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The Effect of Ultraviolet Radiation on the Pattern of Spore Germination in *Funaria hygrometrica* Hedw.

Mary Elizabeth Case
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To the Graduate Council:

I am submitting herewith a thesis written by Mary Elizabeth Case entitled "The Effect of Ultraviolet Radiation on the Pattern of Spore Germination in *Funaria hygrometrica* Hedw.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

Samuel Meyer, Major Professor

We have read this thesis and recommend its acceptance:

Lowell F. Bailey, Royal E. Shanks

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

February 1, 1950

To the C mmittee on Graduate Study:

I am submitting to you a thesis written by Mary Elizabeth Case entitled "The Effect of Ultraviolet Radiation on the Pattern of Spore Germination in Funaria hygrometrica Hedw." I recommend that it be accepted for twelve quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

Samuel L. Meyer
Major Professor

We have read this thesis
and recommend its acceptance:

Lowell F. Bailey

Royal E. Shanks

Accepted for the Committee

C. A. Waters
Dean of the Graduate School

THE EFFECT OF ULTRAVIOLET RADIATION ON THE PATTERN OF SPORE GERMINATION IN *FUNARIA HYGROMETRICA* HEDW.

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

Mary Elizabeth Case

March 1950

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INTRODUCTION

In 1782 Hedwig published a morphological description of spore germination in mosses in his Fundamentum historiae naturalis muscorum frondosorum. Since then others (Schoene, 1906; Brown, 1919; Van der Wijk, 1932; Chalaud, 1932) have described germination of moss spores. In 1906 Schoene described spore germination in Funaria hygrometrica Hedw., the Cord Moss, reporting four general types of germination. This study was undertaken to determine the effect of ultraviolet radiation on the spore germination pattern of F. hygrometrica. No previous work is known on the effect of ultraviolet radiation on germination of moss spores.

SPORE GERMINATION PATTERN IN FUNARIA HYGROMETRICA

Spores of Funaria vary in diameter from 13 to 16 microns (Grout, 1924). In the initial processes of germination, the spores swell to 20 to 25 microns, and chlorophyll develops. With this increase in size, resulting from the absorption of water (Brown, 1919), the spore wall cracks, and a filament protrudes.

In germination of spores of Funaria two types of filaments may develop from the spore, the chloronema and the rhizoid (Schoene, 1906; Brown, 1919). The chloronema (Chalaud, 1932; Van der Wijk, 1932) is a broad filament with many grass-green chloroplasts. The rhizoid is a more slender filament with pale green chloroplasts. The rhizoids are negatively phototropic, and the chloronemata are positively phototropic.

Schoene (1906) described four general types of germination in Funaria based on the time of the appearance of the rhizoids and the chloronemata. These types are: (1) only the chloronema develops from the spore; (2) the rhizoid develops first, later the chloronema; (3) the rhizoid and the chloronema develop at the same time; and (4) the chloronema develops first, followed by the rhizoid. In this study of Funaria, development of untreated spores show that a fifth type should be added in which only the rhizoid develops from the spore.

A. Methods

The spores were grown on 1 percent Benecke's agar, as modified by Wettstein (1924), on petri plates. The capsules were broken into enough water to spread the spores across the surface of the agar. Germination tests were made at room temperatures, varying from 70° to 84° F. Light was supplied for twelve hours each day with a fluorescent lamp. The light intensity varied from forty to seventy foot candles as measured by a Weston Illumination Meter.

Studies of the germination pattern were based on a series of observations made on each spore as germination progressed. A portion of the plate was marked off in sixteen 4 mm. squares with thread. A diagram was made of each square showing the location of the spores within the square so that germination of each spore could be studied. After the initiation of germination observations were made at twelve hour intervals, with final observations on germination at the end of eight days.

B. Observations and Discussion

In untreated cultures spore germination usually began with the appearance of the rhizoids within twenty-four hours after the spores were sown. The chloronemata developed about twelve hours after the rhizoids appeared. At the end of eight days all of the 1407 spores which germinated could be placed in one of the types of germination mentioned above.

