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Biology: Weird Science?

Ao Liu

Elizabeth Stephenson
University of Texas at Arlington

Audrey Hendrickson
University of Texas at Arlington

The Relationship of the Second Light Treatment to Cell Germination and Polarization in *Ceratopteris* Fern Spores

BIO 328D Plant Discovery Lab

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--Ao Liu

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Introduction:

Our research about the *Ceratopteris richardii* fern spores is part of the Discovery Lab in Plant Biology course. We focused on the relationship between the second light treatment and germination as well as polarization of the spores. Several groups of students tested different amounts of light and darkness on the influence of germination rate and the gravity response of the germinated spores.

Based on the results from a previous experiment, we hypothesize that a fixed amount of light is required to initiate germination in *Ceratopteris* spores and spores can stay in darkness for a certain amount of time before the second light treatment is given, which initiats photosynthesis and therefore continue the growth of organs. For *Ceratopteris* spores, the polar calcium current stage starts since the initial light and completes before 24 hours; the nuclear migration stage starts at around 24 hours; the first cell division stage starts at around 48 hours; the rhizoid emergence stage starts from 72 hours. Based on this background information, we designed our experiments using different light treatments in the first two rounds for testing which stage requires the light. Rhizoid polarity is fixed by gravity during the calcium current stage and nuclear migration stage. Different photoreceptors have different effects on the spores, and two main photoreceptors related to this research are phytochrome and chlorophyll. Phytochrome induces gene expression changes, and it can be activated by low-intensity red light, which only requires about half an hour's light treatment. Chlorophyll, on the other hand, requires high levels of red light, which usually requires hours of light treatment. Chlorophyll is required for photosynthesis, so the light and other things such as carbon dioxide will convert to ATP energy to support the development of spores'

growth until rhizoid emergence. This might be the reason why the second light treatment requires a lot of time when compared to the initial light. However, it is not known which photoreceptor is important to which treatment because both gene expression and energy are necessary for germination. There is also another photoreceptor called phototropin, which affects the direction of rhizoids by absorbing unilateral blue light. It would be hard for us to study when phototropin is working based on the limited research time and background information, but it is possible to test how long does it take the gravity to be fixed by observing the direction of rhizoid by flipping the plates at different times.

By carrying out a series of experiments, we tested how much light is needed to initiate germination and when the second light treatment is needed. We were also interested in which photoreceptors played the role in each light treatment, and how the change of light quantity and timing would affect the germination rate and the polarity of rhizoids.

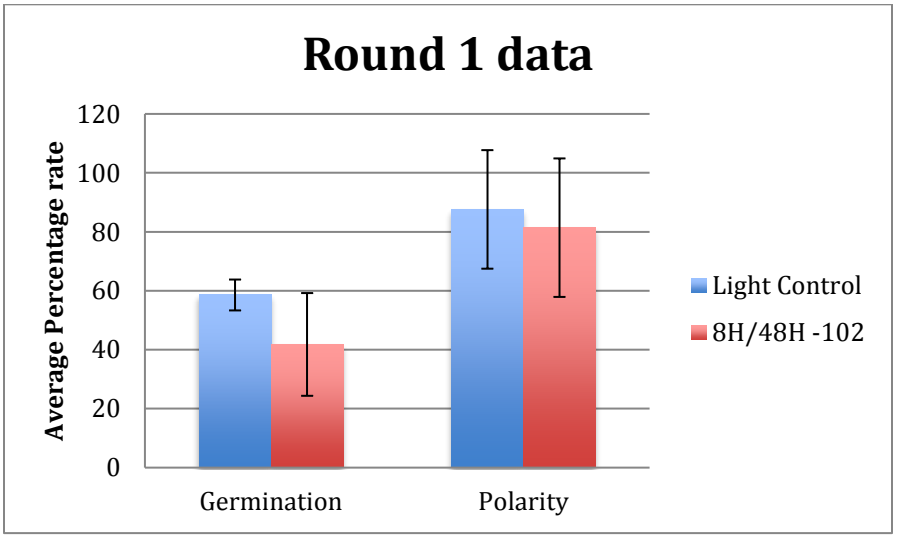
Round 1:

