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The Response of Pilobolus Microsporus to Light Stimulation

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THE RESPONSE OF PILOBOLUS MICROSPORUS TO LIGHT STIMULATION

BY

LURA ELIZABETH PARSONS

THESIS

FOR THE

DEGREE OF BACHELOR OF ARTS

IN BOTANY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Lura Elizabeth Parsons

ENTITLED THE RESPONSE OF PILOBOLUS MICROSPORUS TO LIGHT STIMULATION

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF ARTS

Chas. F. Hottes
Instructor in Charge.

APPROVED: J. Burrill

HEAD OF DEPARTMENT OF Botany

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The Response of Pilobolus microsporus to Light Stimulation.

The spores of Pilobolus are abundantly present in the manure of herbivorous animals. From the fresh manure used with the following experiments, the sporangiophores begin to rise above the substratum in which the mycelium is buried in about a week or ten days, usually appearing in the afternoon. By evening the sporangiophores are frequently one or two millimeters long, their yellow tips are either tapering and pointed, or swollen into rounded knobs. During the night these knobs develop into mature sporangia. Immediately below the sporangium the sporangiophore is swollen by the high osmotic pressure; the force of the ejected water upon the bursting of the membrane immediately below the sporangium hurls the latter for some distance thru the air.

Klein (1872 pp. 306-339) described in great detail the development of the mycelium and sporangia. To this Brefeld ('75 pp. 62-74) added further details of the development of sporangia, and also gave a few physiological observations. In this respect, however, Gräntz has done far more.

The experiments of Gräntz ('98,p.5) were made on Pilobolus microsporus. He cultured Pilobolus on sterilized manure, or agar-agar with manure decoction. With these cultures Gräntz (pp.8-9) observed that in darkness sporangiophores do not appear regularly each morning, as they do in light. The sporangiophores formed in darkness are white, tapering, and half the diameter of those formed in bright light. They grow for weeks (p. 13) without the least

sign of sporangia formation, and occasionally attain the height of 200 mm. Previously Brefeld ('75,pp.76-77) observed that in darkness sterile sporangiophores grew to be 8-10 inches long in from 10 to 14 days.

Gräntz (pp. 11-12) from experimental evidence concluded that the intensity of the light regulated the rapidity of sporangia formation, and so indirectly the length of the sporangiophores since with the formation of the sporangium the growth of the sporangiophores ceases. Thus in intense light the sporangiophores attain a heighth of 1/2 to 1 mm.; in much dimmer light they frequently attain a height of 25 mm. The rate of growth in each case is the same. Since in dim light the sporangia are formed much later than in intense light, the sporangiophores have continued to grow for a longer time and thus attain a greater height .

Gräntz (pp. 14-15) observed in bright light, but with a lower temperature and much moisture, long sterile sporangiophores. He concludes that it is possible that some other factor than the absence of light may be the cause of the elongation and sterility of P. micros. The small diameter (p.16) he shows experimentally, is the characteristic effect of etiolation. Gräntz and Brefeld both experimented to determine the necessary length of time in light to induce the formation of sporangia.

Gräntz (pp. 38-40) found that it requires an exposure of 4-5 hours in diffused light to induce the formation of sporangia on all sporangiophores, regardless of their age or length. An exposure of 3 hours rarely induces the formation of sporangia on all the sporangiophores of a culture; 15 minutes exposure in diffused

light usually induces the formation of a very limited number, while 10 minutes gives no result. On the contrary (p. 41) two minutes in sunlight induces sporangia formation. From his data (p. 45) it is reasonable to suppose that there is a direct proportion between the intensity of the light, and the time of exposure necessary to induce the formation of sporangia.

Brefeld (p.77) claims that an exposure of two hours is necessary to induce sporangia formation on sterile sporangiophores. A longer exposure causes larger sporangia to form on a greater number of sporangiophores. An exposure of this period has no effect on the sporangia formation of the next day.

Gräntz (p. 8) and Brefeld (p. 76) both agree that shutting out the light from a culture of matured Pilobolus delays the liberation of the sporangia. Brefeld says that the time of delay varies with the species.

Gräntz (p. 18) and Brefeld (p. 77-78) agree as to the effect of blue and yellow light on the development of Pilobolus. Behind a copper ammonia solution, or blue light, the sporangiophores develop as in white light; behind a solution of potassium bichromate, or red light, they act as in darkness, that is, lengthen and remain sterile.

The objects of the present investigation were to find whether or not Pilobolus remains sterile in darkness, and if so, how long an exposure to light is necessary to induce the formation of sporangia.

The Pilobolus which appeared in the cultures studied was identified as Pilobolus microsporus according to the descriptions of species by Brefeld (Heft IV.,pp. 69-71).

Material and Methods

The cultures under observation were placed next to the glass on the south, east and west shelves of the greenhouse basement. The shelf on the south side was not shaded from above, for the glass of the greenhouse bent out in such a way that light from above also fell on the cultures. Each shelf could be shaded from direct sunlight by drawing a thin, white, cloth curtain.

Experiment I.-

To determine the period necessary to induce sporangia formation.

Two long shallow wooden boxes were filled with manure, and placdd on the north shelf. Into these on Nov. 6, after letting the manure dry for a day, were pressed 12 crocks and 6 glass jars of the following description:

In the bottom of a dozen cylindrical stone jars were ground two small holes. Into these holes were fitted pieces of lead pipe, which were sealed in and then bent over into a U shape. Thus ventilation was obtained and light excluded.

Three tin cans were treated as the crocks, and then were blackened inside and out.

The glass jars were the same size as the crocks, and in the bottom of each were two small holes for ventilation. Over three of these were placed the blackened tin cans (d^2) and the other three were used for the cultures left constantly in daylight (e). The cultures were exposed to daylight as follows:-

(a) 3 cultures were left in continual darkness.

(b) 3 cultures were exposed to light for an interval of 15 minutes daily.

(c) 3 cultures for an interval of 30 minutes.

(d') 3 cultures for an interval of 60 minutes.

(d^2) 3 cultures for an interval of 60 minutes.

(e) 3 cultures exposed to continual daylight.