The spore germination pattern in untreated cultures is shown in Table I. The largest group consisted of those spores which developed by producing the rhizoid first, later followed by the chloronema. These observations showed 62 percent of the spores developing in this manner. The next largest group was represented by those spores which germinated by producing chloronemata only. This was noted in 33.8 percent of the spores observed. A third type was represented by those spores which germinated by producing rhizoids only. This occurred in 3.5 percent of the spores studied. Although spores of this species normally contain chlorophyll granules (Van der Wijk, 1932), chlorophyll did not form in this third group of spores. No further signs of development beyond the rhizoidal stage were seen even after forty-five days. Only 0.5 percent of the spores germinated by producing both the rhizoid and the chloronema at the same time. The smallest group of spores germinated by producing the chloronema first and then the rhizoid. Observations showed only 0.2 percent in this group.

TABLE I

PATTERN OF SPORE GERMINATION IN FUNARIA HYGROMETRICA

Germination Types	Percentage in Each Type	Number Observed
Rhizoid, then chloronema	62.0 %	873
Chloronema only	33.8 %	476
Rhizoid only	3.5 %	43
Both rhizoid and chloronema	0.5 %	11
Chloronema, then rhizoid	0.2 %	4
Total	100.0 %	1407

THE EFFECT OF ULTRAVIOLET RADIATION ON THE SPORE GERMINATION PATTERN

A. Methods

A 15-watt mercury vapor germicidal tube made by General Electric was used as the source of radiation. This tube was characterized as having at least 65 percent of the radiations transmitted at the wave length of 2537 Å.

The spores were sown on the petri plates in the manner described above. Exposures of the plates to ultraviolet radiation were made for 1, 5, 10, 15, 20, 25, or 30 minute periods. The plates were 13.3 cm. from the tube. Five series were prepared to provide observations on at least one thousand spores from the representative cultures. The ten control cultures, run at the same time, provided data for determining the normal spore germination pattern reported above. The temperature of irradiation varied from 74° to 76° F.

After radiation, the irradiated spores were grown under the same cultural conditions as the untreated spores, and observations were made on germination in the manner described above. The effects of ultraviolet radiation on cell size and the rate of cell division were noted on chloronemata which had developed for five days from treated spores.

B. Observations and Discussion

The effect of ultraviolet radiation on the percentage of germination is shown in Table II and Figure 1. These studies were based on

TABLE II

PERCENTAGE OF SPORE GERMINATION IN UNTREATED AND IRRADIATED CULTURES

Time of Exposure	Percentage of Spore Germination*	Number of Spores
0	94.6 ± 1.5 %	1487
1 min.	98.8 ± 0.18 %	1049
5 min.	96.0 ± 0.34 %	1078
10 min.	58.8 ± 7.4 %	1072
15 min.	12.9 ± 1.7 %	1045
20 min.	7.0 ± 1.5 %	1048
25 min.	0.3 ± 0.02 %	1010
30 min.	0.0 %	1050
TOTAL		8839

*The standard errors were based on ten untreated and five irradiated cultures.