In Round 1, the spores were given 2-8 hours of initial light to test how short of an initial light treatment can support development of rhizoid emergence. They then stayed in darkness until 48 hours before they were given the second light treatment. The control plates were given continuous light. Our group and two other groups repeated the 8 hours of initial light, which was done in a previous experiment to confirm the results. The results shown below in Figure 1 combined all three groups data in this round. The standard deviations are large for mainly three reasons: First, the temperature varied in week 1 and week 2 because the incubator was not set as the same temperature. The lower temperature in week 2 delayed the germination of spores, so the germination rates were

lower in both the control and test plates compared to week 1 at the 78-hour observation. Second, the age of spores used in week 1 and week 2 were different which affected the germination rates. Last, the plates from week 1 were placed in a sealed container while the plates from week 2 were placed in complete darkness. Therefore, the germination rate for 8H/48H[1] plates were lower than 20% during the first observation which occurred around 78 hour but it increased to 60% around 102 hours.

Compared to the germination rate, the downward polarity rate is more stable and constant. Among the germinated spores in 8H/48H, the average percent of downward rhizoids was slightly lower than the control plate. This indicated that 8 hours initial light is enough to fix the polarity with the constant force of gravity. Because the darkness between 8 hours and 48 hours did not have an obvious influence on the polarity, so we concluded that the polarity would not be affected after 8 hours initial light treatment even they were then placed in darkness.

Figure 1. Round 1 data of germination rate and downward rhizoid rate.



[1] *8H/48H stands for the plate given 8 hours initial light, then stayed in darkness until 48 hour before placed back to light. All the description of plates with different light treatment will be using this format: Initial light hour/Back to light hour.

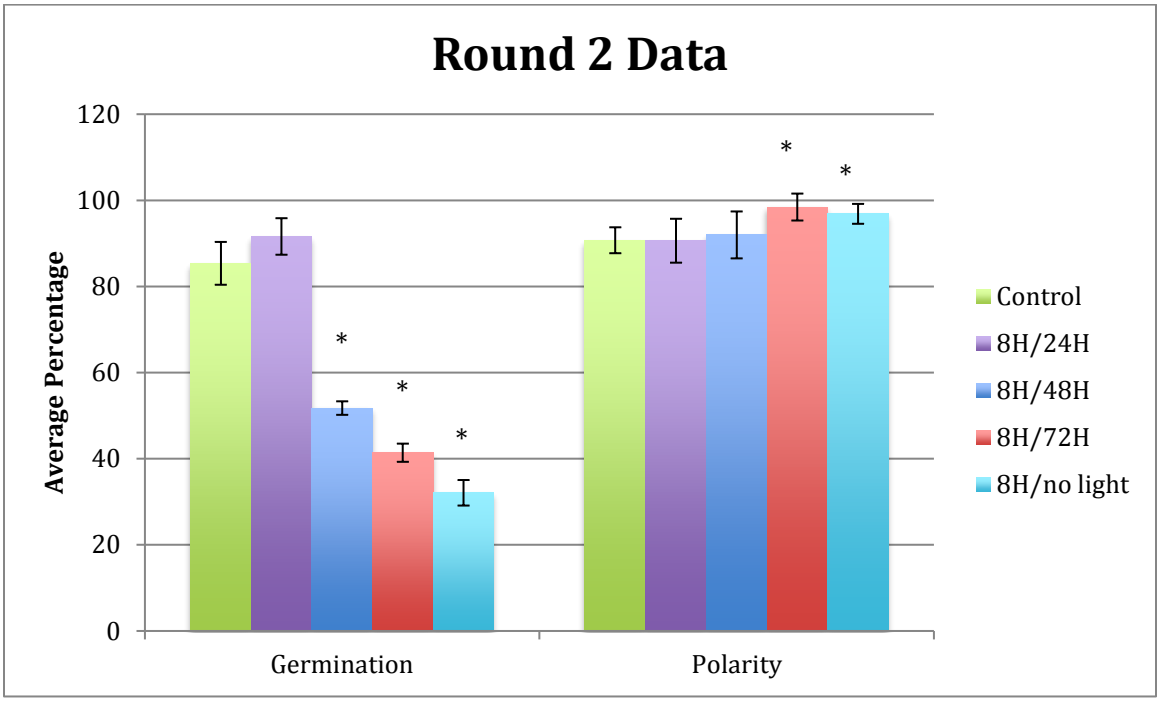
Round 2:

