to assess if variables differed from 0. Additionally, the results of the three tests were indicative of an adequate sample size used; the results from the three tests, p-value outputs are equal or within a p-value of 0.01 difference, showed that there was a sufficient sample size for the survival analysis. Assumptions were checked for the significant models using the R package “survival”: 1) constant hazard ratio over time for each effect via Kaplan-Meir, 2) censoring is random via t-test, and 3) independence of survival times and residuals via scaled Schonfeld residuals with the “cox.zph()” function. Likelihood ratio, Wald, and a log rank tests calculated the significance of the final model.

Developmental stage analyses

Ordered logistic regression was used to compare the progression of the sporophytes through the developmental stage between each treatment. Developmental stage, determined by morphological features based on Table 1 and Figure 1 in Budke et al. 2012 (Figure 2), for each individual was recorded on days 3, 6, 9, 15, 24, and 34. Confidence intervals were added to calculate the significance of the coefficients and then odds ratios were used to exponentiate the coefficient estimates for interpretation.

SECTION 3: RESULTS

Reproductive output and sporophyte development

For the *Funaria hygrometrica* models the response variables of sporophyte growth, capsule length, and capsule weight between treatment 2 and treatment 3 were not statistically significantly different (Figure 3 in Appendix).

The model for sporophyte growth for *Physcomitrium pyriforme* was significant with a p-value < 0.0001. As seen in Figure 4A (Appendix), individuals that had their calyptra exchanged for a larger calyptra (treatment 4) had a larger increase in sporophyte height compared to the manipulated control (treatment 2). On average, the sporophytes grew 0.44 mm taller (standard error +/- 0.17 mm) in treatment 4 compared to treatment 2. In the model for *P. pyriforme* capsule lengths were statistically significant (Figure 4B). The mean capsule length of individuals in treatment 4 was 0.36 mm (standard error +/- 0.03 mm) longer than individuals from treatment 2. *Physcomitrium pyriforme* capsule weight was significant with a p-value < 0.01 (Figure 4C). Starting sporophyte length was highly associated with capsule weight (p-value < 0.01), which made the overall model significant. Individuals in treatment 4 had a capsule weight 0.05 micrograms heavier on average than individuals in treatment 2 (Figure 5 in Appendix), but this difference was not significant with a p-value > 0.05. Capsule weight and initial sporophyte length were positively correlated, and initial sporophyte length was a statistically significant coefficient (p-value < 0.01).

Survival analyses

Funaria hygrometrica: The log-rank test was used to test the difference between treatments 2 and 3 (Figure 6 in Appendix). The model detected a significant difference in terms of survival between the treatments with p-value < 0.02. A multivariate Cox regression was used to calculate the impact of treatment along with starting sporophyte length and the random variables (experimental date, population number, and population date) on the survival. The likelihood, Wald, and log-rank tests each resulted a p-value < 0.02 for the overall model. The model estimated that a smaller calyptra decreased survival probability by 53%.

Physcomitrium pyriforme: The covariates, treatment, and starting sporophyte length, were included in the multivariate Cox regression. The covariates and overall model were not significant. Similarly, the univariate Cox regression examined the treatment variable and concluded that treatment was not significant (Figure 7 in Appendix). The model gave similar output for the likelihood, Wald, and log-rank tests. Similar results from these three tests indicates a sufficient sample size for detecting effects. The model determined that treatments 2 and 4 did not have significant differences in survival.

Developmental stage analyses

Ordinal logistic regression was used to assess each species and developmental stage between manipulated control and the treatments either with smaller or larger calyptra on their apex. Figure 8 (Appendix) illustrates the progress in terms of the average developmental stage between treatments 1, 2, and 3 on days 3, 6, 9, 15, 24, and 34 for *F. hygrometrica*. Figure 9 (Appendix) show the average developmental stage between treatments 1, 2, and 4 in days 3, 6, 9, 15, 24, and 34 for *P. pyriforme*. The treatments were not statistically significantly different from each other for either species.

SECTION 4: DISCUSSION

The overarching theme of maternal care in evolutionary biology can be conceptualized in the maternal moss structure, the calyptra. Previous studies have shown the crucial role the calyptra plays in sporophyte development (Budke et al., 2012; French and Paolillo, 1975*b*); the immature sporophyte apex is protected from desiccation by the calyptra during early development (French and Paolillo, 1975*a*; Budke et al., 2013). Additionally, experiments that involved removing the calyptra displayed abnormal capsule growth or an undifferentiated seta that lacked a proper capsule, indicating that the calyptra plays a critical role in sporophyte development (Paolillo, 1968; Haig, 2013). To further understand the influence of the maternal calyptra, we tested the effect of calyptra size on the sporophyte morphology, development, and fitness by conducting a removal replacement experiment. As stated above, to account for the damages caused by the manipulation experiment, the experimental group was compared to the manipulated control group.