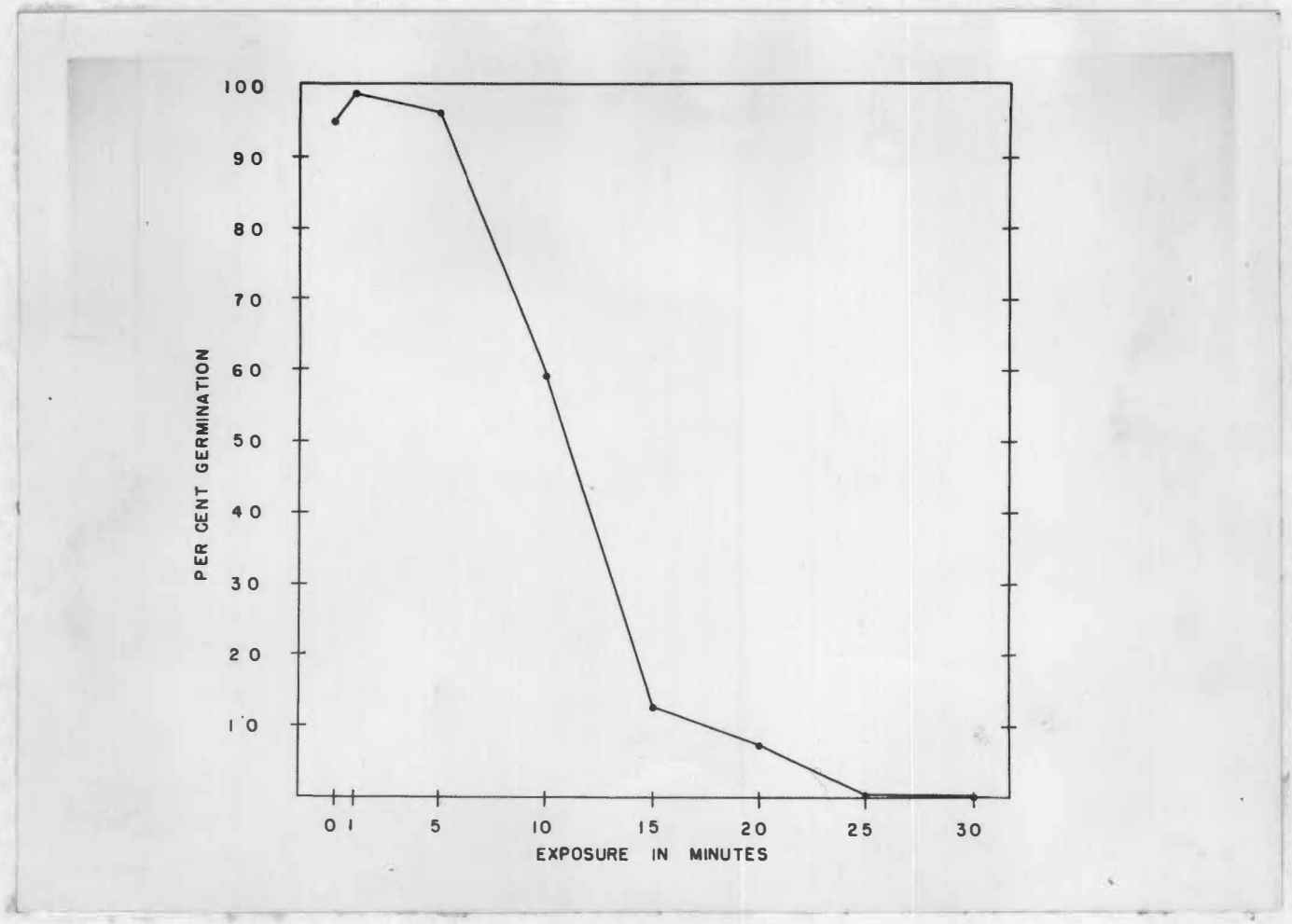

Figure 1. The effect of ultraviolet radiation on spore germination.

8839 spores. Spores which showed any signs of development were considered as germinated. In addition to the types of germination described above, the radiations produced a variation in the development of the spores which was never observed in untreated spores. With an increase in the length of exposure, beginning with the five minute period, spores were seen which became swollen and developed chloroplasts, but never showed further germination. The percentages of germination in Table II include these spores, but they are not included in the spore germination pattern figures, as there was no development of either rhizoids or chloronemata.

Figure 1 shows graphically the effect of ultraviolet radiation on spore germination. The increase in germination from 94.6 percent in the controls to 98.8 percent at one minute exposure to ultraviolet rays was statistically highly significant. This increase showed a stimulation in germination with short exposures to ultraviolet radiation. From one minute exposure the rate of germination rapidly decreased up to fifteen minutes at which only 12.9 percent germination was noted. After the rapid decrease in germination the rate gradually decreased to 0.3 percent at twenty-five minutes. Thus an increase in the length of exposure to ultraviolet radiation produced a decrease in the number of spores which germinated.

The spores in the control and those irradiated for one minute germinated with the appearance of the rhizoid, twenty-four hours after sowing. The chloronemata usually developed about twelve hours later. Those spores irradiated for five minutes germinated twelve hours after

the untreated spores, and those irradiated for ten, fifteen, and twenty minutes were delayed in germination thirty-six to forty-eight hours. Spores exposed for twenty-five minutes to ultraviolet radiation showed a very low percentage of germination after 120 hours.