To avoid the variables we had in round 2, all the plates were kept at 30 degree Celsius in the incubators, and made sure they stayed in complete darkness when the LED white light treatment was not given. To indicate which stage of *Ceratopteris* spore germination is effected by the second light treatment, the length of darkness was modified in round 2. The same eight-hour initial light given to plates with the second light at 24 hours, 48 hours, 72 hours, and one plate without a second light showed a pattern of decreasing germination rates, respectively. As shown in the left part of Figure 2, the result of 8H/24H had a high germination rate, which was close to the control plate. This indicated that the second light treatment is not required before the nuclear migration stage. The 8H/48H plate showed a noticeable decline of germination rate compared to the control plate, which was lower than expected. Before the calculation, we ruled out an outliner of 70.21% by Dixon's Q test. This might be one of the reasons affecting our final result of the 8H/48H plate. We were expecting a germination rate around 80% based on the previous results while our plate in this round only showed around 51.77% germination. Therefore, the 8H/48H plate will be repeated in round 3 to confirm the whether the spores need the energy from light during their nuclear migration stage (24h) or right before the first cell division stage (48h). The significant decline in germination rate in 8H/72H plate implied that light was possibly required before the rhizoid emergence stage. However, we could not identify whether it was the nuclear migration stage or first cell division stage since our 8H/48H plate might not be accurate in this round.

It is also possible that the late light treatment only delayed but did not decrease the germination rate since we observed our plates at 104 hours. By the time of observation, the second light treatment has only amounted to 26 hours, which is not sufficient for germination. The other new plate in this round was the 8H/no light plate. Based on the previous research, no spores should germinate without the second light treatment, but we had around 30% germinated spores. Compared to the germinated spores from 8H/72H plate, the spores on 8H/no light plate were clear instead of green under the microscope, because no light was given to the chlorophyll for photosynthesis. However, since they still germinated without the second light treatment, this suggested that 8 hours initial light had enough effects on both Phytochrome and chlorophyll to support the development of growth. Otherwise, they must have received energy somehow. There was a slight possibility that their gene expression had changed by received natural light during the short amount time during observation at 78 hours. To rule out the possibility that they received energy from natural light during the observation time, they will be kept in darkness until right before observation in round 3.

Similar to the result from round 1, the downward rhizoid polarity rate was high in all plates mainly because the same amount of initial light was given. The result from the t-test showed significant differences between the 8H/72H plate and light control as well as the 8H/no light plate and light control. Both of the plates showed no germination at 78 hours and multiple rhizoids germinated at 104 hours. It was hard to identify which rhizoids were the primary ones; so only spores with all the rhizoids pointing upwards or horizontal were counted as non-downward. Therefore, the results might be higher than the reality since gravity only affects the primary rhizoid.

Figure 2. Round 2 data of germination rate and polarity rate.



After the first two rounds of experiments, we were certain that the 8 hours initial light was long enough to support the germination. However, since there were too many confounding factors for us to accurately identify when the second light was needed, more experiments and studies were required.

Round 3:

In round 3, we performed two different sets of experiments in week 1 and week 2. In week 1, we simply repeated the 8H/48H plate and 8H/72H plate to clear up the uncertainty from round 2. In week 2, we focused on the process of photosynthesis by applying the DCMU to experimental plates.