The two moss species examined in this experiment (*F. hygrometrica* and *P. pyriforme*), have sporophytes and calyptra that differ in size. The taller *F. hygrometrica*, has a larger calyptra, (average calyptra length = 3.57 mm) compared to the shorter *P. pyriforme*, small calyptra (average calyptra length = 1.60 mm). Calyptra size (in this case, small or large) can also represent the amount of protection given by the calyptra. As previously demonstrated, the calyptra impacts sporophyte fitness by dehydration protection strictly provided by the physical properties of the calyptra (Budke et al., 2013). Additionally, Budke and Goffinet 2016 showed that the cuticle on *F. hygrometrica* is thicker than *P. pyriforme*. The larger calyptra size and a thicker cuticle suggest that more protection was provided from desiccation events. Moreover, French and Paolillo (1975*a*, 1975*b*) illustrated through manipulation experiments, that the calyptra is devoid of physiological influence in sporophyte development.

Overall, calyptra size in *Funaria hygrometrica* did not have an effect on sporophyte growth, capsule length, and capsule weight (Figures 3). This suggests that *F. hygrometrica* sporophyte development and size is independent of calyptra size. Sporophytes with smaller calyptra starting at developmental stage 3 and 4 (Figure 2) does not result in decrease in the size of sporophyte morphological features. A follow up experiment may be to carry out this calyptra replacement at an even earlier developmental stage, potentially immediately after stage 2 when the calyptra is first detached from the rest of the sporophyte, but prior to any seta expansion to see if this has an impact on the morphology.

The survival analysis seen in Figure 10 showed a small calyptra had significantly negative effects on sporophyte survival; individuals with a smaller calyptra (treatment 3) had a 53% decrease in survival probability compared to individuals in treatment 2. This may imply that larger calyptra may be necessary for coping with the increased dehydration stress from having a taller sporophyte (Rice et al., 2001; Rice and Schneider, 2004).

The sporophyte apical region consists of the terminal area which gives rise to a capsule and the seta meristem (French and Paolillo, 1975*b*). This apical region is sensitive to desiccation and the calyptra is known to protect this region from dehydration during early developmental stages (French and Paolillo, 1975*a*). The negative effect on sporophyte survival suggests that a smaller calyptra size may be inadequate for protecting the sporophyte from dehydration since larger sporophytes may have larger apical regions. In this experiment, *F. hygrometrica* might need a larger calyptra to cover and protect a larger apical region. Future studies could further investigate the association between the apical region and calyptra size. In contrast, survival analysis for *P. pyriforme* showed that treatment 2 and treatment 4 have similar survival probabilities (Figure 8) possibly due to sufficient level of protection for the apical region.

In *P. pyriforme*, a larger calyptra significantly influenced sporophyte growth and capsule length (Figure 4), but not capsule weight or sporophyte survival. The samples in treatment 4, *P. pyriforme* sporophytes with their calyptra replaced with larger calyptra of *F. hygrometrica*, produced taller sporophytes with longer capsules compared to the manipulated control (treatment 2), as seen in Figure 3. Protection from dehydration stress provided by the calyptra for taller sporophytes, especially during early developmental stages, is known to be essential for their survival and development (Budke et al., 2013). Thus, larger calyptra may have provided additional protection for the sporophytes enabling them to grow taller and to survive exposure to higher levels of desiccation stress as they grew further from protective boundary layer (Rice and Schneider, 2004). Taller sporophytes enable a larger range of distance for spore dispersal (Johansson et al., 2014). Thus, taller sporophytes, especially for soil growing species that do not have the advantage of growing on an elevated substrate such as a tree trunk, have a competitive and potentially evolutionary advantage due to increased dispersal distances. Consequently, any maternal investments in larger calyptra could have a direct impact on increasing sporophyte height, dispersal, and ultimately fitness.

Section 5: Conclusion

The importance of the calyptra for sporophyte development has been well established by past studies (Paolillo, 1968; French and Paolillo, 1975*a*, 1975*b*; Haig, 2013; Budke et al. 2011, 2012, 2013; Budke and Goffinet, 2016). This study further tested maternal impacts by comparatively examining the influence of calyptra size on sporophyte growth and development. In *F. hygrometrica* (species representative of tall sporophytes with large calyptrae replaced with small calyptrae) survival analysis showed that a smaller calyptra negatively affected sporophyte survival, potentially due to the exposure of the sensitive apical region to dehydration stress due to a smaller calyptra providing less physical protection for the apex. Furthermore, sporophyte growth and capsule length in *P. pyriforme* (species representative of short sporophytes with small calyptrae replaced with large calyptrae) increased with larger calyptrae. Having a taller sporophyte, which is associated with larger calyptra in across the Funariaceae (Budke and Goffinet, 2016), is beneficial for spore dispersal with the caveat that dehydration stress increases as the distance away from the maternal gametophyte increases (Rice et al., 2001; Rice and Schneider, 2004). Thus, increased investment by the maternal plant in a larger calyptra can have direct impacts on aspects of sporophyte morphology that it turn influence spore dispersal. Overall, this study expands our observations of the impact of calyptra on sporophyte growth and development.

