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An analysis of the environmental and hormonal effects on the growth and development of the moss Ceratodon purpureus

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Applicant   Megan Brandy Knight

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Read, approved, and signed by:

Thesis adviser(s)   *[signature]*   3/19/09
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Reader(s)   *Thomas E. Dd [signature]*   3 April 2009
                                           Date

Certified by   *[signature]*   6/2/09
                 Director, Honors Program   Date

Level of Honors conferred: University   *Magna Cum Laude*
                           Departmental *High Honors in Biology*

An analysis of the environmental and hormonal effects on the growth and

development of the moss *Ceratodon purpureus*

A Thesis

Presented to the Department of Biological Sciences

College of Liberal Arts and Sciences

and

The Honors Program

of

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In Partial Fulfillment

of the Requirements for Graduation Honors

Megan Knight

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Introduction

Moss is a simple plant that lacks conventional roots, stems, and leaves. This simplicity makes it an optimal choice for developmental research. The true mosses are in the phylum Bryophyta and have a unique life cycle comprised of an alternation of generations. The life-cycle of a typical moss is shown in Figure 1.
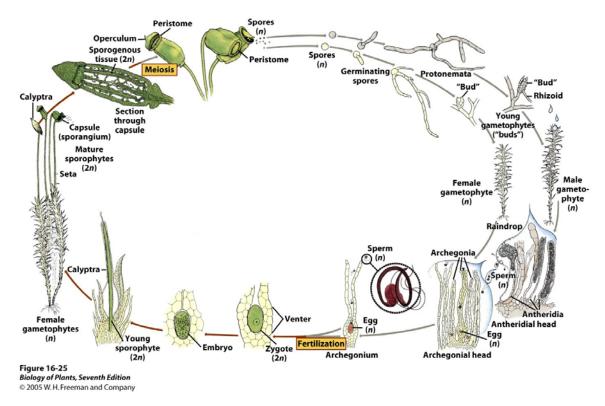

Figure 16-25
Biology of Plants, Seventh Edition
© 2005 W. H. Freeman and Company

Figure 1. Haploid spores germinate to produce protonemata. With an environmental trigger, buds form on the protonemata and grow into gametophytes. If fertilization occurs, a zygote forms in the gametophyte producing a diploid sporophyte, which is dependent on the gametophyte for nutrition. Mature sporophytes produce spores and release them into the environment (Raven *et al.*, 2005).

As Campbell and Reece (2002) explain, a moss spore is haploid (has one set of chromosomes) and germinates to produce a mass of thick, green filaments called a protonema (Fig. 2). Before a bud forms, the chloronemal tip cell of the protonema must differentiate into a caulonemal tip cell and produce a caulonemal filament (Schumaker and Dietrich, 1998). Following this, the hormone cytokinin induces bud assembly in the

caulonemal initial cell (Schumaker and Dietrich, 1998). Buds develop as side branches from caulonemal filaments and these develop into gametophores - leaf-bearing shoots, shown in Figure 3 (Cove *et al.*, 1997). This development of the gametophore, or gamete producing plant, only occurs in the presence of adequate resources and environmental conditions (Campbell and Reece, 2002). The spore, protonema, and gametophore together make up the gametophyte generation. If fertilization occurs, the resulting zygote develops into a diploid sporophyte, which has two sets of chromosomes and is dependent nutritionally upon the gametophyte. A capsule at the tip of the sporophyte stalk produces haploid spores that are immediately released into the environment. Unlike many familiar plants, the diploid generation of a moss is relatively short lived. The single-celled spores that the sporophyte releases face harsh conditions and a changing environment (Campbell and Reece, 2002).
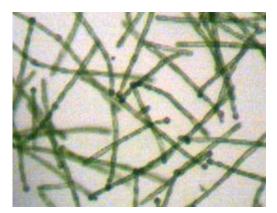

Figure 2. Protonemal filaments of the moss *Ceratodon purpureus* taken on a Bausch & Lomb dissecting microscope with a digital camera.

Figure 3. A gametophore after initial budding of the moss *Ceratodon purpureus* taken on a Bausch & Lomb dissecting microscope with a digital camera.

Plants, unlike animals, are stationary and therefore must acclimate to their changing environment. Such environmental changes affecting development of the spores/mosses may include changes in temperature, light, humidity, pH, and nutrients

(Cove, 1993). These environmental changes affect not only whether the spore will germinate, but also influence moss growth, bud formation, and leaf development. For example, various light conditions, such as red or blue light, have been shown to affect bud formation in the moss *Funaria hygrometrica* (Simon and Naef, 1981). The influence of the environment on moss growth and development has been studied in only a few species; there is a need for further study in this area.

In order to acclimate to environmental conditions, mosses, as well as other plants and animals, utilize hormones to signal changes in growth and development (Swinehart and Dietrich, 2007). Some common hormones present in plants include auxin, cytokinin, abscisic acid (ABA), ethylene, and gibberellin. Of these hormones, ABA is often produced in response to environmental stresses. Most research on mosses and ABA has focused on the function of ABA in acquiring tolerance to environmental stresses (Cove *et al*., 1997). It is thought to have mainly inhibitory functions and may have a role in bud dormancy and inhibition of shoot growth (Cove, 1993).

Since hormones signal important changes, they are often the focus of scientific experiments. Relatively recent research on mosses and hormones has shown conflicting results. Christianson (2000) found that in the moss *Funaria hygrometrica* ABA stopped bud formation, whereas Oliver *et al.* (2004) found that in the moss *Tortula ruralis* ABA was not associated with bud formation. The observations of these two experiments are conflicting because ABA caused two different responses. Further research is needed to investigate the effects of ABA on another species of moss – *Ceratodon purpureus* – to determine the role, if any, of this hormone on growth and development in the majority of mosses.