Cultures (b) (c) and (d') were exposed to light by removing the stone jars for the respective intervals. Cultures (d^2) were exposed to light by removing the blackened tins from over the glass jars. Thus, cultures (d') and (d^2) have the one varying factor of moisture, for (d') is exposed to the drying effects of the air of the greenhouse for sixty minutes daily, (d^2) is protected by the glass jars. In comparing (a) with (d^2) we will see that the moisture relations of the two are exactly alike, the only varying factor in these is that of light, (a) never is exposed to light while (d^2) is exposed for an interval of 60 minutes daily.

Likewise (e) and (d^2) are under the same conditions of moisture, the varying factor being that of light.

All the cultures are subjected to the same degrees of temperature, as the holes in each stone jar allow an exchange of air. The sun never shown directly on the cultures except in the early morn-

ing, and this was not enough to raise the temperature under any.

On Nov. 13, Pilobolus was first observed in cultures (e) and (d'). The table gives in a very condensed form the average results of each set of cultures. Observations and exposures were made daily at 9 a. m. for about two weeks, except on Sundays and on Nov. 15 and 22.

In speaking of the number of Pilobolus present "abundant" indicates the greatest number, and "quite a few", "few", and "very few" decreasing quantities respectively.

Dates	Nov. 13	14	Monday 16
(a) Constant dark			
(b) 15 minutes exposure daily			
(c) 30 minutes exposure daily			Very few 5 mm., fertile
(d) Series I. 60 minutes exposure daily (d^1)	Few, longer fertile	Quite a few, 4-6 mm., slender, fertile.	Quite a few, 10 mm., fertile. Quite a few, 13-20 mm., white pointed tips.
Series II. (d^2)			Quite a few, 2 mm., fertile. Few, yellow pointed tips.
(e) Light cultures	Very few, 2 mm., long, fertile.	Quite a few, 2 mm., fertile. A very few tapering, yellow tips or yellow knobs.	Abundant, 2 mm., fertile. Very few with yellow pointed tips.

17th	18th	19th	20th
Very few, 10 mm., some fertile; others sterile. Yellow at tips.	A few, 10 mm., fertile Few, 15 mm., yellow pointed tips.		Quite a few, 7-10 mm., fertile. Few, 2 mm. and fertile. Few 8-10 mm. with yellow pointed tips.
Few, 7 mm., fertile. Very few, yellow pointed tips.	Quite a few, 5-6 mm., slender, fertile. Very few, longer, yellow pointed tips.	Quite a few, 4-8 mm., slender, fertile. Few, all lengths yellow or white, pointed tips, or yellow knobs.	Few, 3-6 mm., slender, fertile.
Very few, 6-7 mm., fertile.		Few, 6 mm., fertile. Several with pointed yellow tips.	Few, 5 mm., fertile. Very few, 5 mm., slender yellow pointed tips.
Quite a few, 5-10mm., fertile. Quite a few, 15, 20, 25 mm., fertile. Very few of all lengths yellow pointed tips.	Quite a few, 5-6 mm., fertile. Few, 8-10 mm., pointed yellow tips.	Quite a few, 3-8 mm., slender, fertile.	Few, 4-6 mm., fertile. Very few, 7 mm., yellow tips.
A few, 5 mm., fertile. Few, 10 mm., yellow pointed tips, yellow and brown knobs.	Few, 6 mm., fertile. Quite a few, 5-10 mm., long yellow pointed tips or knobs.	Quite a few, 3-7 mm., fertile. Few, 5-8 mm., yellow knobs or pointed yellow tips.	Few, 2 mm., fertile. Very few, longer, yellow tips or knobs.
Abundant, 2-3 mm., fertile. Few, yellow pointed tips or yellow knobs.	Abundant, 2-3 mm., fertile.	Abundant, 2-3 mm., fertile. Few, 2-3 mm., long yellow pointed tips.	Quite a few, 2-4 mm., fertile. Few, yellow pointed tips or knobs.

21st	23rd	24th	25th
Few, 5-6 mm., fertile. Very few, 7 mm., sterile.	Few, 8 mm., fertile. Few, 8 mm., sterile.		Few, 5 mm., fertile.
Very few, 3 mm., fertile. Several sterile.	Very few, 9 mm., fertile, and sterile.	Very few, 4 mm., fertile; one or two sterile.	Very few, 4-5 mm., slender, fertile.
Very few, 4 mm., fertile. Very few, 4 mm. yellow pointed tips.	Quite a few, 1-2 mm., fertile. Very few, 5-7 mm., fertile Quite a few, 8-10 mm. and sterile.	Quite a few, 8-12 mm., slender, fertile. Few, 12 mm., pointed tips.	Very few, 5-8 mm., fertile.
Very few, 4-5 mm., fertile. Several longer and fertile.	Very few, long sterile sporangiophores.		Only several 4-6 mm., fertile.
Quite a few, 1-2 mm., fertile. Very few, with yellow pointed tips.	Quite a few, 1 mm., fertile.		
Quite a few, 2-3 mm., fertile.	Quite a few, 2-4 mm., fertile.	Quite a few, 3 mm., fertile.	Quite a few, 2-3 mm., fertile.

In the absence of sporangia some of the sporangiophores taper to slender yellow tips, a condition which may be seen in normal cultures in the afternoon. Other sporangiophores have on the tips swollen enlargements which are yellow, brownish, or almost black. These are, in fact, sporangia in different stages of maturity.

During the two weeks of observation the cultures in continuous daylight show quite a uniformity as to the length of the sporangiophores and the formation of sporangia.

The sporangiophores of the cultures (a), (b), (c) and (d) vary greatly in length on different days. This variation of length from one day to the next is probably due to some other factor than that of light.

In general the sporangiophores in cultures (e) are shorter than in any other series. An examination of the table from Nov. 17-20 shows that the sporangiophores in (a) are longest, and that the sporangiophores gradually become shorter from (b) to (d). The length of the sporangiophore is proportional to the length of the period of exposure to light, the longest are found in total darkness, the shortest in continual daylight.