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APPENDIX

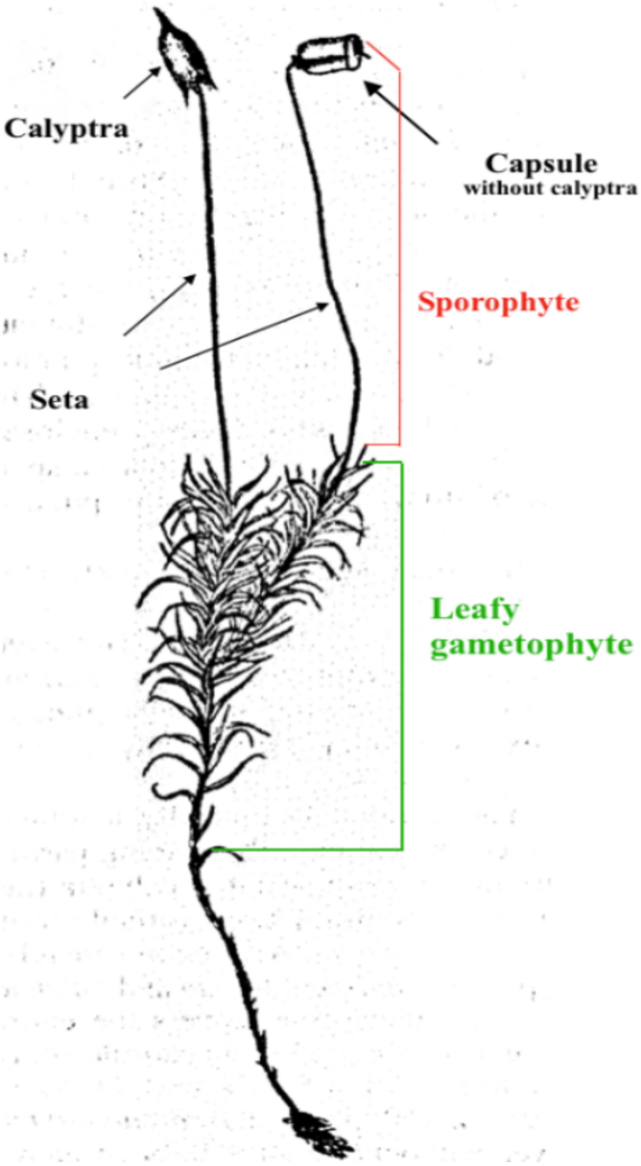

Figure 1: Moss Diagram (modified from Haig, 2013). **Leafy gametophyte**: haploid dominant photosynthetic region with leaf-like structures. **Sporophyte**: diploid offspring. **Seta**: stem-like structures part of the sporophyte. **Calyptra**: protective maternal structure that encompasses the capsule. **Capsule**: sporangium, where spores are produced.

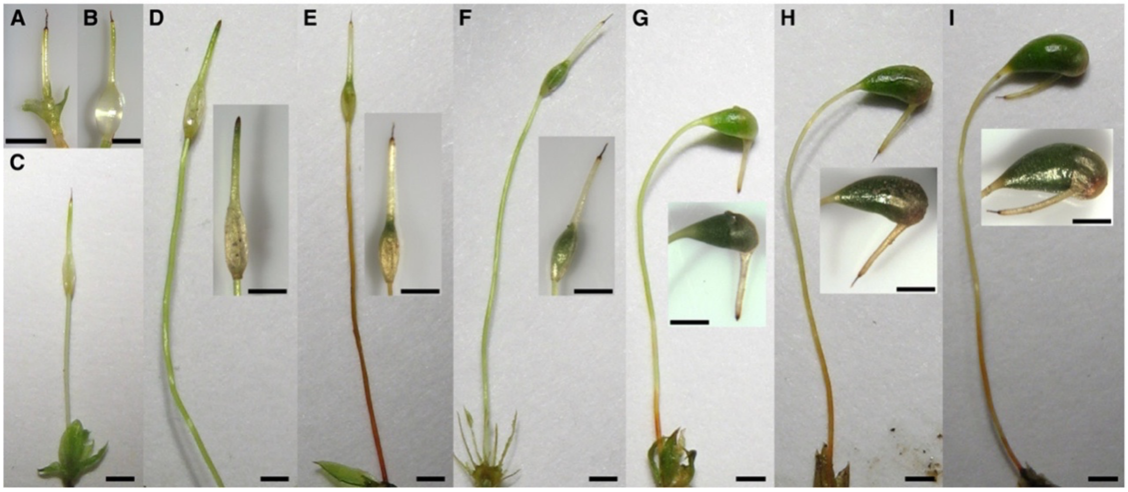

Figure 2: Developmental Stages *Funaria hygrometrica* (modified from Budke et al., 2012). (A) Stage 1, calyptra and sporophyte established and ready to emerge. (B) Stage 2, calyptra with an inflated base (C) Stage 3, sporophyte elongated and spear like <15mm. (D) Stage 4, early developing stages of sporophyte >20mm. (E) Stage 5, early capsule development. (F) Stage 6, capsule fills the inflated base of the calyptra. (G) Stage 7, annulus on capsule is green. (H) Stage 8, annulus on capsule is pink. (I) stage 9 – annulus on capsule is red with visible peristome teeth. Bars = 1mm.

