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Studies on the physiology of germination of spores of *Funaria hygrometrica* (Sibth.)

I. The influence of light on germination with respect to water balance and respiratory processes

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INTRODUCTION

The role of light as an essential or favourable factor to germination of spores of mosses has long been recognized. Among others, Schulz (1902), Listowski (1927), Stephan (1928), Kofler (1959) and Mohr (1959), have devoted studies to this problem. Recently Mohr and Bauer (1959) found that the action of light is associated with the phytochrome system with its characteristic antagonism between near and far red. Notwithstaning numerous studies in this field, the mechanism of the action of light on germination of spores is not yet clear. This is due, on the one hand, to the lack of uniform criteria of germination of spores, or sometimes even complete lack of such criteria, and, on the other hand, to almost complete lack of information about the physiological processes taking place in the early stages of germination preceding bursting of the exosporium and formation of the protonema or rhizoid. This study is an attempt to clarify two fundamental physiological processes, namely the water balance and respiration, with regard to the influence of light on germination of spores.

MATERIAL AND METHODS

Experiments were carried out with spores of *Funaria hygrometrica*.

The material was collected in August in 1960 and 1961 near the locality Sucha in shaded and fairly humid habitats. Large spore capsules were stored in darkness under conditions of room temperature and humidity. In each experimental series 20 randomly selected capsules were used. The spores from the broken capsules were placed in a small weghing vessel, thoroughly mixed with a brush, and sow on suitable media. When necessary to determine the mass of the spores and the water content of air-dried spores, the routine microanalytic method of measuring fresh and dry mass was employed.

In preliminary experiments germination was studied in liquid media in 50 ml flasks stoppered with cotton plugs and holding 25 ml of medium. At intervals, several drops of the suspensions were withdrawn with a pipet and microscopic preparations were made to check the process of germination. In the main series of experiments germination was conducted on Mohr's medium (1959) solidified with 1% agar in Petri plates of 6 cm diameter. To ensure optimal moisture content of the medium and of the atmosphere in the Petri plate, one ml of distilled water was poured into a well in the agar medium. The spores were seeded, not too densely, by means of a brush as uniformly as possible on the surface of the agar medium. When determining the moisture content of the medium glass plates covered with a thin layer of agar medium were placed in a closed weighing vessel over sodium chloride solution of fixed concentration. The spores were seeded out on media which were first adapted during 48 hours to the humidity of the atmosphere as determined by the concentration of the salt solution. All the experiments (except those designed to study the effect of temperature) were carried out in the termostate at 27° (±1°) under 800 lx illumination with two 25 W luminescent lamps giving "daylight" illumination (type 7-61 Telam), or in darkness. Observations of the spores were made microscopically on the Petri plates after placing a glass coverslip on the surface of the agar. The calculations were based on observations of three points on the plates, which were then discarded. The dimensions of the spores were determined with a 40 × objective and K 15 × eyepiece of the microscope. Percentage of germinated spores was calculated from 3 to 7 repeated serial counts of 300 spores each. Spores were considered to be germinated which, besides increased dimensions and green color, showed protrusion of the protonema equal to or greater than ⅓ of the diameter of the spore (Fig. 5c). Beginning of germination was assumed when 1% of the spores had germinated. Oxygen consumption by germinating spores was determined by means of a microrespirometer (Zurzycki 1955, Starzecki 1960). The spores were collected from the agar medium with a brush and placed on the glass stage of the respirometer in a small drop of water. When the measurement was completed, the spores were washed quantitatively with alcohol into a weighing vessel. After evaporation they were boiled with 1 ml of 10% alcohol (with the purpose of dissolving traces of agar which cause the spores to adhere), and spore counts were made in the suspensions. Oxygen consumption was measured in relation to one spore. The number of spores was found from the known volume of the suspension and the number of spores per 1 µl counted in the suspension in a Bürker cytometer.