Week 1

In this week, based on results from the light control plate and 8H/48H plate, the spores were supposedly under good conditions for experiments. Other factors can be

excluded that may cause the low germination rate at 102 hours and higher rates in 120 hours and upwards. Also, the standard deviations for all the experimental plates were lower than 3, which indicated the accuracy of the results.

Repeating of the 8H/48H plate in this round showed an average of 79.18% of germination, which had no significant difference compared to the light control plate. This result was as expected as shown the preliminary study done by Ashley Cannon[2]. We concluded from this that the light between 8 hours and 48 hours was not necessary; this implied the second light would not be effective on the spores before the stage of cell division. As Figure 3-2 shows, the repeated 8H/72H plate had only a 7.82% of germination rate, which was significantly lower than the light control and also lower than the germination rate from round 2 (41.39%). This data displayed the light treatment from 48 hours to 72 hours was necessary to give the spore enough energy for germination. A low germination rate was expected because the energy was necessary for the spores before the rhizoid emergence stage so that they could be germinated. However, germinated spores increased at 120 hours and at 130 hours compared to the 7.82% that was observed at 102 hours. At 120 hours, it showed a significantly higher germination rate, which was approximately from 30-40%. At 130 hours, it showed an even higher germination rate, which was approximately around 60%.

The growing percentage of germinated spores implied that the spores were still developing. This discovery was surprising and maybe contradicted some of the conclusions we had. Since we were certain that the spores need energy before the rhizoid emergence stage, there was a high chance that delaying the second light treatment did not

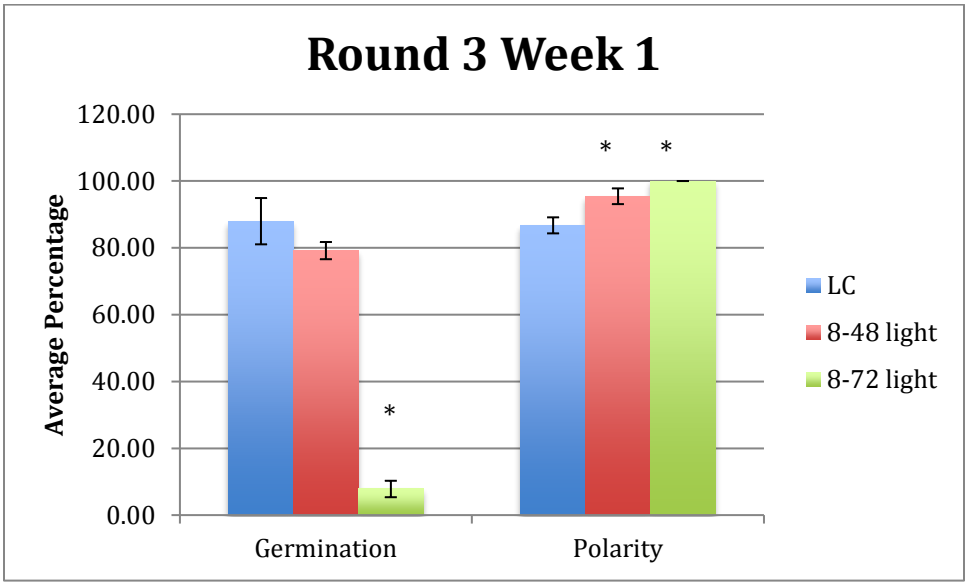
[2] BIO328D syllabus

cause the decrease in germination, but simply postponed the process of spore development. If this were true, then the timing of growing stages for the 8H/72H plate would no longer be the same as the light control. The 8H/48H plate may also have different time pattern of development in this case.

To indicate whether this idea is correct, a different method could be used in further study. Instead of counting the germination rate, the spores could be monitored over time and pictures of the spores can be collected at designated times. The pictures can be compared with a control plate, and the delay period can be observed through similarities and differences of the growth stages. For example, we could use an 8H/48H plate as a control and 8H/72H plate as an experimental plate. Then, take pictures of each plate at 72hr, 96hr, 120hr, etc. Since 8H/72H was given the second light 24 hours later than the 8H/48H plate, the 120hr picture of 8H/72H plate would be alike to the 96hr picture of 8H/48H if the postpone time were also around 24 hours.