Ceratodon has not been documented in scientific literature as thoroughly as other species of moss such as *Funaria* or *Tortula*. Commonly known as fire moss or purple horned moss, *Ceratodon* is often reddish or yellow-brown and the spore capsules are usually purple (Crum, 1983). A picture of the species growing in its natural habitat is shown in Figure 4. *Ceratodon* often grows in tufts and is considered a weed, often thriving in polluted or disturbed areas and frequently invading after a fire (Crum 1983). *Ceratodon* belongs to the class Bryopsida, which also contains the previously mentioned *Funaria* and *Tortula* species. Since hormones and environmental factors both signal changes in moss, this study began with the goal of comparing the responses seen in the presence of each of these individually. This study planned to compare the results of experiments consisting of environmental changes with no external application of ABA to the results of an experiment with the application of ABA but no environmental change.

Figure 4. The moss *Ceratodon purpureus* in its natural environment. Provided by Biopix.dk: JC Shou.

Limited research has been conducted on hormones such as ABA and their effects on different species of moss. By documenting the effect ABA has on a third species of moss, *Ceratodon pupureus*, scientific understanding of how the class Bryopsida reacts

with ABA present will be enhanced. I hypothesize that *Ceratodon* will respond in a similar fashion as *Funaria* (ABA will inhibit bud formation). In addition, I believe I will find that there is a relationship between hormones and the environment because mosses utilize hormones to signal changes in growth and development much like the environment signals changes in growth and development (Swinehart and Dietrich, 2007 and Cove, 1993). If in fact there are similarities between the effects of hormones and environment, it will be indirectly concluded that environmental signals cause release of hormones, which trigger changes in growth and development of the moss.

Methods

Standard media preparation and tissue culture

The medium used to cultivate *Ceratodon purpureus* was made according to directions by Cove (2004), given in Table 1, and consisted of B, C, and D solutions. The medium was solidified with Agargel (Sigma).

Working solution BCD medium   Cove (2004)

    Stock Solution B

              $MgSO_4.7H_2O$ 25 g
              distilled H_2O 1 L

    Solution C

              KH_2PO_4 25 g
              distilled H_2O 500 ml
                    adjust pH to 6.5 with minimal volume of 4 M KOH

    Solution D

              KNO_3 101 g
              $FeSO_4.7H_2O$ 1.25 g

stock solution B   5 ml
stock solution C   5 ml
stock solution D   5 ml

distilled H_2O 1 L
agar   4 g
distilled H_2O 500 ml

Table 1. General moss culture medium consists of three solutions made according to Cove (2004). These solutions were combined with distilled water and agar in the given proportions to form the medium.

A 10 mM Indole-3-acetic acid (IAA) stock solution was added to the medium to dilute it to 0.0073 mM IAA and a 10 mM Benzylaminopurine (BAP) stock solution was added to the medium to dilute it to 0.0089 mM BAP. The pH was then adjusted to 5.8 with 1.0 N NaOH or HCL. Then the medium was autoclaved at 121°C for 25 min. Before pouring, a 1 M $CaCl_2$ stock solution was added to the medium to dilute it to 1 mM. Plates were kept under 16/8 hours fluorescent lights with an intensity of 25 Microeinsteins m^{-2} sec^{-1} and at a temperature of 23°C.

A culture of *Ceratodon purpureus* was obtained from Dr. Mel Oliver at the United States Department of Agriculture in Lubbock Texas. It was kept in a sterile Petri dish containing a cellophane overlay plate throughout the experiment (Cove, 2004). To

transfer samples of this culture to replicate plates, a small amount of *C. purpureus* tissue was cut off from the original culture with a sterile knife and placed in a sterile microtube along with 0.6 ml sterile water. This solution was stirred with the tip of the knife to separate tissue filaments and then poured onto medium in a new plate. The solution was spread over the medium. A plate of *Ceratodon purpureus* cultured by these procedures is shown in Figure 5.
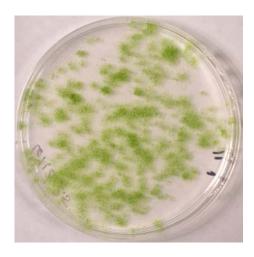

Figure 5. A plate of *Ceratodon purpureus* cultured with the above procedures. Picture taken with a digital camera.

Light intensity experiment

The first experiment varied light intensities in which *C. purpureus* was grown. Medium was made according to the method above and plates were poured and cultured as described above. Three plates were kept at the standard 25 Microeinsteins $m^{-2} sec^{-1}$ light intensity as a control and three plates were each kept in 15, 35, and 45 $\mu E\ m^{-2} sec^{-1}$ light intensity.

pH experiment

The second experiment varied pH concentrations of the medium on which *C. purpureus* was grown. For this experimental set up, medium was made according to the standard method but before the agar was added, the medium was separated into six beakers to allow the pHs to be adjusted separately with NaOH or HCL. The control medium was left at a pH of 5.8. The pHs of the other five media were adjusted to 3.8, 4.8, 6.8, 7.8, and 8.8. Then agar in the appropriate amount indicated by the standard medium recipe in Table 1 was added to each media, the media were autoclaved, and $CaCl_2$ was added to each media to a working concentration of 1 mM. Each of the six media were poured into three plates creating 18 total plates for this experiment. *C. purpureus* tissue was transferred to each of the 18 plates as described by the standard method.

