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THE RESPONSE OF MARCHANTIA POLYMORPHA L.
GEMMAE IN ASEPTIC CULTURE TO
TEMPERATURE, LIGHT, AND SUBSTRATE

by

Robert J. Thullen

B.S.F. Montana State University, 1960

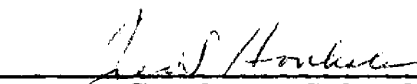
Presented in partial fulfillment of the requirements
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MONTANA STATE UNIVERSITY

1965

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Chairman, Board of Examiners


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ABSTRACT

This experiment was designed to show the response of Marchantia polymorpha L. gemmae grown in aseptic culture to temperature, light, and substrate. In the temperature experiments, the number of rhizoids produced decreased sharply at 26°C. Light intensities used in this experiment had no apparent effect on rhizoid initiation. Any decrease in the number of rhizoids was caused by an accompanying higher temperature and not by light intensity. A change in the position of the light from above the gemmae to beneath it caused a change in the percentage of rhizoids which arose from the upper and lower surfaces, but did not necessarily change the total number. With the light above, gemmae on the surface and gemmae submerged within agar produced 92 and 85% of their rhizoids, respectively, from the surface opposite the light. When the light was positioned beneath the gemmae only 50 and 45% of the rhizoids, respectively, were produced from the surface opposite the light. This appeared to be an auxin induced phenomena. In the former, light and gravity initiated rhizoids from the same side of the gemmae. In the latter, the effects of light and gravity were opposed. Total darkness caused the rhizoids to be produced only by the lower surface regardless of whether the agar was in contact with the upper or lower surface of the gemmae. The substrate therefore had no effect on rhizoid initiation. (The results of the total dark treatment are additional evidence that auxin is involved.)

The substrate was apparently only instrumental in the survival of the rhizoids after other factors had caused their production.

INTRODUCTION

It was observed that gemmae of Marchantia polymorpha L. produced rhizoids from the surface in contact with the soil (Haberlandt, 1914 and Smith, 1955). A gemma was capable of rhizoid production from either surface when young, but from only one surface when it became older (Sinnott, 1960). After the first few rhizoids were produced from the surface in contact with a substrate, the gemma produced rhizoids from the opposite surface when it was rotated 180° (Haberlandt). Light, gravity, and other environmental factors (Sachs, 1882 and Sinnott, 1960) influenced the initial development of rhizoids. Substrate moisture did not seem to be a factor but humidity was influential (Haberlandt, 1914). Gemmae did not produce rhizoids from the side in contact with dry air (Haberlandt, 1914).

Dormancy has not been observed in gemmae of M. polymorpha. However, in thirty years Oppenheimer did not observe a single gemma germinating in a gemmae cup (Moewus and Schader, 1952). There appeared to be a growth inhibitor present in the parent thallus which prevented germination of an intact gemma. When a gemma was removed from the parent thallus, and suitable substrate was found, germination occurred. Freed gemmae were light sensitive (Fitting, 1938 and Lilienstern, 1927) but reaction to light was modified by temperature, substrate, and other environmental factors (Fitting, 1936; 1937; and 1938). Lilienstern observed early gemmae mortality

and she attributed this mortality to strong light intensity (Lilienstern, 1927). Substances such as coumarin and parasorbic acid had been found to inhibit rhizoid initiation (Moewus and Schader, 1952).

Haberlandt (1914) attributed the initiation of rhizoids to a barometric pressure stimulus on plasma and starch grain arrangement which came about through the action of gravity. He further stated that the position of the nucleus predicted the position of the rhizoid on the wall of the cell. The rhizoid usually arose from the center of the outer cell wall. Light, as a stimulus, was not clearly understood; but the effect of light was recognized and light was said to have an influence although not as strong as that of gravity. Haberlandt also investigated the influence of the substrate on rhizoid initiation and concluded that the substrate and moist air were partially responsible for rhizoid production. The influence of the substrate was very limited while the action of dry air was effective in eliminating rhizoid initiation. This conclusion was contrary to the work of Gertz (1926). In experiments with Marchantia and Lunularia Gertz found that 71% of the rhizoids from the dry air side of the gemma provided that this side was dark. (This was an inverted situation with the light beneath the gemma.)