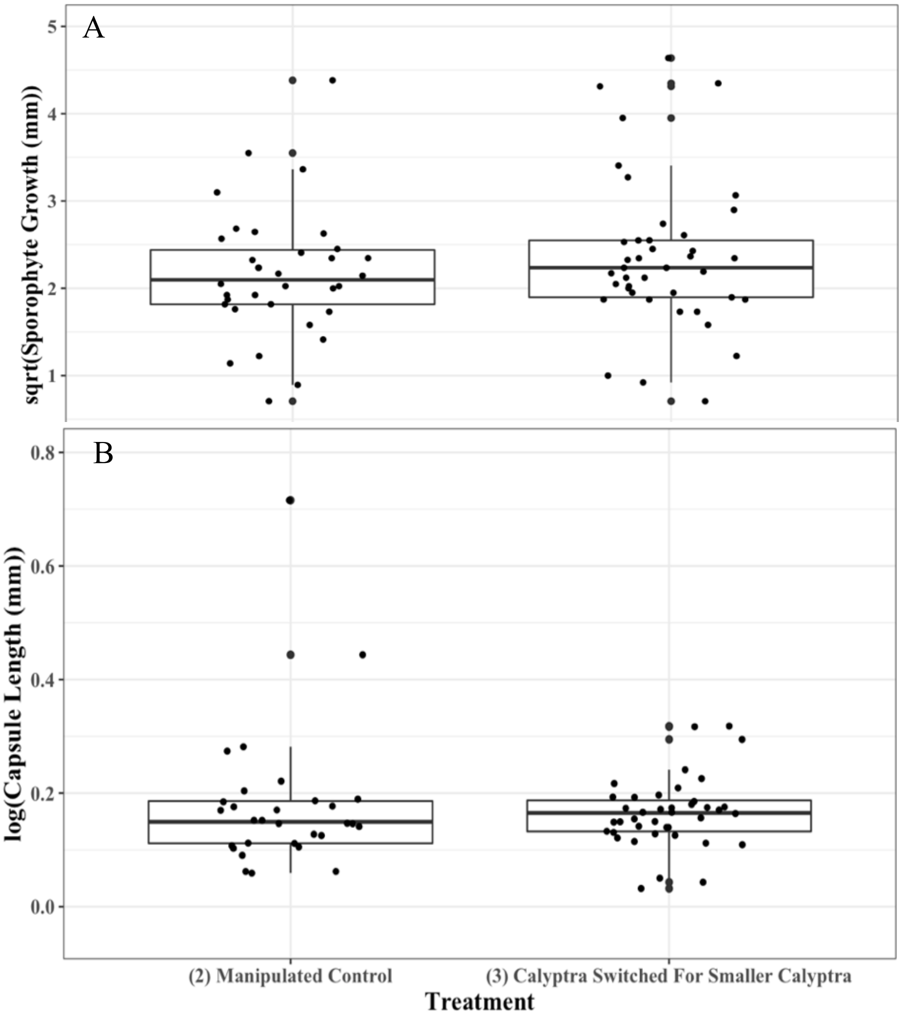

Figure 3: Treatment 2 and Treatment 3. To examine the effect of smaller calyptra size on sporophyte growth, development, and reproductive output, Funaria hygrometrica sporophytes that had their calyptra removed and replaced with the smaller calyptra of Physcomitrium pyriforme (treatment 3). This treatment was compared to the manipulated control (treatment 2) where sporophytes had their calyptra removed and replaced on the sporophyte apex. (A) Sporophyte growth was determined by calculating the difference between the initial sporophyte height and the sporophyte height at the end of the experiment (day 34). Sporophyte growth is not significantly different between the treatments. (B) Capsule lengths between the two treatments are also not significantly different. (C) Comparison of the log-transformed capsule weight. The two groups have similar capsule weight regardless of calyptra size.