Sporangia are developed and ejaculated in every series of cultures, in constant darkness, in the cultures subjected to light for an interval daily, and the cultures constantly in daylight. In every culture, however, each morning some sporangiophores were observed which were either sterile or with immature sporangia.

There is a noticeable difference in cultures (e) and cultures (a), (b), (c) and (d) as to the relative lengths of the fertile and sterile sporangiophores. In the cultures (e) at 9 a. m. the

sterile sporangiophores are about the same length as the fertile. On the contrary, at the same time in cultures (a), (b), (c), and (d), the sterile sporangiophores are usually much longer than the fertile. This points to the fact that growth is more rapid in darkness, or partial darkness, than in light, a fact which Grăntz (p. 12) denies.

Long sterile sporangiophores are usually formed if the regular interval of illumination is omitted. This was observed on Nov. 23, as illumination was omitted on Nov. 22. On Nov. 23 the sporangiophores in (b) are three times longer than on Nov. 21, and many of them are sterile; in (c) they are 2-3 times longer, and more of them are sterile; in (d') all the sporangiophores are much longer and sterile. Sporangia often develop on these long sporangiophores after another day, as the table shows for (d') on Nov.24.

The formation of sporangia in darkness and in cultures illuminated for such short intervals was so contrary to the observations of Brefeld and Grăntz that the apparatus was tested to find whether or not light was entering. Velox paper was left under several stone jars for 24 hours, and on development it showed a strong light test. Light may have entered by reflection through the lead pipes at the top, or got in under the stone jars, which became loosened as the manure became dryer.

Hence, in all the cultures a very small amount of light was constantly present. In addition to this, in all the cultures except (a), there is added the amount of light striking the culture during the interval of illumination. This latter amount must have been perceived for if the interval of illumination is omitted,

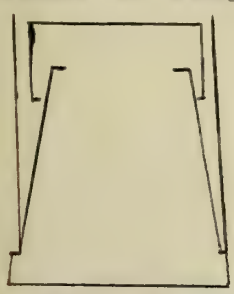
there is a marked effect as noted above.

In accordance with these facts, it is true that the longest sporangiophores are formed in the cultures subjected to the least amount of light,-(a). In cultures (a) sporangia are formed daily in the minute amount of light that in some way entered the cultures. Gräntz (p. 43) says that in weak light an exposure of 15-20 hours is necessary to induce the formation of sporangia on sterile sporangiophores. The above observations show that, if light is necessary, it requires a far shorter length of exposure than Gräntz claims necessary.

Experiment II.-

The two long boxes were again filled with manure, and the tops of three glass jars, similar to those used in Experiment I., and a dozen 4 1/2 inch flower pots were pressed down into the manure. The bottoms of the flower pots had been carefully removed with a chisel, and over the openings were slipped tin caps made in the following manner.- Tin cans were cut down to a height of 1 3/4 inches and in the edge were cut eight slits so as to make four narrow turned in props which prevented the caps from fitting tight to the pots. Thus an interval was left between the cap and the pot for the circulation of air. Over the tin-capped flower pots were slipped cuffs of tin, which were made by melting off the tops and bottoms of larger tin cans. These cuffs fitted tight to the flower pots at the bottom and projected above the tin caps. All the tin was blackened to prevent reflection of light and rusting.

Sand 1/4 inch thick was spread around the base of each flower pot, in order to keep out all light from below. Velox paper, which for 24 hours was left in this apparatus in the sunlight, gave no test for light.



All the cultures were under the same conditions of moisture, as they were side by side in two boxes which were filled at the same time with manure which was uniformly damp. Exposures to light were made by removing the tin caps from the flower pots, and to keep the moisture constant glass plates were laid over the openings.

Because of the arrangement of the apparatus air could circulate and all the cultures were subjected to the same changes of temperature.

The cultures were exposed to daylight as follows:-

(1) 3 cultures under the glass jars constantly in daylight.

(2) 3 cultures exposed daily for an interval of 60 minutes.

(3) 3 cultures exposed daily for an interval of 30 minutes.

(4) 3 cultures exposed daily for an interval of 15 minutes.

(5) 3 cultures exposed daily for an interval of 5 minutes.

(6) 3 cultures left in continual darkness.

Observations and exposures were made daily from Jan. 15 to Feb.19.

Pilobolus appeared for the first time in the cultures in the following order.-

Jan. 25 in (1)
Jan. 27 in (3)
Jan. 29 in (4)
Feb. 1 in (2), etc.

Thus there is no regular sequence of occurrence.

During the four weeks of observation the Pilobolus in cultures (1) varied in length from 2-10 millimeters, the usual length was about 3 or 4 mm. As was observed in Experiment I., sterile or immature sporangia were frequently seen in the morning. These were often mature by evening.

The sporangiophores of cultures (2), (3), (4), and (5) vary greatly in length on different days. One day the sporangiophores are 5 mm. long, and the next 10 or 15 mm.; and every day there may be a number of each length. The condition in every one of these cultures is practically the same. The average length of the sporangiophores is the same in all cultures and sporangia are developed

and ejaculated in each culture.

In cultures (6), in constant darkness, Pilobolus is formed regularly each day. The sporangiophores are 2-4 mm. long with well developed sporangia. The Pilobolus in constant darkness and those constantly in daylight are different only in that the sporangiophores developed in darkness are more slender, and the sporangia are usually liberated in the late afternoon or evening. The sporangia developed in light usually are ejected from 8-11 a. m. This varies, however. Sometimes in all the cultures of every condition of illumination the sporangia are not ejaculated until late in the afternoon. The temperature in the greenhouse was not constant, varying from 10 C at night to 25 and 30 at noon. This delay in ejecting the sporangia was noticed after a cold night when no doubt the temperature became too low for the full development of the sporangia.

As in Experiment I., the omission of the usual interval of exposure was followed the next day by the appearance of long sterile sporangiophores. For example, in cultures (2) from Feb. 1 to 5 there was an abundance of fertile sporangiophores 10 mm. long. The usual intervals of illumination were omitted on Feb. 6 and 7. On Feb. 8 there was an abundance of sterile sporangiophores 12-15 mm. long. The cultures were exposed to light for the usual period on Feb. 8, and on Feb. 9 there were many sporangiophores present and all were fertile. The longest ones present were the sterile sporangiophores of Feb. 8. On each succeeding day these cultures were exposed the proper interval and on each day fertile sporangiophores 5-10 mm. long were observed. Again, on Feb. 14 the period

of exposure to light was omitted and on the following day all the sporangiophores were longer and sterile.