RESULTS

Preliminary experiments*

The preliminary experiments were performed with the object of ascertaining the optimal conditions of germination of the studied spores. The influence of the type of medium, its acidity, temperature and moisture were studied.

Composition of the medium. Germination was studied on three media with different composition.

Table 1

Composition of the mineral media

	Mohr mg	Knop mg	Pirson and Seidel mg
KNO_3	100	–	400
$Ca(NO_3)\cdot 4H_2O$	–	1000	–
$CaCl_2\cdot 4H_2O$	10	–	610
$MgSO_4$	10	250	300
KH_2PO_4	136	250	200
KCl	–	120	–
$MnCl_2\cdot 4H_2O$	–	–	0,3
H_3BO_3	–	–	0,5
$FeCl_3$	–	traces	–
Fe citrate	0,3	–	5
Distilled water ml	1000	1000	1000
pH	5,5	5,7	5,5

The results are summarized in Fig. 1, showing the influence of the composition of the medium on germination. On all three media germination began after about the same time, that is, after 12 to 14 hours. The number of germinated spores then increased in the medium of Knop more rapidly than in the medium of Mohr. Both media, however, may be regarded as being approximately equivalent, enabling full germination of the spores. After 48 hours on Mohr's medium 93.6% of the spores were germinated, and on Knop's medium 95.0%. In further experiments Mohr's medium was adopted, on which germination is more uniform. The medium of Pirson and Seidel was less suitable because on it germination proceeds at a slower rate, and after 48 hours only 69% of the spores germinated.

* The preliminary experiments were carried out by Mgr Paziewski with the author's cooperation, and constitute part of a diploma thesis presented at the Department of Plant Physiology, WSP, in 1961/62.

Acidity. Studies on the influence of acidity on germination were performed by adjusting the medium of Mohr to various pH in the range from 4.3 to 8.1 by means of combinations of KH_2PO_4 and K_2HPO_4, or KH_2PO_4 and H_3PO_4 at constant concentrations of PO_4 ions and other ingredients of the medium. The results illustrated in Fig. 2 show that the rate of germination is slower in acid media, or even completely inhibited. Nevertheless, in the pH range 5,5—8 germination was practically identical and in the further experiments the reaction of Mohr's medium was adjusted to this level of acidity. Slight changes in pH did not affect the course of germination.

Temperature. The influence of temperature on germination of the studied spores in shown in Fig. 3, from which it may be seen that the rate of germination was highest at 29°. The extreme temperatures

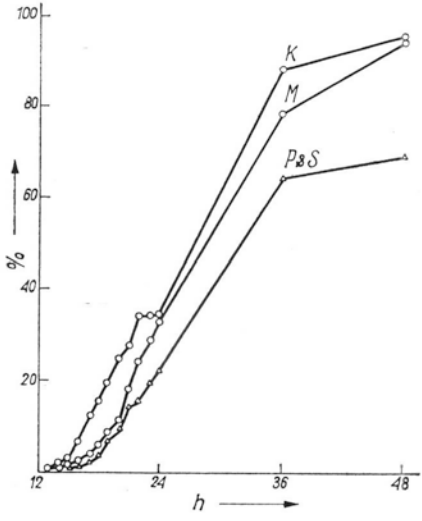

Fig. 1. Effect of composition of the medium on germination

K — Knop's medium; *M* — Mohr's medium; *P&S* — Pirson-Seidel medium; x-axis — time in hours; y-axis — germination of spores.