The effect of ultraviolet radiation on the germination pattern is shown in Table III and Figures 2 and 3. The number of germinated spores which developed by producing only a chloronema increased with an increase in the length of exposure. After twenty minutes exposure to ultraviolet radiation, only the chloronemata developed from the spores. No other germination types were represented. Spores which developed rhizoids, later followed by chloronemata, decreased in number with an increase in time of exposure. After ten minutes exposure, only 1.6 percent of the spores germinated in this manner in comparison with the development of 62 percent of the untreated spores. Two of the germination types in the lower percentage groups showed a slight increase with radiation. The development of the chloronemata and then the rhizoids showed an increase with 1.3 percent after five minutes exposure in comparison with 0.2 percent in untreated spores and 0.6 percent in spores exposed for ten minutes. Spores which developed by producing both rhizoids and chloronemata at the same time increased from 0.5 percent in the controls to 1.5 percent after fifteen minutes exposure. The germination type in which only rhizoids appeared decreased from 3.5 percent in the untreated spores to 0.3 percent after ten minutes exposure. Apparently the longer exposure of spores to ultraviolet radia-

TABLE III

THE EFFECT OF ULTRAVIOLET RADIATION ON THE PATTERN OF SPORE GERMINATION

Time of Exposure	Germination Types										Total Number of Spores
	Rhizoid, Then Chloronema		Chloronema Only		Chloronema, Then Rhizoid		Both Rhizoid and Chloronema		Rhizoid Only		
	%	No.	%	No.	%	No.	%	No.	%	No.	
0	62.0	873	33.8	476	0.2	4	0.5	11	3.5	43	1407
1 min.	36.8	383	59.6	622	0.3	1	0.9	6	2.1	12	1039
5 min.	5.1	51	91.5	940	1.3	22	0.9	7	0.4	5	1027
10 min.	1.6	11	96.7	558	0.6	2	0.8	5	0.3	1	577
15 min.	0.0	0	98.5	69	0.0	0	1.5	1	0.0	0	70
20 min.	0.0	0	100.0	63	0.0	0	0.0	0	0.0	0	63
25 min.	0.0	0	100.0	3	0.0	0	0.0	0	0.0	0	3
30 min.	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0
Total											4186

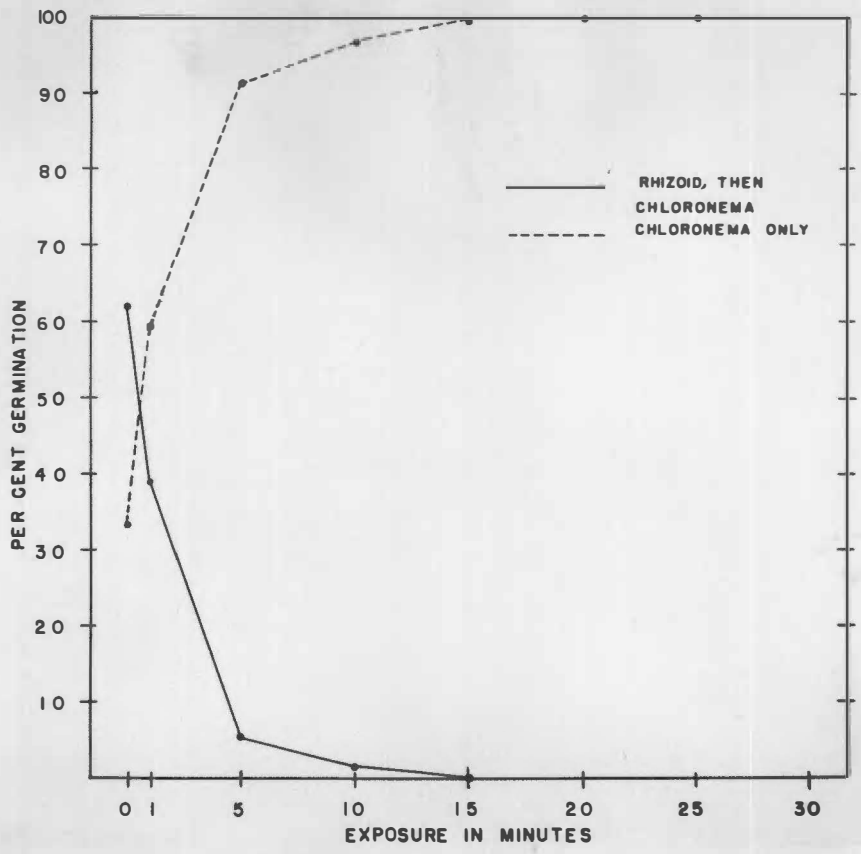

Figure 2. The effect of ultraviolet radiation on the spore germination pattern as indicated by the development of (1) the rhizoid and then the chloronema, and (2) the chloronema only.