Gertz performed many experiments to test the influence of environmental factors on rhizoid initiation. By exposing opposite sides of a gemma to different stimuli, he found that the rhizoids were

produced in varying percentages from each side. The results of Gertz's experiments left little doubt that gravity, light, substrate, and moist air influenced rhizoid initiation; but because of a lack of adequate humidity and light controls the effect of the environmental stimuli was not clearly understood.

The following experiments were designed in an attempt to clarify the confusing results of past experiments and to add additional information about the initiation of rhizoids during germination of M. polymorpha gemmae. The objectives of this study were to determine the effect of temperature, light and substrate on rhizoid initiation of Marchantia polymorpha L.

METHODS

Because of the ease in handling and manipulation, agar was selected as the cultural medium. On a suggestion from Dr. C. C. Gordon¹, commercial orchid agar was tried and found satisfactory. Both the stock cultures and the experimental cultures were grown on this medium. The agar was made in one liter batches according to the manufacturer's directions and poured into stock culture jars and 18 x 150mm test tubes for experimental use.

Gemmae to be used in the experiments were grown under sterile conditions. The stock cultures were started with Marchantia polymorpha L. gemmae (Ammons, 1940) from the botany greenhouse on the Montana State University campus. Early experiments showed a need for contaminant-free plant material and a way to sterilize gemmae was sought. Although references in the literature frequently gave methods of keeping the medium free of contaminants, there was no mention of sterilization of gemmae until the work of Kaul, Mitra, and Tripathis (1962).

The sterilization method of Kaul et al, was not used as it was not discovered until my experiments were nearly completed. However, the following method was found satisfactory. The gemmae used to start stock cultures were sterilized for 3 to 4 minutes in a solution

¹Dr. C. C. Gordon, Assistant Professor, Botany and Microbiology Department, Montana State University.

of 5% commercial sodium hypochlorite bleach plus one drop of Tween detergent. The sterilized gemmae were then transferred aseptically into sterile culture jars containing orchid agar. These stock cultures were kept under 24 hour illumination. The lighting consisted of a 150 watt incandescent bulb at a distance of 15 inches (Anthony, 1962) preceded by 4 days of low intensity light (Lilienstern, 1927) from a bulb of the same wattage at 5 feet. Because of the lack of room temperature controls, the temperature was kept less than 26°C. Experience showed that temperatures above 26°C often proved fatal to gemmae, and also to mature thalli. Even though the gemmae were paler in color than usual, the cultures seemed to be quite healthy and vigorous at 25°C.

The last stock culture was placed 15 inches from a 150 watt incandescent light source with no initial subdued light period and no adverse effects were noted. Three weeks after the culture had been started, the gemmae grew into healthy and normal looking thalli. Unlike the previous stock cultures which were kept at 25°C, this latest culture was placed in a room at 19 to 20°C.

Ideally one gemma was placed in a test tube, but sometimes two or more gemmae would be inadvertently placed on the agar. If two or more gemmae were placed in a test tube, they were separated to lessen any possible interaction. Because of the short growing period involved in the experiment, there should have been no competition

between gemmae in the same test tube. Surface grown gemmae were placed on the agar surface with a small platinum spatula. Submerged gemmae were placed either in slits made in the agar with the spatula or were covered with small pieces of agar taken from the edge of the medium. Sometimes it was necessary to cover the slits with pieces of agar to insure that the gemmae were submerged.

The experiment comprised 15 treatments. Light intensity and position, temperature, and gemmae substrate relationship were varied to determine the effect on rhizoid initiation and production. Illumination with light of three different intensity ranges; high, medium, and low intensity; was used in the experiment (See Table 1). A complete summary of the experimental conditions can be found in the appendix.

When counting rhizoids, a polarized light microscope was used and the dense rhizoid tips were counted to avoid confusion. It was possible to count accurately both the upper surface and the total number of rhizoids without turning over the gemma. However, when it was necessary to invert the gemma, the gemma was first mounted between two cover glasses. In this way, the unit was turned over without disturbing the mounted gemma.

Although most microscopic work was done with living specimens, fixed material was examined. The microtechnique procedures were predominantly those of Sass (1958) and Johannson (1940).