ABA experiment

The third experiment varied abscisic acid (ABA) concentrations of the medium on which *C. purpureus* was grown. A 1 mM stock ABA solution was made with 25 mg ABA, 500 µL 95% EtOH, and 11.5 ml sterile water. This solution was distributed into 2 ml eppendorf tubes and kept frozen until needed. For this experiment, the basic medium described earlier was made but the medium was divided into separate beakers before the agar was added. Five separate beakers were used and one marked as the control had no added ABA. ABA was added to the other four beakers to create solutions of 0.04 µM, 0.4 µM, 4 µM, and 40 µM ABA. Then the pH of each media was adjusted to 5.8 and agar was added to each solution in the appropriate amount indicated by the basic medium recipe in Table 1. Each solution was then autoclaved, $CaCl_2$ was added to a working concentration of 1 mM, and each of the five media were poured into three plates giving

15 total plates for this experiment. *C. purpureus* tissue was transferred to each of the 15

plates in the method described previously.

Data analysis

Data for each of the three experiments was recorded and analyzed in the same

way. Recording began three days after media preparation and tissue culture. For instance

if tissue culture took place on Friday, data recording began on Monday. This was done to

identify filaments that may die in response to the tissue culture and to only allow data

collection on live filaments. On day 1 of data recording, three protonemal filaments per

plate were identified and marked for data collection. The length of each of these

filaments was measured on day 1 and then daily for five days. Measurements were made

using a 1.3M Pixel Moticam 1000 camera attached to a Bausch & Lomb dissecting

microscope. Motic Images Plus software was calibrated to enable precise measurements

of filaments through the use of micrographs (pictures taken on a microscope) generated

by the Moticam (Fig. 6). The number of branches on each of the three filaments was also

recorded each day for five days. Data on the length and branching of three filaments were
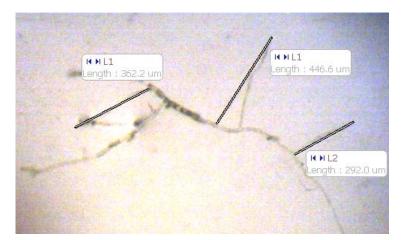
taken for each plate.

Figure 6. Motic Images Plus was used to take pictures of filaments, as seen
through the Bausch & Lomb dissecting microscope, and transfer them to the
computer program for analysis.

On each day, the mean length in µm per plate was found by averaging the lengths of the three filaments. Then the change in length per day for each plate was found. This gave a standardized number so that all plates could be compared based on their daily growth. The same analysis was done for change in the number of branches per day. Since there were replicate plates for each condition, the mean change in length and the mean change in branches were found for each. For example, the mean change in branches per day were found for 0 µM ABA, as well as for 0.04 µM, 0.4 µM, 4 µM, and 40 µM ABA.

On day 5 of data recording, ten colonies per plate were identified and marked for data collection. I anticipated a correlation between colony size and bud production so the ten identified colonies consisted of five large and five small colonies. These colonies were chosen at random. Then, 14 days from the date of culture, the number of buds per colony was counted and averaged for each plate. The data from the three experiments was compared and examined for similarities in trends.

<u>Results</u>

Light intensity experiment

Light intensity was varied to determine its influence on the growth of *C. purpureus*. The mean growth rates were -21 µm/day for 5 $\mu E \, m^{-2} \, sec^{-1}$, 187 µm/day for 15 $\mu E \, m^{-2} \, sec^{-1}$, 299 µm/day for 25 $\mu E \, m^{-2} \, sec^{-1}$, 196 µm/day for 35 $\mu E \, m^{-2} \, sec^{-1}$, and 307 µm/day for 45 $\mu E \, m^{-2} \, sec^{-1}$. Figure 7 shows that filaments in 25 and 45 $\mu E \, m^{-2} \, sec^{-1}$ of light intensity showed the highest growth rates (line slopes) over the 5 day period. However, filaments in 15 and 35 $\mu E \, m^{-2} \, sec^{-1}$ of light intensity also showed a steady increase in growth (Fig. 7). The mean change in length per day decreased as a result of filament death for filaments grown under 5 $\mu E \, m^{-2} \, sec^{-1}$ light intensity (Fig. 8). No

increase or change in branching occurred in filaments under 5 µE m^{-2} sec^{-1} light intensity (Fig. 9). The greatest change in length was observed in the control group, 25 µE m^{-2} sec^{-1} intensity, closely followed by the highest light intensity tested, 45 µE m^{-2} sec^{-1} (Fig. 8). Along with a great daily change in length, the control group also had the most branching observed over the 5 day period (Fig. 9). Filaments grown in 15 and 35 µE m^{-2} sec^{-1} of light intensity were similar in their mean change in length per day, which was around 180 µm (Fig. 8). Filaments in 35 and 45 µE m^{-2} sec^{-1} of light intensity had a similar amount of branching take place (Fig. 9). The mean change in branching was slightly lower for filaments in 15 µE m^{-2} sec^{-1} (Fig. 9).

The trend seen for number of buds was that plates in 25, 35, and 45 µE m^{-2} sec^{-1} of light intensity showed the highest mean number of buds per colony (Fig. 10). The error bars show that there may not be a difference between these light intensities (Fig. 10). Plates in 5 and 15 µE m^{-2} sec^{-1} of light intensity both had a lower mean number of buds per plate seen in (Fig. 10). However, looking at each plate separately (Fig. 11) it is noticeable that one plate seems to be an outlier in both 35 and 45 µE m^{-2} sec^{-1}, even though ten colonies per plate were counted. These outliers contributed to the high mean number of buds seen in each of the 35 and 45 µE m^{-2} sec^{-1} categories (Fig. 11). When looking at the size of the colonies, a higher mean number of buds per colony was seen for large colonies compared to medium colonies (Fig. 12).