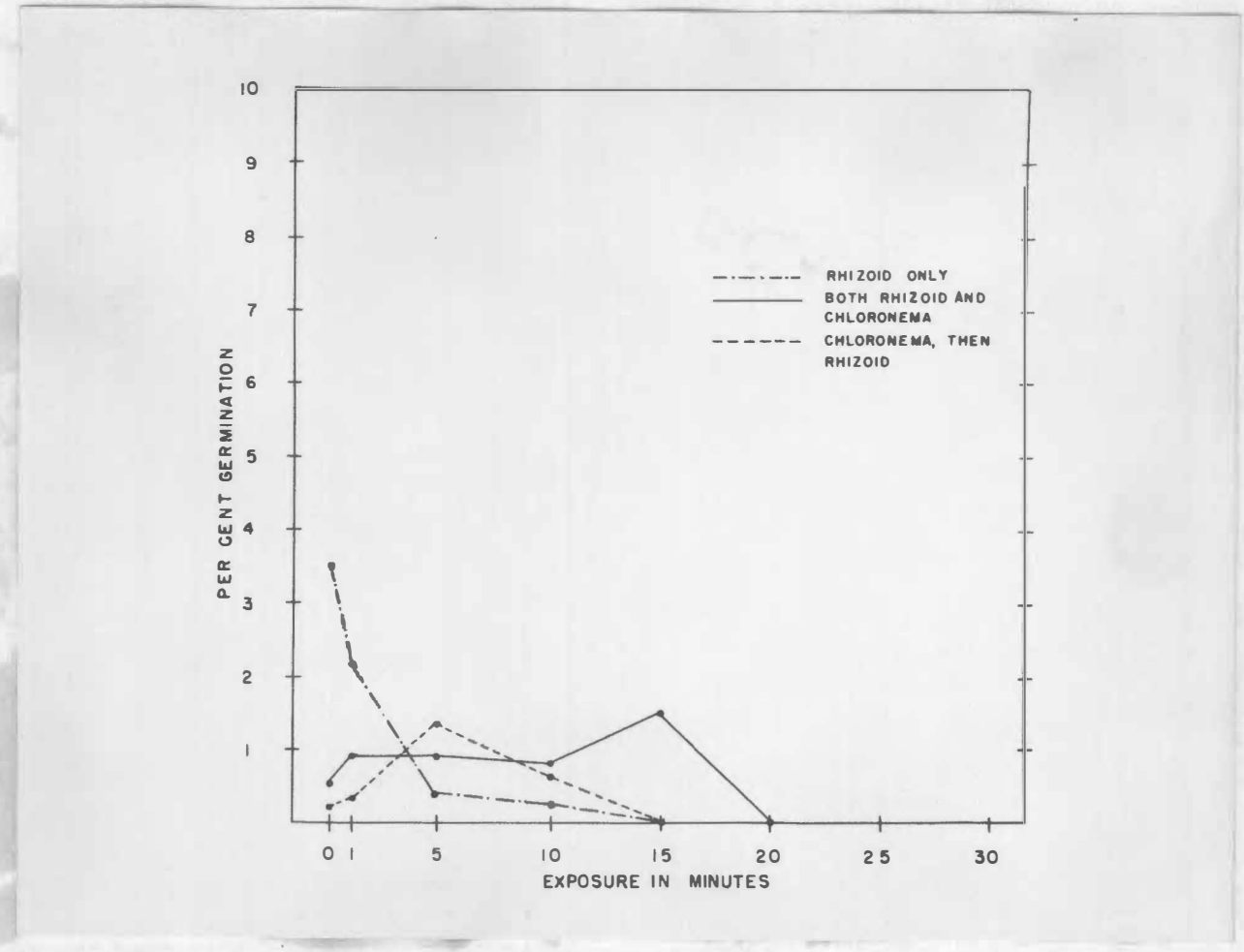

Figure 3. The effect of ultraviolet radiation on the spore germination patterm as indicated by the development of (1) the rhizoid only, (2) both rhizoid and the chloronema, and (3) the chloronema and then the rhizoid.

tion hindered the development of the rhizoids to a greater extent than the development of the chloronemata.