TABLE 1
LIGHT INTENSITY RANGES

	5 day growing period	2 day growing period		
	High	High	Medium	Low
1st 24 hr	45 ft-c*	90 ft-c	45 ft-c	10 ft-c
2nd 24 hr	90	120	60	25
Remain- der	120			

Continuous illumination

*Foot candles.

RESULTS

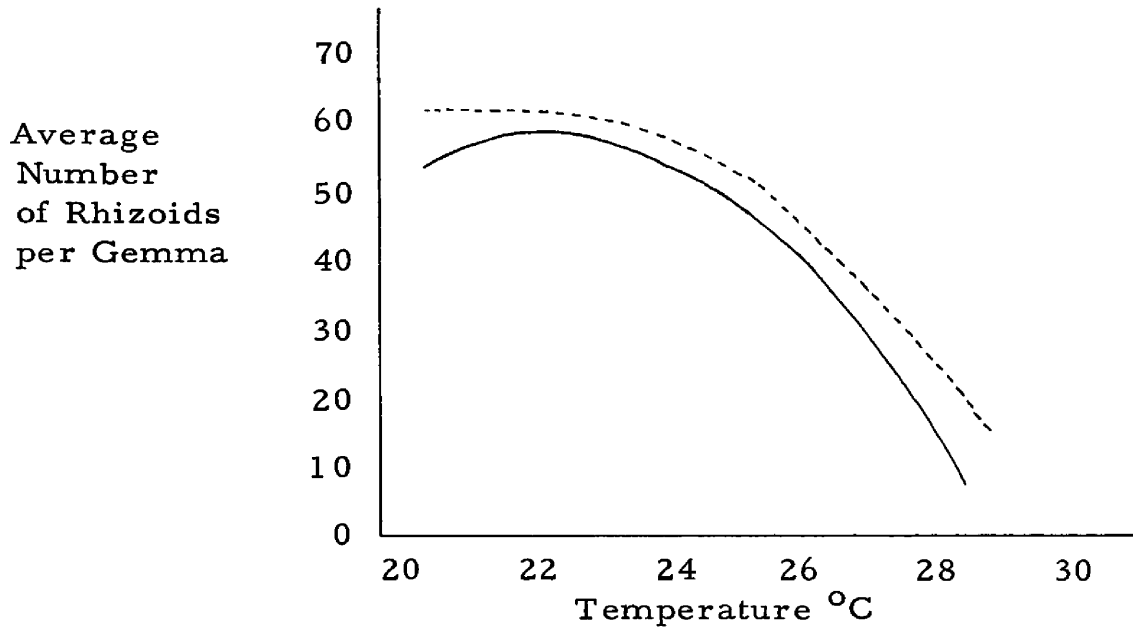
At approximately 26°C, there was a rather sharp decrease in the number of rhizoids produced (See Figure 1). The decrease was seen in the submerged gemmae as well as those grown on the surface of the agar. However, a decrease in the number of rhizoids was not the only effect of high temperature. At 28°C those gemmae which did produce rhizoids had only atypical or abnormally shaped rhizoids.

At temperatures below 26°C the rhizoids were long, straight, tapering slightly over their entire length to the region of the tip, and the tip rounded off in what appeared to be a hemisphere. Above 26°C the rhizoids were short, 1/4 normal length or less; with greater taper than normal; and the tip was bulbous. The upper surface rhizoids grown at the higher temperatures showed a very sharp curvature in response to gravity. At 28°C, these upper surface rhizoids grew downward immediately after clearing the surface of the gemma (See Figure 2). However, at temperatures less than 26°C the upper surface rhizoids arched more gently, and usually started a downward path some distance from the gemma. The effect occurred regardless of whether the gemma was surface grown or submerged in the agar.

During the same set of experiments, an inverted treatment (Treatment L) produced no rhizoids. While the room temperature was 28°C, the temperature near the gemmae was suspected to be higher than 28°C due to a black cloth which was used to eliminate reflection.