This same effect was observed on cultures (5) if the regular interval of exposure to light was omitted.

Experiment III.-

The jars and flower pots were arranged as before in the two long boxes of manure, which were placed on the south shelf of the greenhouse. From March 1 to 30, except on March 21, 24 and 28, observations and exposures were made as follows:-

(1) 3 cultures under glass jars constantly in daylight.
(2) 3 cultures exposed for an interval of 10 minutes.
(3) 3 cultures exposed for an interval of 8 minutes.
(4) 3 cultures exposed for an interval of 5 minutes.
(5) 3 cultures exposed for an interval of 3 minutes.
(6) 3 cultures left in continual darkness.

The results of this series of observations are about the same as in Experiment II. For the first week or ten days the sporangiophores in all the cultures were about 2 mm. long. In constant light and in continual darkness the sporangiophores continued to be about this length. In the other cultures (2), (3), (4), and (5), after March 17 the sporangiophores are longer. They never exceed 10 mm. in length, and this length usually occurs the day after an omission of the regular periods of illumination.

In this series the sporangiophores never are as long as in Experiment II. The light on the south shelf is far more intense at 9 a. m. than on the west shelf, where Experiment II. was conducted. During the period of exposure the curtains were withdrawn and sunlight struck the cultures. As noted before, the sporangiophores in Experiment II. were frequently 15 mm. long. In this Experiment, however, although the periods of exposure are shorter,

the light is more intense. As a result, the sporangiophores are never as long as 15 mm.

Sporangia develop regularly each day under every condition of illumination and in constant darkness. The sporangia developed in light and in darkness are about the same size, and differ only in the time of day at which they are ejaculated, as mentioned in Experiment II.

The sporangia formed in cultures (2), (3), (4), and (5) are of about a uniform size, but smaller than those which develop in constant darkness, or constant daylight.

The effect of omitting a period of illumination was observed frequently in this experiment, as an examination of the table will show. As noted in Experiment II., the result of such an omission is longer, sterile sporangiophores. For example, in cultures (2) on March 20 the fertile sporangiophores were from 1 to 5 mm. long. On March 21 there was no exposure to light, and on March 22 the sporangiophores were sterile and from 5 to 10 mm. long. These long sterile sporangiophores were growing on the very places in the cultures where on March 20 there had been shorter, fertile sporangiophores. A few fertile sporangiophores, 2 mm. long, were observed to be growing on places where there had never before been any Pilobolus.

Likewise on March 24, the cultures were not illuminated. On March 25 in cultures (2), (3), (4), and (5) almost all the sporangiophores are longer and sterile. These, again, are growing just where on March 23, the Pilobolus had been shorter and fertile.

In other places where there had never been any growth of Pilobolus are a few fertile sporangiophores, 1 mm. long.

If the long sterile sporangiophores are illuminated for the usual period, they become fertile in one or two days. Thus for several days following an omission of Illumination there may be seen unusually long, fertile sporangiophores. These sterile sporangiophores have been observed to develop sporangia without any illumination: and sometimes sporangia are never developed. In the latter case it is as if all the energy has been expended in elongation, and there is none left to form sporangia.

March	10th	11th	12th
Constant light daily.	Few, 1-3 mm., fertile.	Quite a few, 2 mm., fertile.	
10 minutes light exposure, daily.			
8 minutes exposure.	Few, 2 mm., slender, fertile.	Few, 2 mm., slender, fertile.	Few, 2 mm., slender, fer-fertile.
5 minutes exposure.			
3 minutes exposure.	Very few, 1-2 mm., fertile.		
Constant dark.	Very few, 4 mm., fertile.		

13th	14th	15th	16th
Quite a few, 2 mm., fertile. Very few, longer with pointed yellow tips.		Few, 2 mm., fertile.	Very few, 1 mm., fertile.
	Few, 2 mm., fertile.	Very few, 2 mm., fertile.	Quite a few, 1-2 mm., fertile.
Few, 1-2 mm., slender, fertile.	Quite a few, 2 mm., fertile.	Quite a few, 1-2 mm., fertile.	Few, 1-2-3 mm., fertile.
			Very few, 1 mm., fertile.
		Very few, 1-2 mm., fertile.	Few, 1-2 mm., slender fertile.
Very few, 1-2 mm., fertile. Several with yellow knobs on tips.	Few, 2 mm., fertile.	Few, 1-2 mm., fertile.	Few, 1-2 mm., fertile.

17th	18th	19th	20th
Few, 1-2 mm., all have yellow knobs on tips.		Few, 2 mm., fertile.	Very few, 1-2 mm., fertile.
Few, tips with yellow or brown knobs.	Few, 1-2 mm., fertile. Very few, 5 mm., fertile.	Very few, 4 mm., tips tapering and yellow.	Few, 5 mm., slender fertile. Several 1 mm., fertile.
Few, 1-2 mm., tips tapering and yellow, or with hard yellow knobs.	Few, 1-2 mm., fertile. Very few, 5 mm., fertile.	Very few, 2 mm., fertile. Several with yellow knobs on tips.	Very few, some 4-5 mm., others 1-2 mm., all fertile.
Very few, yellow knobs on tips.	Very few, 5 mm., fertile.		Very few, 3-5 mm., fertile.
	Very few, 1-2 mm., fertile.	Very few, 1-2 mm., fertile. Several have yellow knobs on tips.	Very few, 1 mm., fertile.
Quite a few, 1-2 mm., few fertile; others with yellow knobs on tips.	Few, 1-2 mm., fertile.	Abundant, 2 mm., fertile.	Abundant, 2 mm., fertile.