pH experiment

pH was varied to determine its influence on the growth of *C. purpureus*. The mean growth rates were 357 µm/day for a pH of 3.8, 280 µm/day for a pH of 4.8, 342 µm/day for a pH of 5.8, 369 µm/day for a pH of 6.8, 322 µm/day for a pH of 7.8, and 151

μm/day for a pH of 8.8. Moss filaments exhibited positive growth rates at all pH levels, with filaments grown in a pH of 8.8 clearly showing the least growth (Fig. 13). Figure 14 shows the lowest mean change in length per day for filaments in a pH of 8.8. All other pH environments had similar mean changes in length per day (Fig. 14). Filaments in an environment of 3.8, 5.8, and 6.8 showed the highest mean change in branches per day, relative to the other groups and considering the wide range of error for the 3.8 and 5.8 groups (Fig. 15). From the control group (5.8) and higher, in more basic environments the mean change in branches declined (Fig. 15). Filaments grown in a pH of 4.8 showed an abnormally low amount of branching compared to the other groups (Fig. 15).

The mean number of buds per colony was the greatest for the two lowest pH groups (3.8 and 4.8), as can be seen in Figure 16. The smallest mean number of buds per colony was seen for colonies in a pH environment of 5.8 and 6.8 (Fig. 16). Colonies grown in a pH of 7.8 produced an intermediate mean number of buds while those in a pH of 8.8 had a smaller mean number of buds (Fig. 16). However, this mean number of buds for colonies in a pH of 8.8 was higher than colonies in a pH of 5.8 or 6.8 (Fig. 16). It is important to note that one plate in the 3.8 group is an extreme value because it shows a uniquely high mean, which undoubtedly raised the 3.8 group's mean value (Fig. 17); however, this is shown with the error bars in Figure 16. Another notable feature in Figure 17 is that groups 6.8 and 8.8 both had one plate with no buds, which lowered both groups' mean number of buds per colony. Considering colony size, a higher mean number of buds per colony was seen for large colonies compared to medium colonies (Fig. 18).

ABA experiment

ABA was varied to determine its influence on the growth of *C. purpureus*. The

mean growth rates were 356 μm/day for 0 μM ABA, 241 μm/day for 0.04 μM ABA, 283

μm/day for 0.4 μM ABA, 209 μm/day for 4 μM ABA, and 303 μm/day for 40 μM ABA.

The control group, which had a 0 μM ABA concentration, showed the highest growth

rate as well as the greatest length each day (Fig. 19). Figure 20 clearly shows that the

mean change in length per day for the control group was greatest. Considering error, all

other ABA concentration groups had a similar mean change in length (Fig. 20). While

filaments in the control group showed the greatest growth, they showed the least amount

of branching (Fig. 21). All ABA concentrations showed similar mean changes in

branching, when considering error (Fig. 21).

Figure 22 suggests that the optimum ABA concentration for bud production is 0.4

μM. The trend seen in Figure 22 is that the mean number of buds per colony declines in

both directions from that middle value of 0.4 μM. Another notable factor is that the

control group had the lowest mean number of buds per colony (Fig. 22). One plate in the

control group had a low mean number of buds per plate; however, this is not an extreme

value (Fig. 23). No other oddities or outliers are seen when looking at the individual

plates (Fig. 23). This suggests that the means for each group are good representations of

the population. When looking at colony size, a higher mean number of buds per colony

was seen for large colonies compared to medium colonies (Fig. 24).
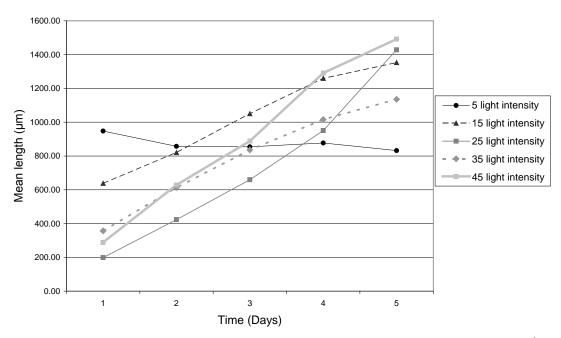
Figure 7. *C. purpureus* moss filaments were grown under light intensities ranging from 5 to 45 $\mu E\ m^{-2}\ sec^{-1}$ and were measured over 5 days. The mean length for all filaments grown under each light intensity is shown here.
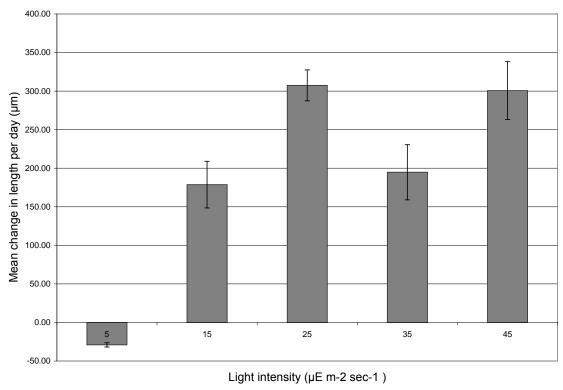