After the development of the chloronemata for five days, measurements were made to determine any differences in the width of cells, the length of individual cells in the filament, and the number of cells in the filament from untreated and irradiated spores. Table IV gives the results of these measurements.

Cells in the controls were somewhat more narrow than the cells which developed from spores exposed to ultraviolet radiation. The individual cells of chloronemata from spores treated for five minutes averaged shorter than cells from untreated spores. The cells in the chloronemata from spores treated for five and twenty-five minutes showed little difference in length. Thus the peak in the retardation of cell elongation was reached after five minutes exposure. The average number of cells in the chloronemata increased from 3.2 cells in the controls to 4.3 cells in the filaments from spores exposed for ten minutes. Radiation exposures up to ten minutes produced an increased rate of cell division with a retardation of cell elongation. The decrease in the average number of cells in the chloronemata at exposures longer than ten minutes indicated that the peak in the stimulation of cell division occurred in the range represented by the ten minute exposure.

In working with the daily cumulative effect of ultraviolet radiation on the development of fern prothallia from spores, Charlton

TABLE IV

DIFFERENCES IN THE GROWTH RATE OF CHLORONEMATA FROM TREATED AND UNTREATED SPORES*

Time of Exposure	Ave. No. of Cells in the Chloronemata	Number Counted	Ave. Length of Individual Cells in the Chloronemata	Number Counted	Width of the Chloronemata
0	3.2	100	123.8	100	7.5
1 min.	3.5	100	109.4	100	7.5
5 min.	3.8	100	83.5	100	7.5
10 min.	4.3	100	72.0	100	7.5-10
15 min.	1.5	20	64.8	20	15
20 min.	2.4	14	90.6	21	7.5-15
25 min.	1.6	12	72.0	18	7.5-15

*Measurements were taken five days after the appearance of the chloronemata. All measurements are in microns. Because of the lower percentage of germination, fewer chloronemata were measured in the plates exposed for 15, 20, and 25 minutes.

(1938) found a similar shortening of cells. This effect was attributed to the disturbance of hormone activity within the cells by ultraviolet radiation.

The activity of ultraviolet rays upon a cell may be a direct function of the absorption of radiation energy by specific biochemical substances (Duggar, 1936; Ellis and Wells, 1941; Lea, 1947; Foster, 1949). The wave length predominantly used, 2537 Å, was within the range highly absorbed by nucleic acids (Hollaender and Emmons, 1939). The related evidence suggested that the effect of radiation on the rate of germination, on the decrease in germination, and on the spore germination pattern, may be due to the absorption of energy by substances within the spore, thus altering the physical and chemical processes involved in germination and growth. The effect of ultraviolet radiation on spore germination can not be attributed to any effect of the radiations on the agar, which might have later affected germination and spore development, since radiation of plates and the subsequent germination of untreated spores on those plates produced germination results similar to the control plates.

SUMMARY

The results of this investigation on the effect of ultraviolet radiation on the pattern of spore germination in Fumaria hygrometrica indicate the following conclusions:

1. In addition to the types of germination described by Schoene, a fifth type is noted in which only the rhizoids develop from the spore.

2. Spore germination is delayed with increased exposure to ultraviolet radiation.

3. This delay in germination increased with the length of exposure.

4. After a slight stimulation of germination at one minute exposure to ultraviolet radiation, spore germination decreases with an increase in length of exposure until complete inhibition of germination is reached after thirty minutes exposure.

5. With an increase in the time of exposure, a change in the germination pattern occurs characterized by a marked increase in the proportion of spores which germinate by producing chloronemata only and a decrease in the number of spores which germinate by producing rhizoids, followed by chloronemata.

6. Exposures to ultraviolet radiation produce a retardation of cell elongation in chloronemata.

7. One to ten minute exposures to ultraviolet radiation stimulate cell division in chloronemata; exposures for fifteen minutes or

longer retard cell division.

8. The effect of ultraviolet radiation on the rate and percentage of spore germination and on the spore germination pattern may be due to the absorption of energy by biochemical substances within the spore, thus altering the physical and chemical processes involved in germination and growth.

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