22nd	23rd	25th	26th
Quite a few, 2 mm., fertile.	Quite a few, 1-2 mm., fertile.		Few, fertile but not mature.
Few, 2 mm., fertile. Quite a few, 5-10 mm., slender and sterile.	Few, 2-3 mm., fertile. Few, 8-9 mm., and sterile.	Few, 3-5 mm., and sterile.	Abundant, 2-5-6 mm., fertile.
Very few, 1-2 mm., fertile.	Few, 2-5 mm., fertile but not mature.	Few, 3 mm., and sterile. Few, 1 mm., and fertile.	Abundant, 2-5 mm., fertile.
Few, 3-7 mm., fertile but not mature.	Very few, 4-5 mm., slender, fertile. Some have yellow knobs on tips.	Quite a few, 3-5 mm., and sterile. Few 1 mm., fertile.	Quite a few, 3-4-5 mm., fertile. Very few sterile.
Few, 2-3 mm., fertile.	Very few, 1-2 mm., slender, fertile.	Quite a few, 1 mm., fertile. Very few, 7 mm., and sterile.	Few, 1-4 mm., fertile. Few, 5-8 mm., fertile.
Abundant, 2 mm., fertile.	Abundant, 1 mm., fertile.	Very abundant, 1 mm., fertile.	Few, 1-2 mm., fertile.

27th	29th	30th	31st
	Few, 1 mm., fertile.	Few, 1 mm., fertile. Several with tapering yellow tips.	
Quite a few, 2-5 mm., fertile.	Quite a few, 3-5 mm., sterile. Few 3 mm., fertile.	Quite a few, 4-5 mm., fertile some not mature.	Quite a few, 1-2 or 3-4 mm., fertile. Very few, 8-10 mm., fertile.
Few, 3-5 mm., fertile. Several 1 mm. fertile.	Quite a few, 3-5 mm., sterile. Few, 1 mm., fertile.	Quite a few, 5 mm., fertile. others with yellow tips or yellow knobs.	Few, 2-4 mm., fertile. Quite a few 5-8 mm., fertile. Few, 1 mm., fertile.
Abundant, 3 mm., fertile.	Few, 3-7 mm., sterile.	Few, 3-6 mm., fertile. Few, 3-4 mm., and sterile.	Quite a few, 3 mm., fertile.
Few, 5 mm., fertile. Abundant, 1 mm., fertile.	Few, 3-6 mm., fertile; few sterile. Very few, 1 mm., fertile.	Few, 5-6 mm., fertile.	Quite a few, 3-4 mm., fertile.
Few, 1 mm., fertile.	Few, 1 mm., fertile.	Few, 1 mm., fertile.	Few, 1 mm., fertile.

1st	2nd	3rd
	Few, 1 mm., fertile.	Few, 1-1 1/2 mm., fertile.
Quite a few, 2-4-6 mm., fertile. Very few, 7 mm. with brown knobs.	Abundant, 2-4-5 mm., fertile. Few, 1 mm., fertile.	Quite a few, 2-4 mm., fertile.
Abundant, 1 mm., fertile. Few, 3-4 mm., fertile. Very few, 6-8 mm., sterile.	Abundant, 2-3-5 mm., fertile. Very few, 7 mm. fertile. Few, 1 mm., fertile.	Quite a few, 1 mm. fertile. Few, 2-5 mm., fertile.
Very few, 2 mm., fertile.	Quite a few, 1-2-3 mm., fertile.	Very few, 1-3 mm., fertile.
Few, 2 mm., fertile.	Very few, 1-2 mm., fertile.	Very few, 1 mm., fertile.
Few, 1 mm., fertile.		Few, 1 mm., fertile.

On March 17, as the table shows, the Pilobolus in every culture was immature in the morning. The sporangiophores were tapering and yellow, or little yellow knobs were on the tips. The cause of this immature condition in all the cultures may have been that of temperature. On that day and on the previous day the room had been without heat.

The observations of these three experiments conclusively show that, the conditions of temperature and moisture being the same, in darkness fructification occurs regularly, the length of the sporangiophores being the same as in light. The sporangia developed in darkness are regularly ejaculated, altho some hours later than those developed in light.

Brefeld (pp.76-77) and Gräntz (p.13) agree that in darkness no sporangia are formed, and that the sporangiophores greatly elongate. Gräntz (p. 9) says that the sporangiophores developed in darkness are one half the diameter of those in light, the tips are white and there is not the least sign of sporangia formation.

The observations of the foregoing experiments agree with those of Gräntz that the sporangiophores in darkness are more slender. Granting that in the dark cultures of Experiments II. and II. some light is admitted, the results differ from those of Gräntz (p. 43). He says that in dim light an exposure of 10, 15 or 20 hours is necessary for the formation of sporangia. If this length of time is necessary, mature sporangia would not be observed each morning, a condition which was observed in Experiments II. and III. Very frequently also, at 5 p. m. sporangiophores are observed with immature sporangia already formed.

Gräntz (p.11) also says that in dim light sporangiophores elongate to 25 mm. In Experiments I. and II. the sporangiophores in darkness never exceeded 4 mm.; hence if light is present, the sporangiophores do not elongate. If these cultures are in complete darkness, sporangia do form in darkness, on sporangiophores which are of the same average length as those developed in light.

This fact that the formation of sporangia is not dependent on light is further shown by the fact that many sterile sporangiophores frequently occur in cultures continually in daylight. In every culture, no matter what the conditions of light, there are usually each morning many sterile sporangiophores. As mentioned in Experiment III., March 17, in every culture many sterile or immature sporangiophores were observed. The sterility of Pilobolus is not due to the absence of light, a fact which Gräntz (p. 16) said might be true. He, however, never observed Pilobolus fertile in darkness.

In the course of these observations no long sterile sporangiophores were found in constant darkness. Consequently many of the experiments of Gräntz and Prefeld could not be repeated. Elongated sporangiophores are found only in the cultures which are illuminated for short periods a day. As mentioned before, if one of the regular intervals of exposure to light is omitted, the sporangiophores become longer and sterile.