Figure 8. Filaments of *C. purpureus* were grown under light intensities ranging from 5 to 45 $\mu E\ m^{-2}\ sec^{-1}$ and were measured over 5 days. The mean change in length per day was calculated for each light intensity.
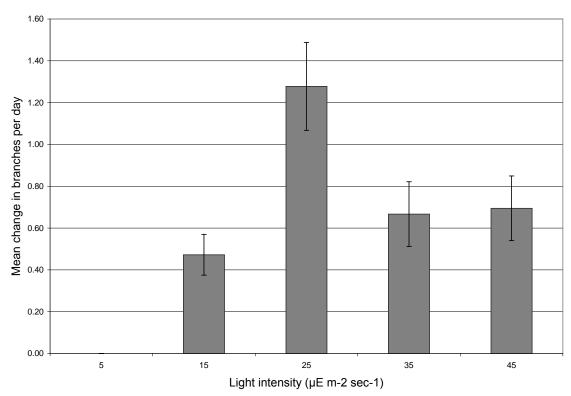
Figure 9. Filaments of *C. purpureus* were grown under light intensities ranging from 5 to 45 µE m^{-2} sec^{-1} and the number of times each filament branched was counted over 5 days. The mean change in branches per day was calculated for each light intensity.
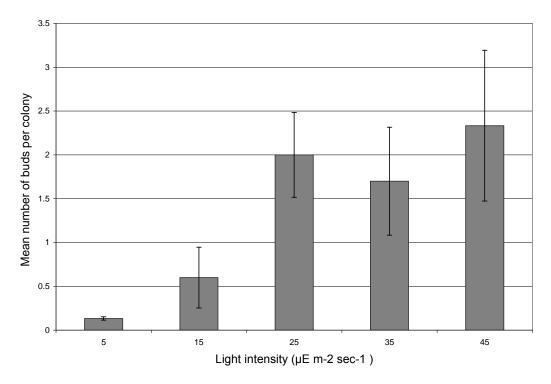
Figure 10. Filaments of *C. purpureus* were grown under light intensities ranging from 5 to 45 µE m^{-2} sec^{-1}. Buds were counted in 10 colonies per plate on day 14. The mean number of buds per colony was found for each light intensity.
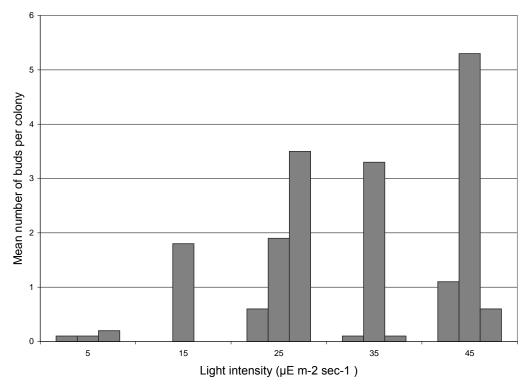
Figure 11. Filaments of *C. purpureus* were grown under light intensities ranging from 5 to 45 μE m^{-2} sec^{-1}. Buds on filaments of *C. purpureus* were counted in 10 colonies on each plate on day 14. The mean number of buds per colony was found for each plate and these means are shown here for each light intensity.
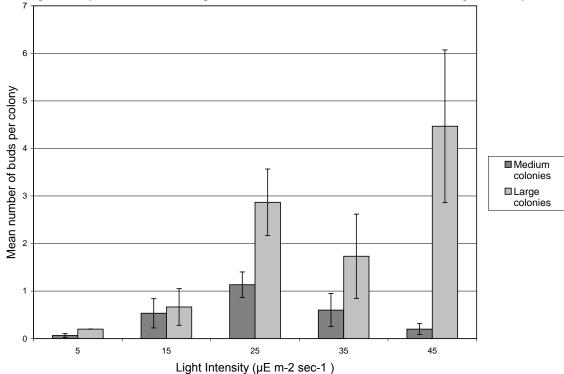

Figure 12. *C. purpureus* filaments were grown under light intensities ranging from 5 to 45 μE m^{-2} sec^{-1}. Buds were counted in 5 medium and 5 large colonies per plate on day 14. The mean number of buds per colony was found for each light intensity and colony size is distinguished here.
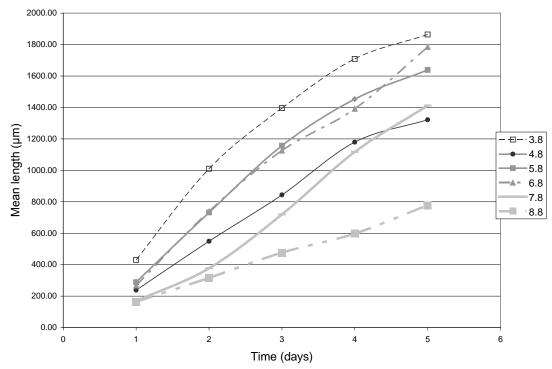
Figure 13. C. purpureus moss filaments were grown on media with pH values ranging from 3.8 to 8.8. Filaments were measured over 5 days. The mean length for all filaments grown on each pH is shown here.
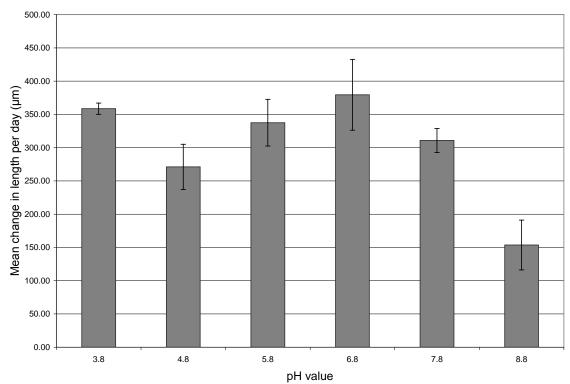