The downward polarity rate for three plates showed a slight increasing pattern, which may suggest as the second light treatment decreases, downward polarity rate increases. However, based on our results of 8H/48H and continuous light plates from round 1 and round 2, there were no such consistent patterns. The 8H/72H plate had a 100% downward polarity rate mainly because their germination rate was only around 8%. So this low germination rate would not be strong enough to present a firm conclusion. Since we focused more on the germination rate, the polarity data were not included in Figure 3-1 even though the t-test showed all of three had significant differences between each other.

Figure 3-1. Round 3 week 1 data of Germination rate and Downward polarity rate.

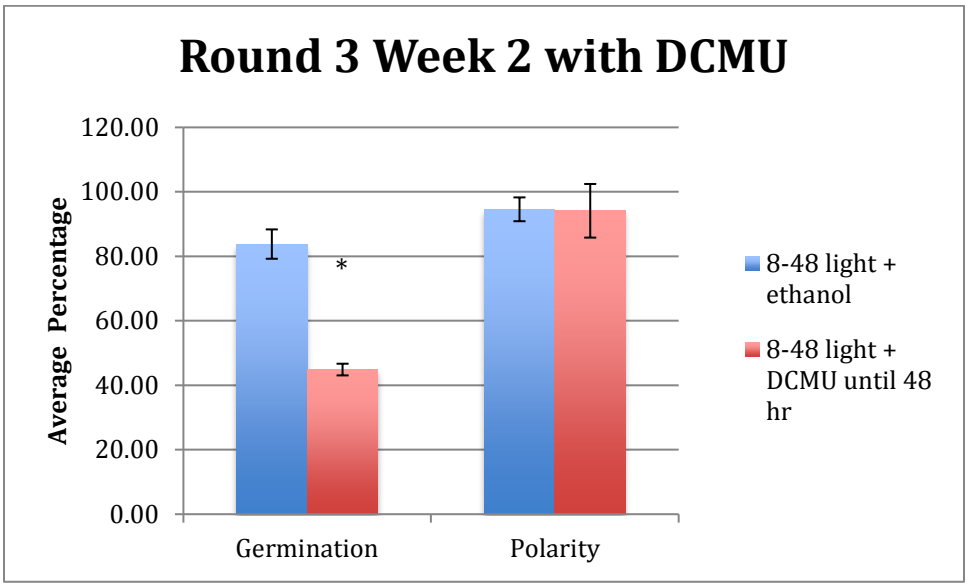


Week 2

Because of the limited time of research, we focused on what time period photosynthesis has effect on the germination in week 2. The control plate in this round was given light at 8H/48H with ethanol, and the experimental plate had DCMU presented from 0 to 48 hours under the same light treatment. The downward polarity rates were almost identical as expected, but the germination rate showed a significant difference. Since DCMU blocked photosynthesis, the result displayed that photosynthesis during the initial eight hours of light had an effect on the germination rate if the second light was given at 48 hours. The control plate had around 80% of germination rate, while the plate with DCMU only had a 44.87% as the Figure 3-2 shows. Photosynthesis in the initial light treatment may not be necessary for the germination because there was still a significant amount of germinated spores when photosynthesis was blocked. However,

photosynthesis during the 8 hours of initial light affected the control plate by producing a higher germination rate.

Figure 3-2. Round 3 week 2 data.