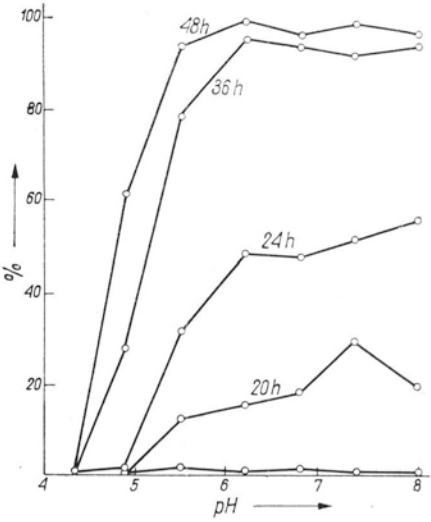

Fig. 2. Effect of acidity of the medium on germination of spores; x-axis — pH; y-axis — percent germinated spores after different intervals of time.

at which germination takes place were 14° and 35°; at these temperatures after 48 hours only 1—2% of the spores germinated. Below the optimal temperature germination was retarded, although finally complete germination was obtained in a wide range of temperatures. Further experiments were carried out at 27°. Since deviations of temperature may have a distinct influence on the rate of germination, the experiments were conducted in an termostate which enables strict control and stabilization of the temperature factor.

Moisture. The course of germination on an agar medium at different degrees of dessication of the medium is illustrated in Fig. 4. The curve shows that the spores germinate when the water content of the

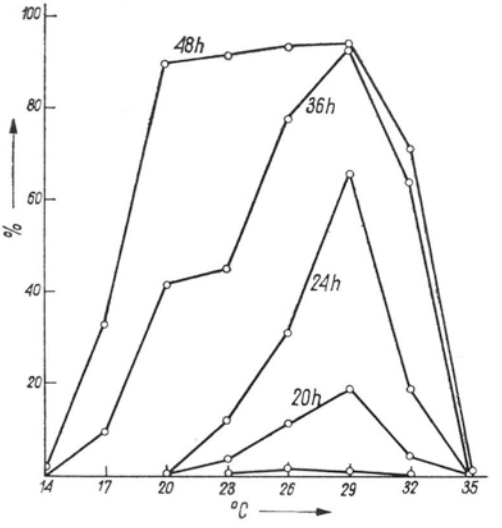

Fig. 3. Effect of temperature on germination of spores. x-axis — temperature in °C, y-axis — percent of germination.

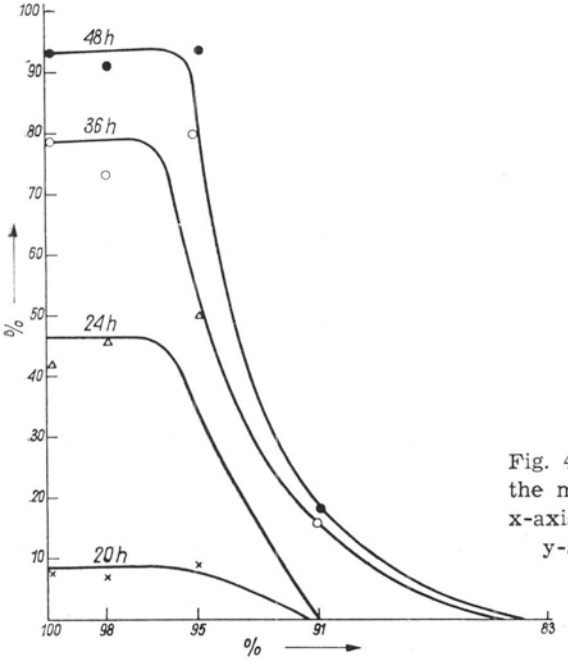

Fig. 4. Effect of moisture content of the medium on germination of spores. x-axis — relative humidity of air; y-axis — percent of germination.

medium is between 98.58% and 91.67% and relative humidity of the air between 100% and 95%. At lower levels of moisture germination is inhibited. At 83% relative humidity of the air, germination ceases altogether.

Absorbtion of water during germination

Air-dried spores of *Funaria hygrometrica* show a mean water content of 18.2% (mean of five measurements ranging between 10.96% and 24.42%). Air-dried spores have the appearance not of regular spheres, but more often of rotating ellipsoids or spindles with gently rounded ends (Fig. 5*a*). Two dimensions can be distinguished — width and breadth. The mean of 300 measurements showed: length 16.52 μ, breadth 13.52 μ.