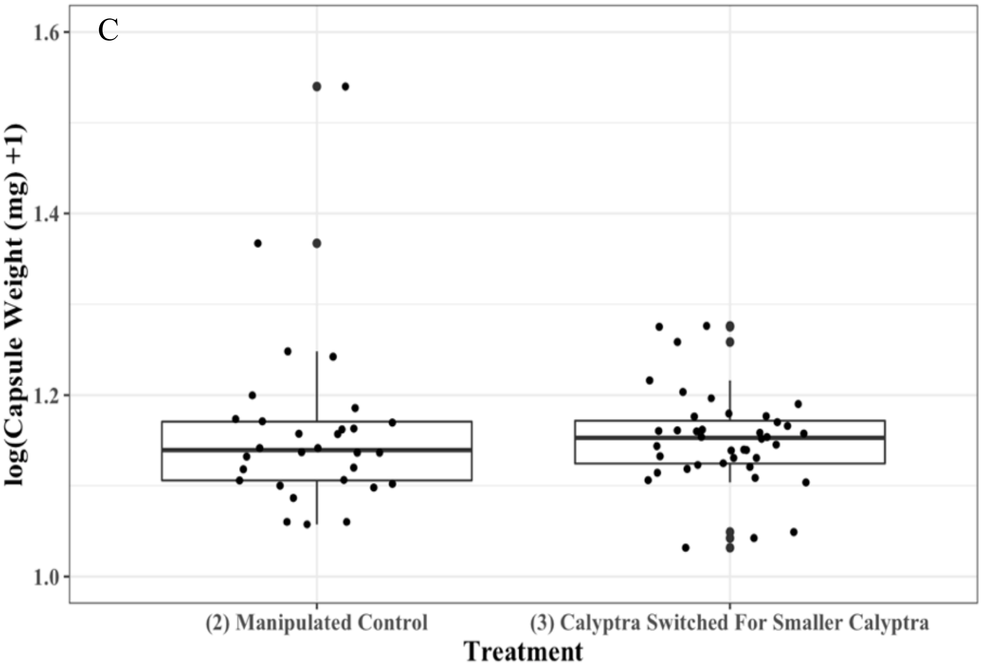

Figure 3 continued

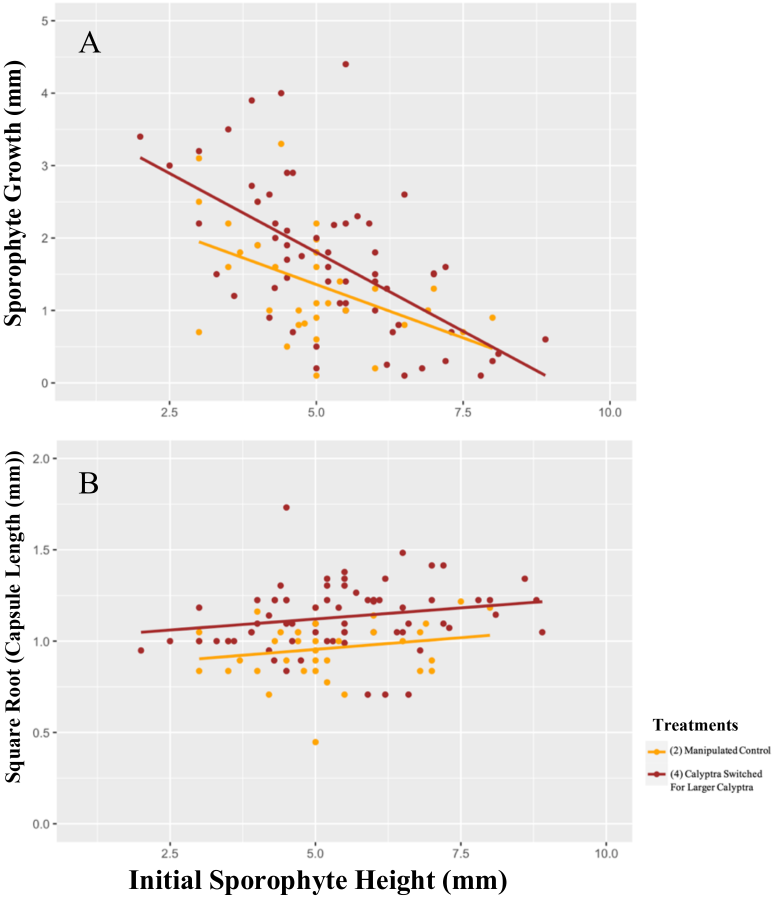

Figure 4: Treatment 2 and Treatment 4. To examine the effect of larger calyptra size on sporophyte growth, development, and reproductive output, *Physcomitrium pyriforme* sporophytes that had their calyptra removed and replaced with the larger calyptra of *Funaria hygrometrica* (treatment 4) were compared to the manipulated control (treatment 2) where sporophytes had their calyptra removed and replaced on the sporophyte apex. (A) Sporophyte growth was determined by calculating the difference between the initial sporophyte height and the sporophyte height at the end of the experiment (day 34). The data show that the group with larger calyptra had a statistically significantly higher growth rate as compared to the group with smaller calyptra. (B) Square root transformed capsule length between the two treatments are statistically significant. (C) Comparison of the log-transformed capsule weight. Individuals that started with initially taller sporophyte height in treatment 4 had heavier capsules which influenced the model to be statistically significant.