Round 4:

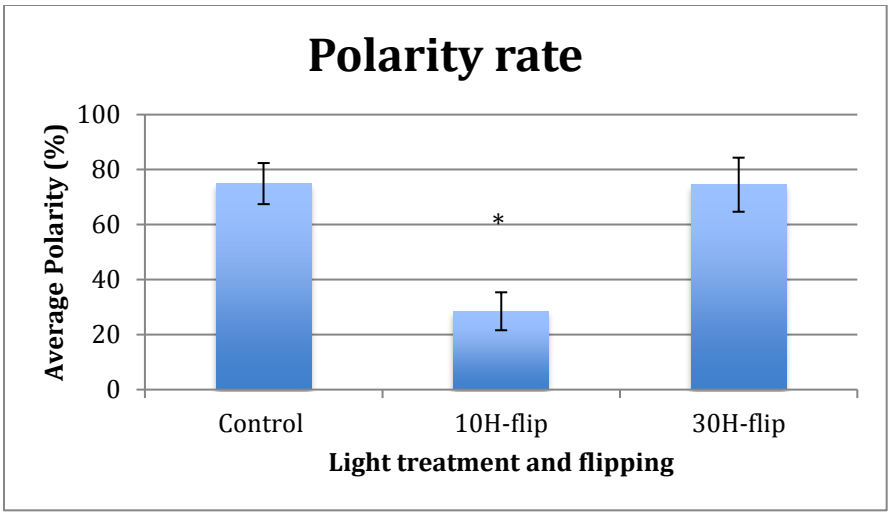
In the previous rounds of experiments, our group focused on the germination rate of the *Ceratopteris* fern spores when the second light treatment was given at different times. Based on the results of these experiments, we were certain that the light treatment between 8 and 48 hours was not required when the plates were under good conditions. From our experiment in round 3 week 2 with DCMU, our data implied that the photosynthesis did happen between 0 and 48 hours and would affect the germination rate. However, other groups' data showed the opposite conclusion so this may be an aspect for future study. However, we decided to focus on the polarity rate in our final round.

The previous research suggested that the effect of gravity would be fixed at some point between 10 to 32 hours, with the constant gravity being presented before the fixation. To minimize the variables of light effects, we gave all the plates 8 hours of

initial light, and then darkness until the 48 hours before they were given the second light. The control plate was placed with constant gravity pointing downward at all times. The first experimental plate was flipped 180 degrees at 10 hours, and the second plate was flipped at 30 hours. We hypothesized whether there was a significant difference of polarity rate between the experimental plates and the control plate; this would indicate that the polarity has not been fixed at the time when plate was flipped if there is a significant difference. The germination rates were around 60% for all three plates; we found out later that the fun of incubator was not working which give a lower temperature environment to the spores, which might be the main reason caused the lower germination rates. As a result, we discarded the germination rates data for this round. The polarity rates for both the light control and 30H-flipped[3] plate were around 75% downward[4], which indicated the effect of gravity was fixed before 30 hours. However, the 10H-flipped plate showed only a 28.485% of downward polarity rate, which was significantly different from the control plate. This result implied that the effect of gravity has not been fixed by the time of 10 hours. Despite the overall lower germination rates in this round, it would not affect our conclusion about gravity fixation. There were some errors for the plates on week 2, so we rejected the data. For further studying, it would be interesting to modify the flipping time and see whether the gravity fixation has connections to the spore growth of development.

Figure 4. Round 3 data of the downward polarity rates.

[3] 30H-flipped plate refers to the experimental plate that was flipped 180 degrees at 30 hours.
[4] "Downward" refers to the original orientation of the spores at 0 hours.



Conclusion:

Overall, there were a few conclusions on different aspects based on the experiments our group presented during this semester. First of all, a plate given 8 hours of initial light, then darkness until 48 hours and return to light shows no difference than a control plate given continuous light. The experimental plates given 8 hours initial light imply that the light before cell division stage was not necessary. Second, if the spores were given 8 hours initial light, it appears that photosynthesis may not be necessary for germination, but it does give a higher germination rate. Last, the effect of gravity on spores is not fixed at 10 hours but would be fixed before 30 hours under the condition of 8H/48H light.

For the purpose of achieving higher accuracy in further study, we could eliminate some preference bias, and set a standard counting and identifying method.