FIGURE 1

TEMPERATURE EFFECT ON THE
PRODUCTION OF RHIZOIDS



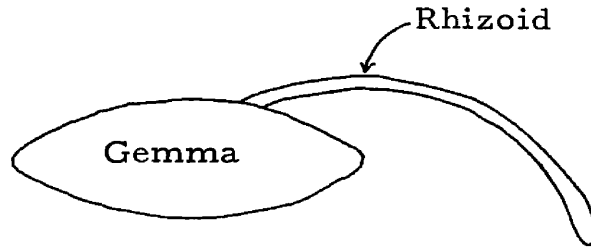
A 5 day growing period with continuous illumination from above. (See Table 1)

----- Gemmae grown beneath agar surface.

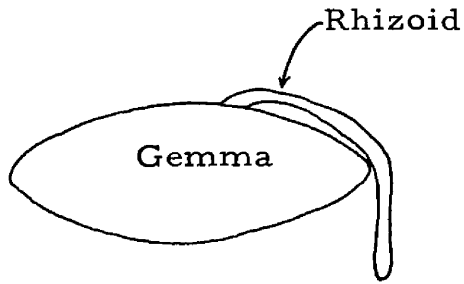
————— Gemmae grown on the agar surface.

FIGURE 2

CURVATURE OF RHIZOIDS AS EFFECTED
BY TEMPERATURE



Temperature less than 26°C

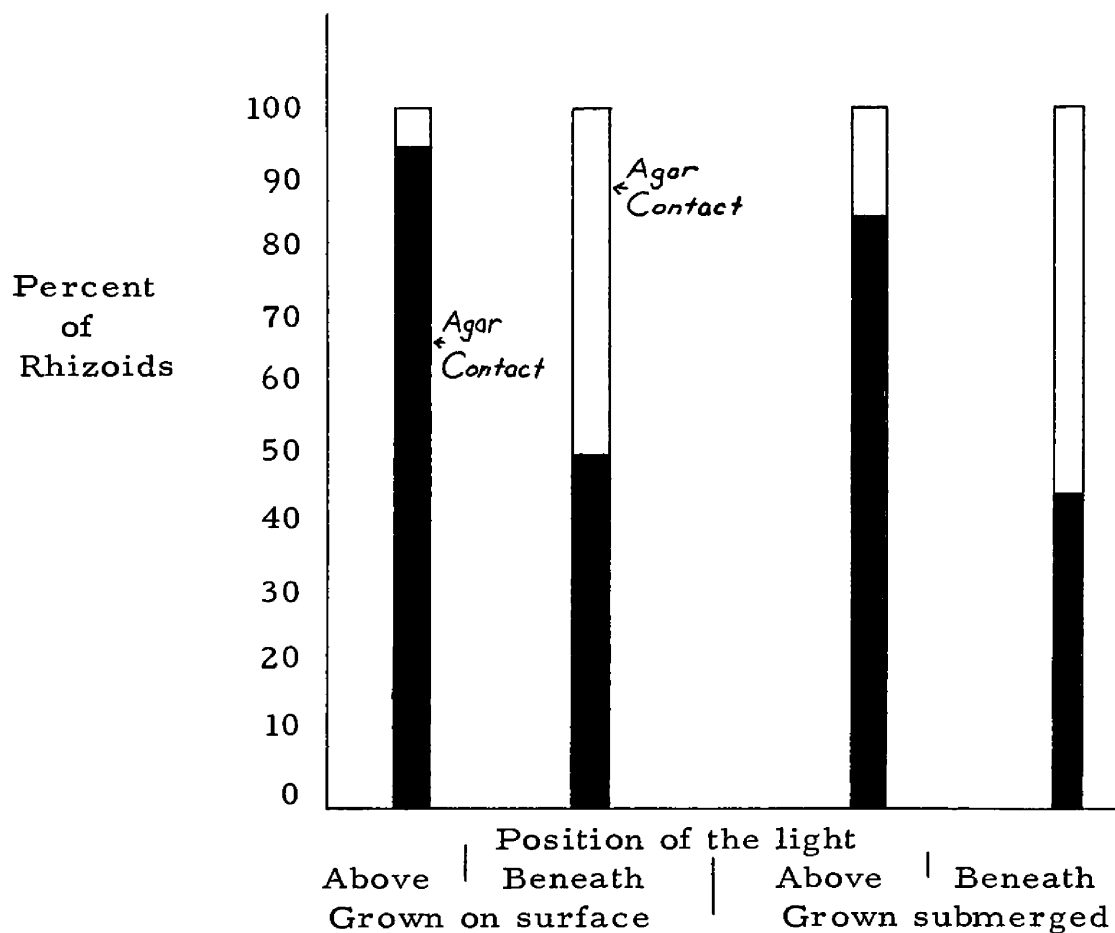


Temperature greater than 26°C

The effect of substrate was tested to a limited extent. Observations made on germinating gemmae by the author before this experiment showed that some rhizoids formed from the side of the gemmae not in contact with the substrate. The logical question was whether the number of rhizoids would increase if the gemmae were surrounded by substrate. There was an increase of less than 10% caused by the substrate when the gemmae were submerged within the agar (See Figure 3). In treatment Q, where no light was used and the agar was in contact with the upper surface only, 100% of the rhizoids were produced from the lower surface. Here substrate contact had no effect.

When the position of the light was changed so that the illumination came from beneath the gemmae, a change in the number of rhizoids coming from each surface was observed. When the gemmae were surface grown with the light coming from above, the gemmae had nearly all the rhizoids coming from the surface not facing the light. On the average 92% of the rhizoids were found to appear from the side of the gemmae facing downward and farthest from the light (See figure 3). The 8% of the rhizoids growing from the light facing surface might have been induced by light being reflected from the surface of the glass test tube and from the agar. Such a conclusion was suggested by the dark treatments (Treatments P and Q) where 100% of the rhizoids grew from the downward facing surface. By changing the position of

FIGURE 3
EFFECT OF LIGHT, GRAVITY, AND
MOISTURE



The light area represents the percent of the rhizoids coming from the surface of the gemma facing upwards.

The dark area represents the percent of the rhizoids coming from the surface of the gemma facing downward.

the light to beneath the gemma, about 50% of the rhizoids now arose from the side of the gemma facing downward and the light, and 50% of the rhizoids arose from the surface of the gemmae facing upward and opposite the light source. When the gemmae were submerged within the agar a like change occurred. With the light above the submerged gemmae, about 85% of the rhizoids were produced from the surface of the gemmae facing downward and opposite the light (See Figure 3). When the light was placed beneath the submerged gemmae, about 45% of the rhizoids arose from the downward and light facing surface, and 55% of the rhizoids appeared from the surface facing upward and opposite the light.