Figure 14. C. purpureus filaments were grown on media with pH values ranging from 3.8 to 8.8 and were measured over 5 days. The mean change in length per day was calculated for each pH.
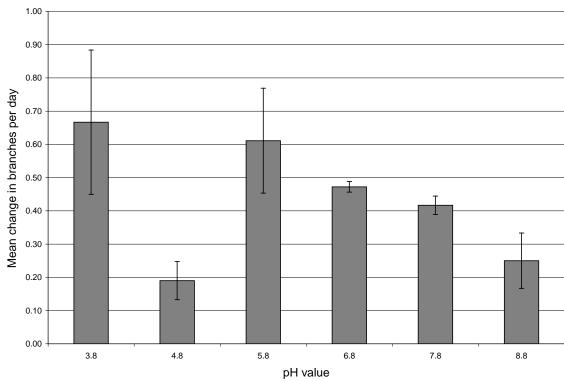
Figure 15. C. purpureus filaments were grown on media with pH values ranging from 3.8 to 8.8 and were measured over 5 days. The mean change in branches per day was calculated for each pH.
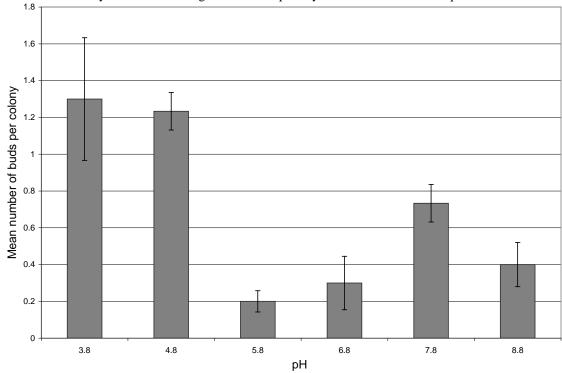

Figure 16. C. purpureus filaments were grown on media with pH values ranging from 3.8 to 8.8. Buds were counted in 10 colonies per plate on day 14. The mean number of buds per colony was found for each pH.
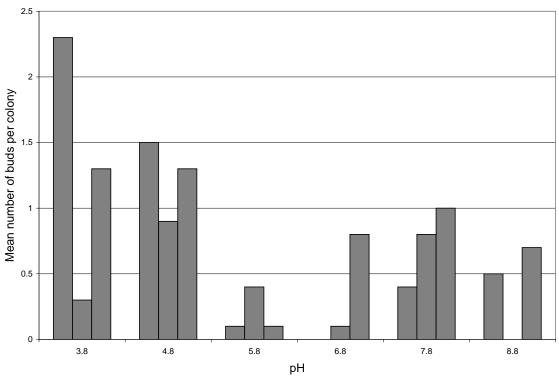
Figure 17. C. purpureus filaments were grown on media with pH values ranging from 3.8 to 8.8. Buds were counted in 10 colonies per plate on day 14. The number of buds per colony was averaged for each plate and these means are shown here for each pH.
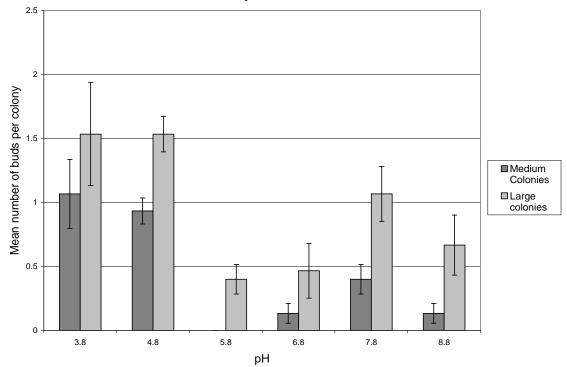

Figure 18. C. purpureus filaments were grown in pH environments ranging from 3.8 to 8.8. Buds were counted in 5 medium and 5 large colonies per plate on day 14. The mean number of buds per colony was found for each pH and colony size is distinguished here.
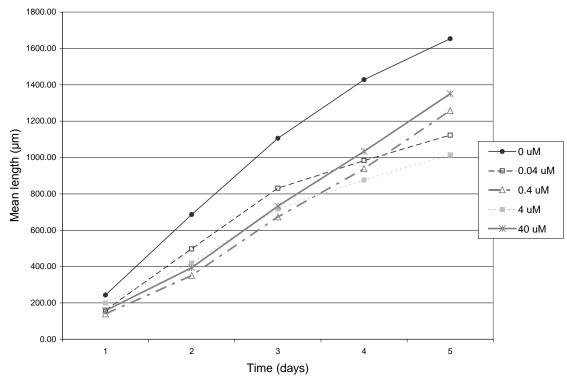
Figure 19. *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM and were measured over 5 days. The mean length for all filaments grown in each ABA concentration is shown here.

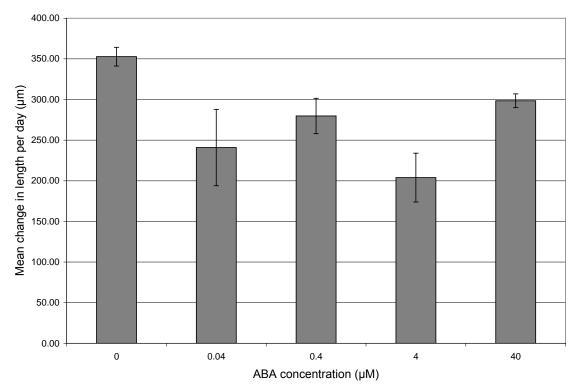
Figure 20. *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM and were measured over 5 days. The mean change in length per day for *C. purpureus* filaments was calculated for each ABA concentration.
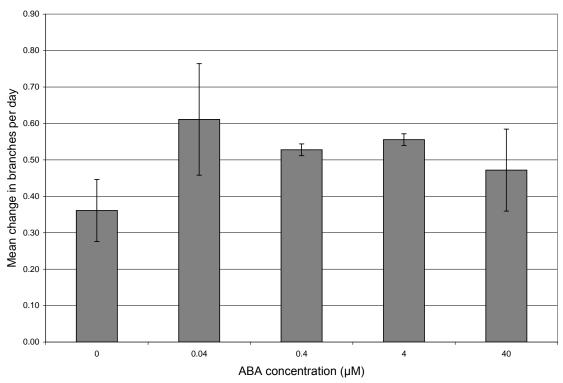
Figure 21. *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM and were measured over 5 days. The mean change in branches per day for *C. purpureus* filaments was calculated for each ABA concentration.