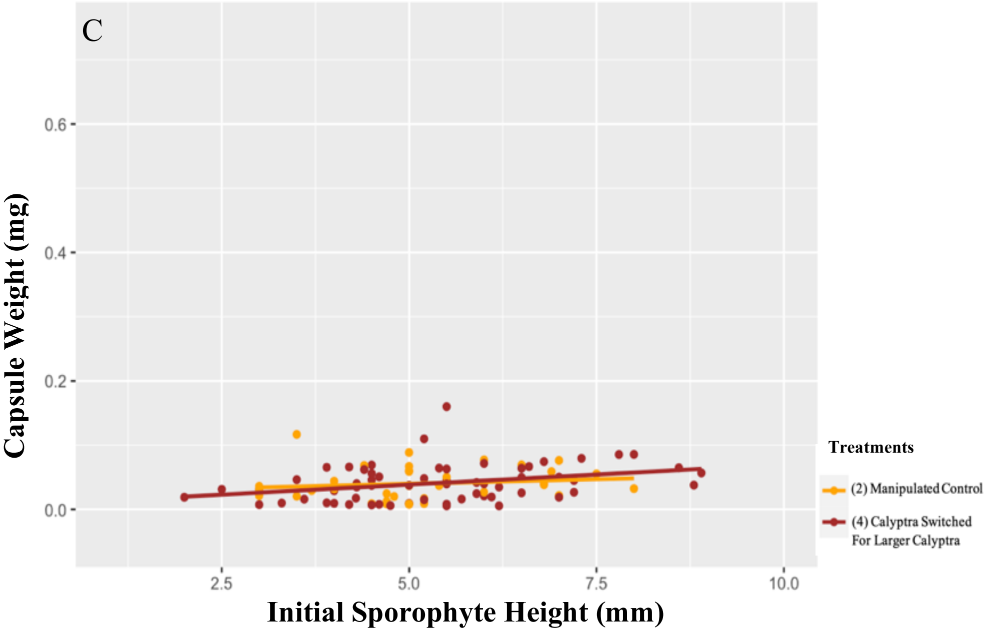

Figure 4 continued

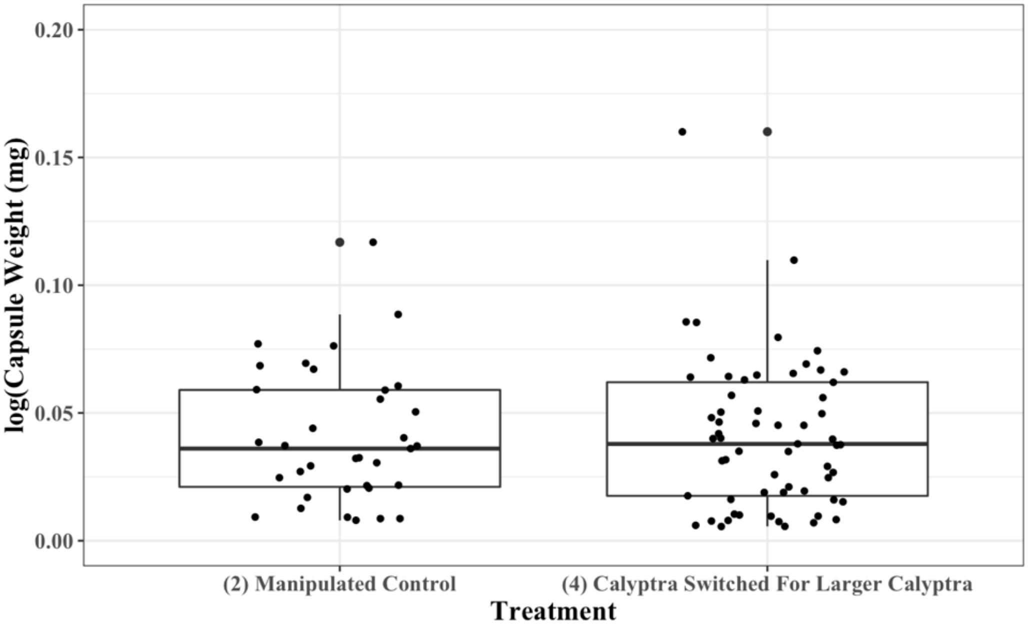

Figure 5: *Physcomitrium pyriforme* Capsule Weight.Comparison of Physcomitrium pyriforme capsule weight between the manipulated control group (treatment 2) and the experimental group (treatment 4), individuals with their calyptra replaced with a larger calyptra. Individuals that started with taller sporophyte height in treatment 4 had heavier capsules which influenced the model to be statistically significant. The figure shows that the two treatments have similar capsule weight.