DISCUSSION

The mortality of gemmae observed under experimental conditions by Lilienstern (1927), which she attributed to high light intensity, was questionable. The light, in Lilienstern's experiments, was sunlight at a south window in April. The intensity of this sunlight may have been less than that found in nature during the summer in some areas inhabited by Marchantia polymorpha. After a forest fire M. polymorpha would grow if the area had sufficient moisture (Wilson and Loomis, 1952). The difference between the forest conditions, after a fire had denuded the land, and Lilienstern's experiments was probably temperature. Lilienstern gave no temperature data. The test tubes used in her experiments, when placed in the sun, probably heated more than the cool moist habitat where M. polymorpha is found in nature.

Temperature, as the cause of gemmae mortality, was also suggested by the complete lack of survival of the gemmae occurring in Treatment L where the temperature was suspected to be above 28°C. In Treatment L two consecutive 24 hour periods of reduced light intensity, 45 and 90 ft-c, were used, followed by 3 days of 120 ft-c. Other gemmae which grew under the same light source but at a slightly lower temperature, here the recorded temperature was 28°C, did not die but produced some rhizoids. The last stock culture, as noted in the methods, was placed 15 inches from a 150 watt bulb yielding

132 ft-c at a temperature of 19 to 20°C with no period of reduced light intensity and it did not show abnormal development.

Experimentation by Haberlandt (1914) attributed rhizoid initiation to environmental influences upon the nucleus and starch grains of the cell. Haberlandt proposed that rhizoid initiation was established by the action of the nucleus and statolithic starch in cells predestined to produce rhizoids. Smith (1955) also stated that some cells were predestined to produce rhizoids. "Gemmae lodging on soil develop rhizoids from colorless cells on the face next to the soil." Such colorless cells (it was assumed that colorless meant the lack of chloroplasts) had not been observed during the author's experiment. Although some cells looked colorless, upon closer examination the cells did contain chloroplasts. No sectioned gemma showed cells which were free of chloroplasts in the cup or before germination. Some rhizoids were observed with chloroplasts, and it was suspected these chloroplasts migrated from the cell which produced the rhizoid. However, rhizoid-producing cells were predetermined to a limited extent; rhizoids were produced only by the cells centrally located on the surface of the gemma. Only a few rhizoids were observed growing from the edge of the gemma and then never from the vicinity of the apical initials.