In these intermittent lighted cultures there seems to be a correlation between the length of the exposure and the intensity of light. That is, the sporangiophores in the 10 to 3 minute cultures of Experiment III. are shorter in the strong diffused light of the south shelf, than the sporangiophores of the cultures of Experiment

II. which are exposed to weaker diffuse light for longer intervals.

In constant dim light, as noted in Experiment I., the sporangiophores are longest. These, however, are fertile and appear each morning.

The appearance of elongated sporangiophores in dim light agrees with the statementsof Grăntz (pp. 13 & 14). He says that the intensity of light indirectly regulates the length of the sporangiophores and gives the results (p.11) of an experiment to show that in dimmest light the sporangiophores are longest. He claims, however, that the elongation is due only to the fact that dim light delays the formation of sporangia. The observations recorded of the experiments of this investigation show that the light relations affect primarily the length of the sporangiophores.

It is possible that Brefeld's and Grăntz's results are due to the fact that the cultures, which they thought were in absolute darkness, were exposed to faint light. The Pilobolus stimulated by very dim light, expended all its energy in elongating the sporangiophores so that no sporangia were formed. Such a condition was observed in Experiments III.and II.

In the three experiments discussed above besides light, there has been the varying factor of moisture. In Experiment I. when the cultures were exposed to light there was a change in the moisture contents of the cultures, for the covers were entirely removed. In cultures (d) this difficulty was overcome, but the sporangiophores elongated as in (d), altho not as much. In Experiment II. when the tin caps were removed, glass plates were laid over the top to prevent any change in moisture contents. Nevertheless, the

sporangiophores elongated.

In a series of cultures in light, but under varying degrees of moisture, no difference in the length of the sporangiophores could be observed.

If moister or dryer air should influence the length of the sporangiophores there would be a great difference in length between Pilobolus grown under crocks in darkness and Pilobolus growing in the open air of the greenhouse. There is, however, no noticeable difference. There seems no doubt that the elongated sporangiophores are caused by a change in light relations.

In constant darkness and in light similar sporangiophores are observed. In cultures exposed 60 minutes daily, and in cultures exposed to light 3 minutes daily much longer sporangiophores are observed. These facts lead one to reason that there may be a period of illumination long enough and a period short enough when the length of the sporangiophores would not be affected.

As the table indicates, on March 22, after an omission of illumination on the previous day, the sporangiophores in the 3 minute cultures are not elongated as in the 10, 8 and 5 minute cultures. The sporangiophores continue to be the same length as they had previously been and the same length as those in light or darkness. Thus, we may conclude that the Pilobolus in the 3 minute cultures had not yet perceived any light stimulus.

A few days later, on the day following the omission of the regular 3 minute period of illumination, the sporangiophores are elongated. Thus, not until the cultures had been subjected to light 3 minutes daily for two weeks, was there any indication that

the Pilobolus had been affected by the light. Hence it is reasonable to suppose that the period of exposure to light in which no lengthening of sporangiophores would occur is less than 3 minutes.

For the first ten days in all the cultures of Experiment III. the sporangiophores are the same length. On March 20 the elongated sporangiophores in cultures illuminated 10, 8 and 5 minutes daily, indicate that the Pilobolus has perceived the light stimulus. When the light stimulus has been perceived energy is expended in lengthening the sporangiophores or "reaching the light". If this light stimulant is omitted from any culture at the regular period of illumination, the elongation of the sporangiophores continues. The omission of the regular illumination is in fact a further stimulus, and no energy is expended toward sporangia formation but all toward elongation.

As previously mentioned, the long sterile sporangiophores always appear where there had previously been shorter fertile sporangiophores. On the same day and in the same culture there may occur short fertile sporangiophores, but these grow on spots where there had never been any Pilobolus. This same phenomena was observed on cultures which were put in darkness after they had produced normal Pilobolus for one or two days.

There seems to be an "endgram" or "memory" shown in these instances. This may be the explanation for the appearance of long sterile sporangiophores when a regular period of illumination is omitted.

In exposing the cultures to light daily, it will be noted that the exposures were usually made at 9 a. m. daily. At that time the

sporangiophores of the next day had not appeared. Hence that part of the growth that is affected by light is not the tips of the sporangiophores, but some part of the mycelium.

Gräntz (pp.45-56) says that the sporangiophores of Pilobolus elongate if the cultures be placed in darkness after 2 or 3 hours' growth in light.

If a normal culture, which has sterile sporangiophores 1 to 2 mm. high at 5 p. m. is put in darkness, there is no elongation of the sporangiophores. This was repeated frequently and at no time were results similar to Gräntz's obtained.

If a normal culture is placed in darkness in the morning, there is no elongation of sporangiophores until the second day. A culture, which had normal Pilobolus growing on it, was put in darkness at 9 a. m. Wednesday. The next day the Pilobolus was about the same length as on a culture in light. On Friday the Pilobolus was twice as long as that growing in light, and many were sterile. On the next day all the sporangiophores were longer and sterile. On Monday the sporangiophores were long; some were sterile and just as many were fertile. In the same culture on another spot were a few fertile sporangiophores, 1 mm. long. On each succeeding day a few short fertile sporangiophores were observed on one side of the culture, and on the other side a few which were longer and mostly fertile.

If, after a week in darkness, a culture which has had sporangiophores 15 mm. long, be put back in light, all the sporangiophores on the second day are 2 mm. long.

As pointed out in Experiment I. the rate of growth of the sporangiophores in cultures continually in daylight is slower than

in the other cultures. Gräntz says (pp.16 & 17) that the rate of growth of the sporangiophores is the same under all conditions of illumination.

In the three experiments recorded, a great difference was often observed in the length of the sporangiophores in several cultures side by side; one is continually in strong diffused daylight; another is illuminated a short period a day; and another is constantly in dim light. If such cultures be observed in the afternoon, small yellow sporangiophores may be seen. Very often no sporangiophores can be observed in the afternoon. In the morning, however, short fertile and sterile sporangiophores are present in the culture continually in strong diffused daylight. In the other two cultures both the fertile and the sterile sporangiophores are frequently three to five times as long. Hence in dim light, or in intermittent Illumination the rate of growth of Pilobolus is much more rapid than in bright light.