Figure 22. *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM. Buds on filaments of *C. purpureus* were counted in 10 colonies per plate on day 14. The mean number of buds per colony was found for each ABA concentration.
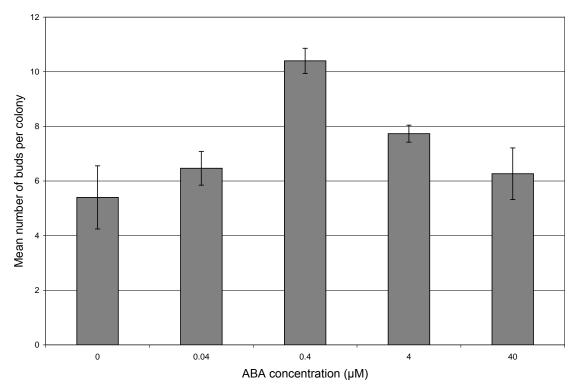
Figure 23. *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM. Buds on filaments of *C. purpureus* were counted in 10 colonies per plate on day 14. The mean number of buds per colony was found for each plate and these means are shown here for each ABA concentration.

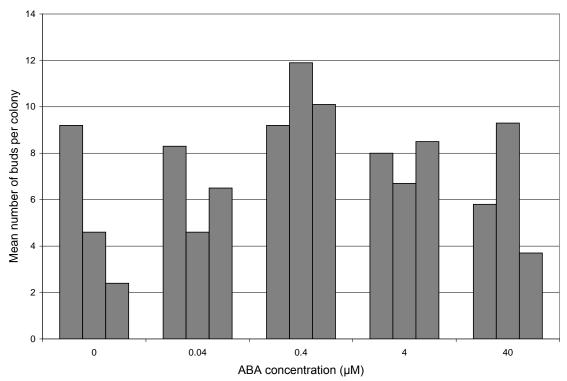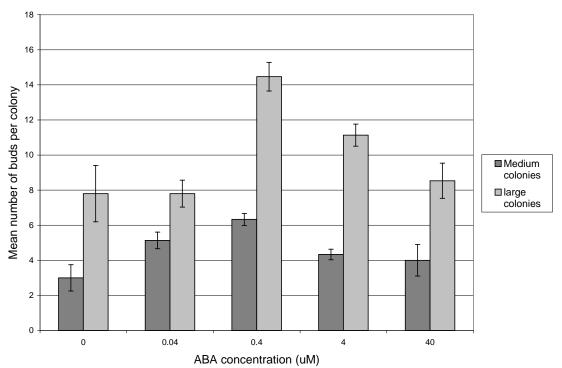*Figure 24.* *C. purpureus* filaments were grown on media with ABA concentrations ranging from 0 μM to 40 μM. Buds were counted in 5 medium and 5 large colonies per plate on day 14. The mean number of buds per colony was found for each ABA concentration and colony size is distinguished here.

Discussion

This study showed that environment is a major factor in growth and development of the moss *Ceratodon purpureus*. Growth declines with a lower or higher light intensity than the optimal 25 μE m^{-2} sec^{-1} and branching is greatly reduced in altered light intensities. Growth and branching also declined with extremely high pH values. The quick increase in growth from a pH of 5.8 to 6.8 could be accounted to the acid growth hypothesis, which states that an increase in acidity in a cell allows for cell expansion and elongation (Campbell and Reece, 2002). Then perhaps at a higher pH of 7.8 or 8.8 the cell is under too much stress and growth declines, possibly due to protein destruction in such a basic environment. Bud formation was clearly affected by both light intensity and pH. Lower light intensities inhibited bud formation and higher intensities had the opposite effect while any pH other than the control (a pH of 5.8) increased bud formation. The environment signals changes in growth and development according to Swinehart and Dietrich (2007) and Cove (1993), therefore this difference from the controls was expected.

This study began with the hypothesis that *Ceratodon* will respond in a similar fashion as *Funaria* (Christianson, 2000), which means ABA will inhibit bud formation. Therefore, I expected that with increasing concentrations of ABA, bud formation would decline. In addition, I expected to find a relationship between hormones and the environment because mosses utilize hormones to signal changes in growth and development much like the environment signals changes in growth and development (Swinehart and Dietrich, 2007 and Cove, 1993). Expecting to see similarities between the effects of hormones and environment, I anticipated to indirectly conclude that

environmental signals cause release of hormones, which trigger changes in growth and development of the moss.

Since hormones and environmental factors both signal changes in moss, this study began with the goal of comparing the responses seen in the presence of each of these individually. The data from the experiment testing ABA concentrations does not support the original hypothesis that with increasing ABA concentrations, bud formation is inhibited. In fact, the opposite was observed: the control plates with no ABA showed the fewest buds. Consequently, now three different observations of the effects of ABA have been seen with three different species of moss. In *Funaria* ABA inhibited bud formation, while in *Tortula* ABA had no effect on bud formation, and in *Ceratodon* ABA increased bud formation (Christianson, 2000 and Oliver, 2004). It is possible that *Ceratodon* simply behaves differently under the same experimental conditions. This study has clearly shown that exogenous ABA does influence the growth and bud formation of *Ceratodon*. The next step would be to test whether endogenous ABA and an ABA signaling pathway is present in this species of moss. This pathway has not been found in the moss *Tortula* (Cove, 1993). Nevertheless, more experimentation clearly needs to be conducted on additional species in the class Bryopsida in order to determine how the majority of the species in this class respond to ABA.