Observations on gemmae which were allowed to grow longer than five days indicated that all the rhizoids produced by the gemmae them-

selves were formed by the fifth day. All rhizoids which arose after this time appeared from the newly formed thallus tissue and not from the original tissue of the gemma.

On the basis of data from the light position experiments, it appeared that rhizoid initiation may be mediated by auxin. Light, when directed at the gemmae from beneath, caused about 50% of the rhizoids to be initiated from the surface opposite the light and the effect of gravity. This was in contrast to 92% of the rhizoids which were produced from the surface farthest from the light when the light was above the gemmae. In the former, the action of light and gravity on the gemmae was opposed. In the latter, light and gravity were both acting upon the gemma to produce rhizoids from the same side. This effect may have been due to auxin migration, destruction of auxin on the lighted side, a decrease in the sensitivity of the tissue on the lighted side, or some combination of these effects (Sinnott, 1960). In experiments conducted in the absence of light, all of the rhizoids were produced by the downward facing surface (See Treatments P and Q). In the above experiments the auxin appeared to have been acted upon only by gravity resulting in rhizoids from only the lower surface.

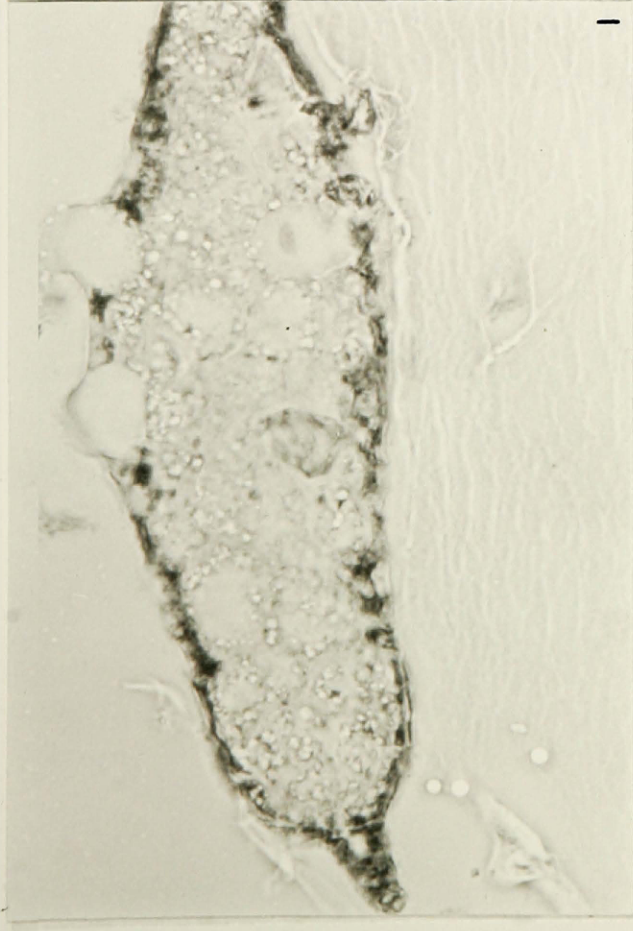
Although the data on substrate effect was limited, the conclusion was that substrate had very little or no influence. A gemma seemed capable of rhizoid production with equal success whether a surface was in contact with the agar or not. Evidence was seen in Treatment

Q where the agar contacted only with the upper surface and the rhizoids were all produced by the lower surface. My observations supported the conclusion of Haberlandt (1914) that something other than substrate contact was necessary for rhizoid formation. Haberlandt stated that dry air will stop rhizoid initiation and substrate contact was not very influential in rhizoid initiation.