Gräntz (pp. 12-13) says that upon the formation of the sporangia, the sporangiophores cease to elongate. All observations during this investigation agree with those of Gräntz. This explains the appearance on the same day of such different lengths of fertile sporangiophores of a culture. A difference in sensitivity to light, or of physiological conditions of the mycelium, causes a difference in the time of the formation of the sporangia, and consequently a difference in the lengths of the sporangiophores.

The tips of the sporangiophores are heliotropic. This was frequently observed in the afternoon. The yellow tips of the sporangiophores are always turned towards the window or the strongest

light. If in the late afternoon a culture is turned around so that the tips point away from the light, the next morning all the Pilobolus is again pointed toward the light. This was tried by placing at 5 p. m. a strongly bent culture of Pilobolus in a dark box with the tips all turned from a single slit where the light was entering. The next morning all the Pilobolus was turned toward the slit. In order to test the heliotropism of Pilobolus which had matured sporangia developed, the cultures were subjected to strong unilateral light.

At 8.30 a. m. a straight growing culture of fertile sporangiophores was placed 2 feet from a strong electric light in a dark room. The culture was exposed for one hour and then put in darkness. There was no effect from this unilateral light. Other trials were made at a distance of 1 foot for 1 1/2 hours with no effect.

The growing tip is the zone of heliotropic irritability, as Grăntz observed (p.31), and after the sporangia are developed they are not heliotropically affected by light.

Monochromatic Light Screens.

Colored fluids, made according to the following formulae, are used in ground parallel walled Soyka flasks. The flasks are of such a size that the fluid is one centimeter thick.

The fluids were tested spectroscopically, and are found to break up the spectrum into bands of violet, blue, green, orange, and red light. With each fluid all other light rays, except those of the one color, are absorbed; hence these fluids are monochromatic light screens.

Violet. (See Pennington, 1892)

To 100 cc.of a saturated coppersulphate solution, [made by dissolving 100 grams of coppersulphate in 250 cc.of water] add .01 gram of Hoffmann's Violett, which is dissolved in 2 cc. of 95 per cent alcohol. To this add 10 cc. of water to prevent crystallization at low temperature.

Red.

Dissolve .5 gram of safranin in 250 cc. of water.

Green.

Make a solution of solidgrün by dissolving .25 grams solidgrün in 200 cc. of water. Add to 50 cc. of this solution 50 cc. of a saturated solution of coppersulphate. To this mixture now add .15 grams of tartrazine dissolved in 40 cc. of water.

Orange. (See Nagel, 1898)

Make a solution of copper acetate by dissolving 14 grams of copper acetate in 200 cc. of water and acidify with acetic acid. To 70 cc. of this solution add 28 cc. of the solution of safranin as made above. Dilute this with 28 cc. of water.

Blue.

Dissolve 1.5 grams of Echtgrün bläul in 200 cc. of water. To 30 cc. of this solution add 60 cc. of the concentrated copper sulphate solution. To this mixture add .02 grams of rhodamine dissolved in 40 cc. of water.

The effect of monochromatic light on the development of Pilobolus microsporus.

Two long boxes, similar to those used in the other experiments, were filled with manure. The open ends of a number of cylindrical tin cans, about three inches high, were pressed down into the manure. The small sealers in the bottoms of these cans had been melted out. On the bottoms, around the circular openings thus made, were soldered several rivets to prevent the caps, which were next put on, from fitting down tight.

The caps were made of cans about a half inch larger in diameter, and were cut shorter than the base-cans. The sealers, the same size as those in the base cans, were melted out and over the openings were sealed the Soyka flasks containing the colored fluids.

When the caps are put over the cans the cultures below are subjected to light of different wave lengths. No white light can enter the cultures directly because of the arrangement described, or by reflection, as all the tin was painted a dead black. Sand was put between the cans and around the bottoms of each as in the previous experiments. This apparatus was tested with Velox paper, and found not to admit white light.

The space left between each can and its cap allows air to circulate. Thus all the cultures are under the same conditions of temperature and moisture.

Three cultures are subjected to white light, the flasks being filled with distilled water; three are subjected to blue light; three to violet; three to green; three to orange; and three to red light. Observations were made daily at 9 a. m. and at 4 p. m. from February 19 to March 13.

On March 2, 9 a. m., fertile sporangiophores, 1 to 1 1/2 millimeters long, were observed in one of the cultures under white light.

On March 3, Pilobolus was observed in three cultures in red light, one culture in violet light, two cultures in white light, two cultures in blue light, one culture in orange light, and one culture in green light. The sporangiophores were fertile and about the same length in all the cultures.

At 3.30 p. m. the cultures were examined to see if the sporangia had been thrown off. In the cultures in white light approximately one half of the sporangia had been thrown off. Under violet light all the sporangia had been ejaculated; under red light most of the sporangia were still on the sporangiophores; under blue, orange, and green light a few sporangiophores still bore sporangia.

As in the previous experiments, on some mornings all the sporangia are immature, or the sporangiophores taper to slender yellow tips.

The Pilobolus in no culture was ever longer than 3 or 4 millimeters. Throughout the three weeks of observation the length of

the sporangiophores is almost uniform in all the cultures. Sporangia are developed under all the colors, and they are similar to those formed in white light. The time at which the sporangia are thrown off varies quite widely. It was observed in white light as frequently as in colored light, that at 4 p. m. some sporangiophores still bore sporangia.

The Pilobolus continued to grow under red, violet and blue light rays longer than under orange and green light. The table also shows that Pilobolus continued to grow longer in red and blue light than in violet light.

The daily number of cultures in which Pilobolus is growing from March 3 to 10.

March	3	4	5	6	8	9	10
Red	3	2	3	3	1	0	1
Violet	1	2	2	2	0	1	3
White	2	3	3	2	1	1	1
Blue	2	3	3	2	1	0	2
Orange	1	1	3	2	1	1	1
Green	1	1	1	1	2	1	1

A similar series was observed from March 17 to April 17.

On March 27 Pilobolus appeared first in cultures under green and violet light. These observations, together with those in other similar series, show that the color of the light does not regulate in any way the culture in which Pilobolus first appears.