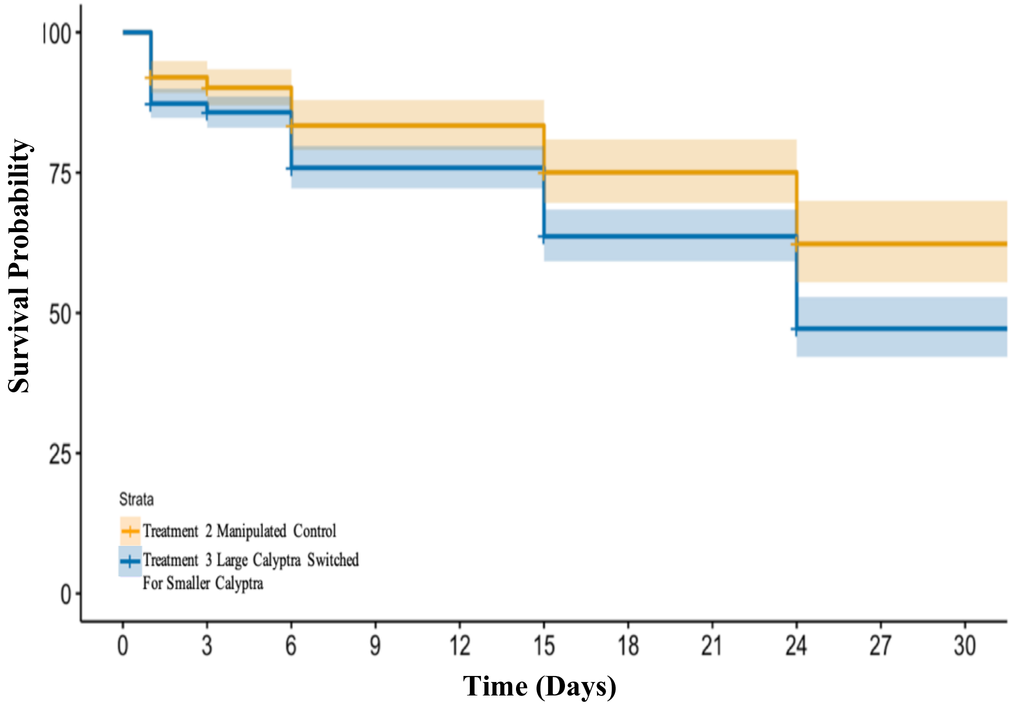

Figure 6: Survival Analysis *Funaria hygrometrica*.Survival probability of *Funaria hygrometrica* sporophytes in treatments 2 (manipulated control) and 3 (experimental group). The control group, treatment 2, had a significantly higher survival probability than the individuals with smaller calyptra (treatment 3). Treatment 3, smaller calyptra decreased survival probability by 53%.

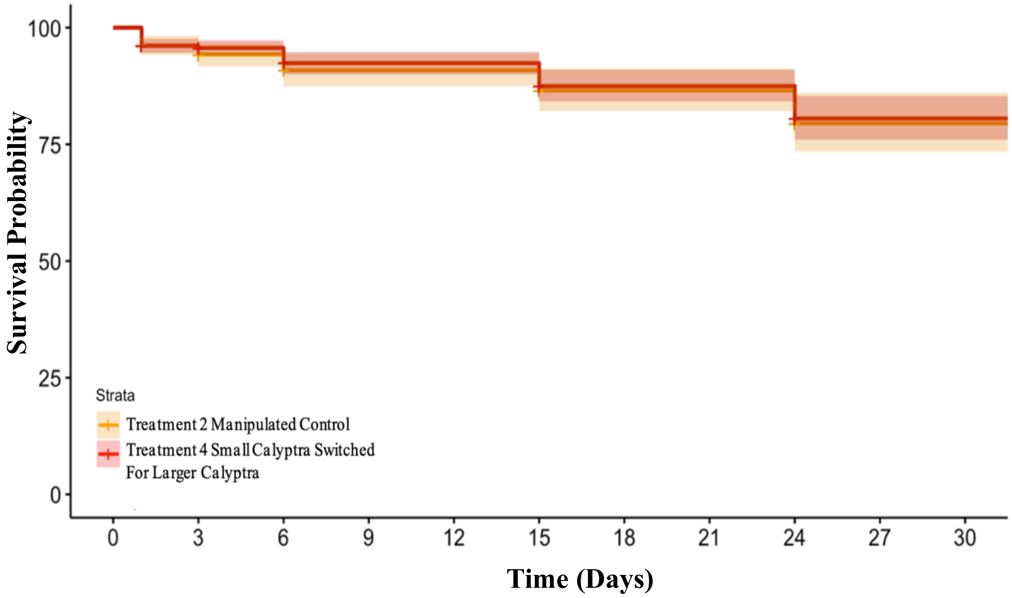

Figure 7: Survival Analysis *Physcomitrium pyriforme*.Survival probability of *Physcomitrium pyriforme* sporophytes in treatments 2 (manipulated control) and 4 (experimental group). Treatments 2 and 4 had similar survival probability.