In nature the initiation of rhizoids is probably an auxin effect. The auxin may be acted upon by gravity and light producing rhizoids from the surface where the auxin is concentrated. Few or no rhizoids are produced from the surface where the auxin is reduced below some critical quantity. Such factors as humidity and substrate determine the survival of rhizoids arising under various environmental conditions. Rhizoids growing into a dry environment become dessicated and die, but the initial stimulus is probably still in effect on rhizoid-producing cells. Such dessicated rhizoids were observed in several experiments.

APPENDIX
PHOTOGRAPHS

- Photo 1. A cross section of a gemma, harvested 4 days after planting, showing 2 upper surface rhizoids. The substrate, in this and succeeding photos, was orchid agar.
- Photo 2. A stock culture containing an excellent growth of Marchantia polymorpha, which was started from a single gemma.
- Photo 3. A cross section of a 6 day old gemma showing an upper surface rhizoid.
- Photo 4. A cross section of a 2 day old gemma showing a lower surface rhizoid.



SUMMARY OF THE TREATMENTS

Gemmae on agar surface		
Light from above		
	% Rhizoids from the surface of the gemma facing the light	% Rhizoids from the surface of the gemma not facing the light
	Light intensity:	
	First 24 hrs.	45 ft-c
	Second 24 hrs.	90 ft-c
	Remainder	120 ft-c
	Harvested on fifth day	
Treatment A		
temp. 28°C	60	40
Treatment B & C		
temp. 26°C	16	84
Treatment D		
temp. 20 to 22°C	11	89

	Light intensity:	
	First 24 hrs.	90 ft-c
	Second 24 hrs.	120 ft-c
	Harvested after 48 hrs.	
Treatment E		
temp. 16 to 19°C	77	93

	Light intensity:	
	First 24 hrs.	45 ft-c
	Remainder	60 ft-c
	Harvested after 48 hrs.	
Treatment F		
temp. 25°C	22	78

	Light intensity:	
	First 24 hrs.	10 ft-c
	Remainder	25 ft-c
	Harvested after 48 hrs.	
Treatment G		
temp. 25°C	15	85

Summary Con'd

Gemmae grown submerged
Light from above

<u>% Rhizoids from the surface of the gemma facing the light</u>	<u>% Rhizoids from the surface of the gemma not facing the light</u>
--	--

Light intensity:

First 24 hrs.	45 ft-c
Second 24 hrs.	90 ft-c
Remainder	120 ft-c

Harvested on fifth day

Treatment H temp. 28°C	53	47
Treatment I temp. 26°C	18	82
Treatment J temp. 20 to 22°C	32	68

Light intensity:

First 24 hrs.	90 ft-c
Second 24 hrs.	120 ft-c

Harvested after 48 hrs.

Treatment K temp. 16 to 19°C	15	85
------------------------------------	----	----

Summary Con't

Gemmae on agar surface

Light from beneath

<u>% Rhizoids from the surface of the gemma facing the light</u>	<u>% Rhizoids from the surface of the gemma not facing the light</u>
--	--

Light intensity:

First 24 hrs.	45 ft-c
Second 24 hrs.	90 ft-c
Remainder	120 ft-c

Harvested on fifth day

Treatment L
temp. 28°C
suspected to
be higher)

0 covered with a
black cloth 0

Treatment M
temp. 26°C

26 74

Light intensity

First 24 hrs.	90 ft-c
Second 24 hrs.	120 ft-c

Harvested after 48 hrs.

Treatment N
temp. 16 to
19°C

49 51

Summary Con't

Gemmae grown submerged

Light beneath

<u>% Rhizoids from the surface of the gemma facing the light</u>	<u>% Rhizoids from the surface of the gemma not facing the light</u>
--	--

Light intensity:

First 24 hrs.

90 ft-c

Second 24 hrs.

120 ft-c

Harvested after 48 hrs.

Treatment O
temp. 16 to
19⁰C

42

58

Gemmae on agar surface

Agar below

<u>% Rhizoids from the surface of the gemma facing upward</u>	<u>% Rhizoids from the surface of the gemma facing downward</u>
---	---

Grown in the dark

Harvested on fifth day

Treatment P
temp. 25⁰C

0

100

Gemmae on agar surface

Agar above

Grown in the dark

Harvested on fifth day

Treatment Q
temp. 25⁰C

0

100

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