As in the first series, the abundance of Pilobolus in each

culture varies from day to day. The Pilobolus formed under every color is uniform except that in white light the sporangiophores frequently are a little longer and more swollen.

The table shows for each color the daily number of cultures in which Pilobolus is growing. As all the cultures did not begin to have Pilobolus growing on the same day, the diagrams do not show the relative daily abundance of Pilobolus under each color. For example, on April 1 the cultures under orange light show an abundance of growth of Pilobolus. On April 2, Pilobolus is most abundant under violet light; on April 5 under white light, and on no day was there an abundance of growth in all the cultures of green, red or blue light.

Daily number of cultures in which Pilobolus grows.

March	29	30	31	April 1	2	3	5	7	8
Red	0	0	0	1	2	2	2	1	1
Violet	3	3	3	3	3	2	2	1	1
White	2	0	1	1	2	3	3	3	3
Blue	1	0	0	1	2	1	1	2	0
Orange	1	2	2	2	3	3	3	1	0
Green	1	1	0	1	1	1	2	1	1

The results of these two series of observations and of several other series which followed show that under red, orange, green, violet and blue light rays Pilobolus is formed abundantly. The sporangiophores under each color are of about the same length and diameter. Under all these colors sporangia of the same size are formed and ejaculated. The latter frequently does not take place

until the late afternoon or evening.

Brefeld (p. 77) and Grăntz (p.18) agree that under red light the sporangiophores of Pilobolus microsporus elongate and are sterile, or act as in darkness; under blue light Pilobolus develops as in white light.

Brefeld (Heft III., 1872, p. 96; Heft VIII., 1889, pp. 286, 280, 285) and Grăntz (pp. 19, 29) agree as to the effect of the light of different wave lengths on several species of Coprinus. They say that red-yellow light acts as darkness, and the blue light as white light. In darkness the stalks elongate and the caps never mature.

Georg B. Lakon (1905, p.162) observed Coprinus plicatilis, a species which Brefeld says acts in red light as if in darkness. Lakon says that red and blue light affect this species somewhat differently, but that the difference is due to the moisture contents of the air, which are different under red and blue light. He regulated the moisture contents and could in this way exactly reverse the results under red and blue light. Hence, he claims that the different colors of light in themselves do not affect the growth of Coprinus plicatilis.

In these experiments on Pilobolus all the cultures are under the same conditions of moisture and temperature. The arrangement of the apparatus allows a circulation of air, this and the fact that the cultures are all protected by the curtain from the direct rays of the sun, keeps the cultures at the same temperature. All the cultures are side by side in two boxes, the moisture of which is uniform.

Coprinus and Pilobolus have frequently been studied as to the effects of light on their growth and development. Gräntz and Brefeld agree in many particulars as to the reaction of these fungi to different stimuli.

Lakon has shown that Coprinus plicatilis is not affected by the different wave lengths of light, and this investigation shows that Pilobolus microsporus is also unaffected by different wave lengths of light.

Heliotropic action of different colors.

In order to test the heliotropic powers of each wave length, several methods were used. The simplest method, and the one which gave the best results, was to paste over each flask a black paper in which was a single long slit.

If the flasks, so covered, were put as before over cultures which had been growing for some time under the respective colors, the results were the same as when put over fresh cultures grown in white light. In the latter case each color apparatus was put over a culture which for several days had had good growths of Pilobolus.

Such a change from white light to colored light, or in other words, from strong light to much dimmer light, usually caused lengthened sporangiophores. Occasionally these elongated sporangiophores were sterile until the second day.

Following are the details of one or two such trials.-

Orange.

April 20.

Over a culture which had fertile Pilobolus, 1 mm. long, was put a flask of orange color. Over the flask was pasted a black paper with one long slit in it.

April 21.

9 a. m. Pilobolus is 5-6 mm. long, all the sporangiophores are fertile and turned toward the slit in the paper.

4 p. m. Many sporangia are on the flask at the slit, although many had not been ejaculated.

April 22.

9 a. m. Sporangiophores are 7 mm. long, fertile and pointing toward the slit.

April 23.

9 a. m. Sporangia are found only under the slit.

Green.

April 19.

5 p. m. A culture with an abundance of yellow tipped sporangiophores pointing toward one side, over this is put a green flask as above.

April 21.

9 a. m. Sporangia are found only on glass under the slit in the paper. These are the sporangia formed on the sporangiophores observed at five p.m. on April 19.

Red.

April 19.

5 p. m. The same methods are followed as in green above.

April 21.

9 a. m. The sporangia are all over the surface of the flask and on the black tin. An examination of the

sporangiophores shows that they are turned in all directions, even pointing directly toward the tin walls.

Tests with violet and blue flasks gave results similar to the green and orange tests. These tests were repeated with the same results each time.

Thus it is shown that orange, green, blue, and violet cause heliotropic bending in Pilobolus. With red, however, there is no such irritability.

Brefeld and Gräntz did not use monochromatic light, and so their results with red-yellow light cannot be compared with the above. Brefeld (p.77) uses the terms "gelben Lichte". In this light he says there is no formation of sporangia, but an intense positive heliotropism is shown. Gräntz (p. 18) says that both red and blue rays cause heliotropic bending. In what Brefeld calls "yellow" and Gräntz "red" there is a mixture of the red and yellow wave lengths, and it is on the latter no doubt that the heliotropic irritability depends.

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Materials Used for Color Screens.

Kupfersulfat,
Chemische Fabrik C. A. F. Kahlbaum, Berlin, S. O.

Hoffmann's Violett,
Dr. Th. Schuchardt, Görlitz.

Safranin wasserl,
Dr. G. Grübler & Co., Leipzig.

Solidgrün,
Dr. G. Grübler & Co., Leipzig.

Tartrazine,
National Aniline and Chemical Co., Chicago.

Mandarin,
National Aniline and Chemical Co., Chicago.

Echtgrün bläul,
Farbenfabriken vorm. Frieder. Bayer & Co., Elberfeld.

Rhodamine,
Elmer & Amend, New York.

Copper acetate, pure cryst.
Bausch & Lomb Optical Co., New York.

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