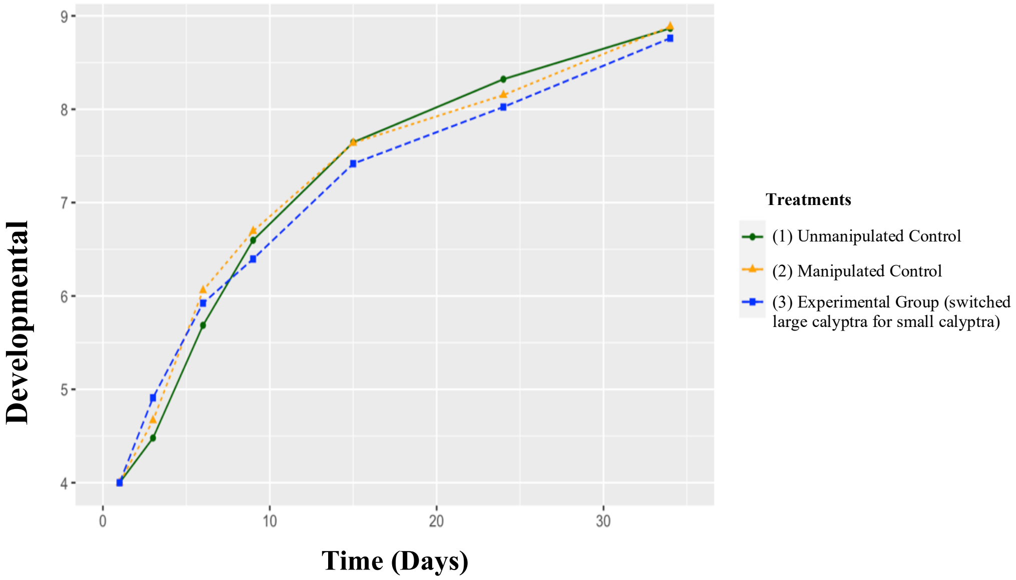

Figure 8: Developmental Stages *Funaria hygrometrica*.Average developmental stage of *Funaria hygrometrica* in treatments 1 (unmanipulated control), 2 (manipulated control), and 3 (experimental group). Developmental stages were recorded on days 3, 6, 9, 15, 24, and 34 post manipulation. Treatments 2 and 3 did not have significantly different developmental trajectories.

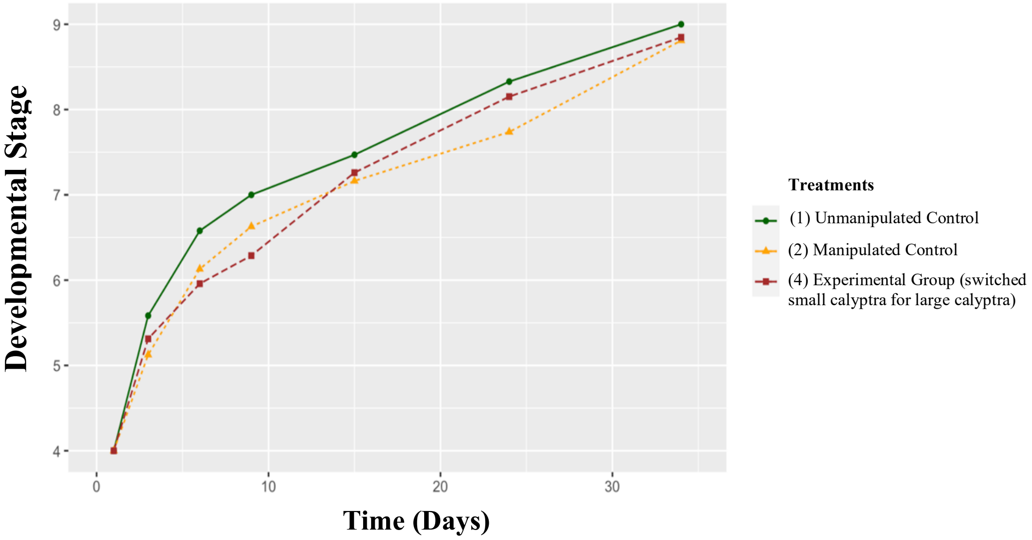

Figure 9: Developmental Stage *Physcomitrium pyriforme*. Average developmental stage of *Physcomitrium pyriforme* in treatment 1 (unmanipulated control), treatments 2 (manipulated control), and 4 (experimental group). Developmental stages were recorded on days 3, 6, 9, 15, 24, and 34 post manipulation. Treatments 2 and 4 are not statistically significant in developmental progress.

VITA

Alessandra Marie Cabrera Aromin graduated with a Bachelor of Science in Environmental Science – Biology from Montana State University, Billings in 2016. From 2016 to 2018, she worked in research under the guidance of a graduate student, Mandy Slate, in Dr. Ragan Callaway’s plant ecology lab at the University of Montana. In 2018, she joined Dr. Jessica Budke’s lab at the University of Tennessee and in May 2021, she received a Master of Science in Ecology and Evolutionary Biology.