When looking at growth and branching as well as bud formation, there is some evidence that branching may be associated with bud formation. In many of the plates from these three experiments, an increase in branching of filaments was associated with a higher number of buds. Also, growth tended to be greatest in the control groups meaning that in normal optimal conditions, growth will continue rapidly. However, when

conditions become harsh, growth declines and resources are transferred to bud production and branching. Plausibly, this transfer to bud production is initiated by a release of ABA by the moss.

Since ABA plays a role in adapting to stresses, perhaps it initiates bud formation in *Ceratodon* in order to quicken sexual reproduction and spore production when adversity from stress is present; i.e. initiating reproduction in a time when there is a pressure to survive. This new hypothesis is supported by the light and pH experiments because the environmentally stressful situations such as a pH of 3.8 or a light intensity of 45 $\mu E\ m^{-2}\ sec^{-1}$ initiated an increase in bud formation. Comparing the control in the ABA experiment to plates where ABA was added, it is noticeable that ABA in general lowers the mean change in length, increases the mean change in branches, and increases bud formation. I saw the same results with a higher or lower pH, which leads to the conclusion that environmental signals may cause a release of hormones such as ABA, which in turn may trigger changes in growth and development of the moss. However, the light experiment does not sufficiently validate this statement because while a lower mean change in length was observed, some of the altered light intensities showed a higher mean change in branching. Perhaps branching is not related to the interaction of hormones and environmental factors in *Ceratodon*. To determine this, more experiments should be conducted specifically on branching and its relationship to ABA and environmental factors in this species. At the beginning of this experiment, I hypothesized that I was going to find an association between hormones and the environment. This was supported in part and may have some validity, but more research needs to be completed before this statement can be confirmed.

The results of the ABA treatments were unexpected. ABA did not inhibit bud formation but instead all treatments of ABA had a higher mean number of buds per colony than the control group with no added ABA. There are some potential explanations for these results. Cytokinin initiates bud formation and was added to all media used for all experiments in this study. Christianson (2000) has investigated its interaction with ABA previously and found cytokinin has two interactions: one when cytokinin is first perceived and the second when buds first initiate. Christianson has shown that these two interactions use different receptors. In his experiments with *Funaria*, ABA inhibits bud formation well after the initial perception of cytokinin, therefore only interfering with the second perception of cytokinin. At least in *Funaria* it has been shown that ABA is not a competitive inhibitor of cytokinin because ABA does not share either of cytokinin's two receptors (Cove *et al*., 1997 and Christianson, 2000). However, Christianson (2000) ends his study by saying that the interaction between cytokinin and ABA may be a mixed-competitive interaction, one that is neither non-competitive nor uncompetitive.

Subsequently, perhaps the reaction in *Ceratodon* is competitive and maybe *Ceratodon* does not naturally produce ABA; it was instead responding to what it perceived was an increase in cytokinin. It is possible also that *Ceratodon* evolved with a different response to ABA and has receptors for cytokinin and ABA unlike those present in *Funaria*. Since the effects of ABA are concentration dependent (Christianson, 2000), another plausible explanation is that bud inhibition would occur at higher ABA concentrations than were tested in this study. Perhaps *Ceratodon* requires a higher concentration of ABA than was demonstrated by Christianson (2000) to affect *Funaria*. This should be further studied with higher concentrations of ABA.

An additional explanation for ABA increasing bud formation may be that calcium was added to the medium used in this experiment. Before a bud forms, the chloronemal tip cell must differentiate into a caulonemal tip cell and produce a caulonemal filament and then cytokinin induces bud assembly in the caulonemal initial cell (Schumaker and Dietrich, 1998). Buds develop as side branches from caulonemal filaments and these develop into gametophores - leaf-bearing shoots (Cove *et al.*, 1997). While it is clear that cytokinin can stimulate the formation of a bud from a caulonemal initial cell, there are many questions still remaining about cytokinin-induced signaling such as where is endogenous cytokinin made and where does the perception that leads to bud assembly take place? Is it perceived intracellularly? In this present study, $CaCl_2$ was added to the medium before pouring plates. It has been shown in several studies that calcium plays an important role in bud formation (Schumaker and Gizinski, 1995 and 1996). Calcium acts as an intracellular messenger in cytokinin-induced bud formation and at the time of bud formation, channels become more sensitive to increases in cellular calcium (Schumaker and Gizinski, 1995 and 1996). However, Christianson (2000) did not add calcium to the medium used when ABA's inhibition of bud formation was seen in *Funaria*. Perhaps calcium is involved in inhibiting ABA's action and further experimentation should be conducted.

Future studies on this issue should include larger sample sizes. This preliminary study looked for trends seen in growth and bud formation in *Ceratodon* under the influence of various light intensities, pH environments, and ABA concentrations. Further experiments must include statistical testing to detect statistical significance of the data. Such analysis would require an increase in resources. In addition, statistical testing using

nested ANOVA's (light intensity, pH, and ABA) would be necessary to detect the

treatment effect among the variables and the variability among measurements for

individual plates. Additional studies could address changes in patterns of cell expansion,

elongation, and division to determine the mode of branch elongation and division

(Schumaker and Dietrich, 1998). In addition to the environmental conditions documented

in this study, it would most certainly be beneficial to compare the effects of ABA with

the effects of temperature on *Ceratodon* because ABA has been shown to induce freezing

tolerance in the moss *Physcomitrella patens* (Cove, 1993).

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