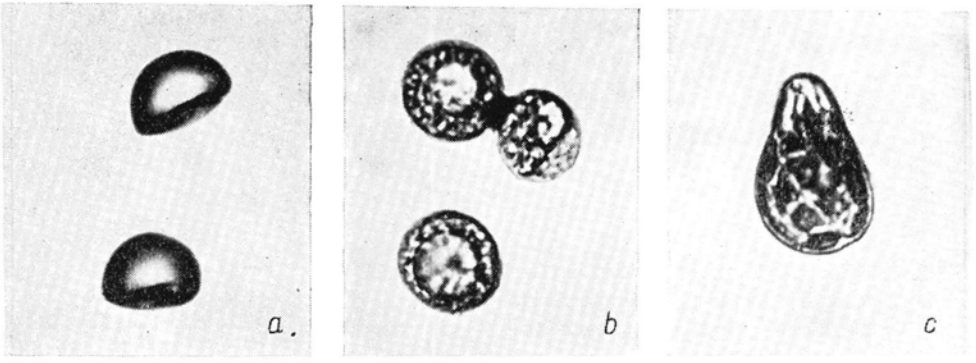

Fig. 5. Spores of *Funaria hygrometrica*:

a — air dried; *b* — after the ist phase of swelling; *c* — germinated (criterion of germination).

Assuming, for the sake of simplification, that the spores have the form of rotating ellipsoids, their volume calculated from these two dimensions would be 1580 μ^3. When the spores are placed in conditions favoring germination, that is, on medium with sufficient moisture content, a number of processes leading to germination takes place. One of these processes, manifested by changing shape and dimensions of the spores, consists in water absorption. Within 10—15 minutes after the spores are transferred to agar medium, their dimensions increase distinctly, accompanied by a tendency to rounding and shortly resulting in the spores assuming approximately spherical shape. This phenomenon is plainly discernible in a series of photograms. Rapid increase in size and equalization of length and breadth of the spores within 10 minutes is apparent in the attached diagram. Toward the end of the first phase of swelling a distinct vacuole may be seen (Fig. 5*b*). After further elapse of time, between 1/2 and 10 hours after the spores were transferred to the medium, a stationary phase ensues, during which the dimensions of the spores increase very little. During the third phase, between the 10th and 16th hours, the spores again increase in size at a somewhat diminished rate before bursting of the exosporium. In order to obtain a series of photograms, the above-described processes were studied at first in a moist chamber on the microscope stage continuously illuminated with a micro-

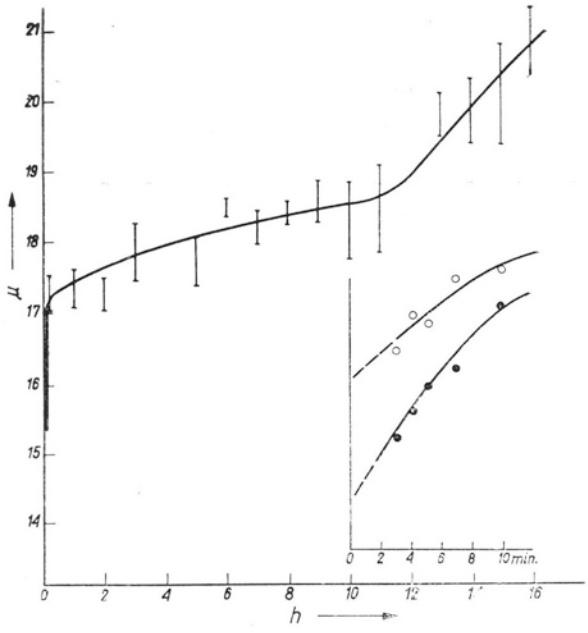

Fig. 6. Changing dimensions of swelling spores. x-axis — time in hours and minutes; y-axis — dimensions of the spores.

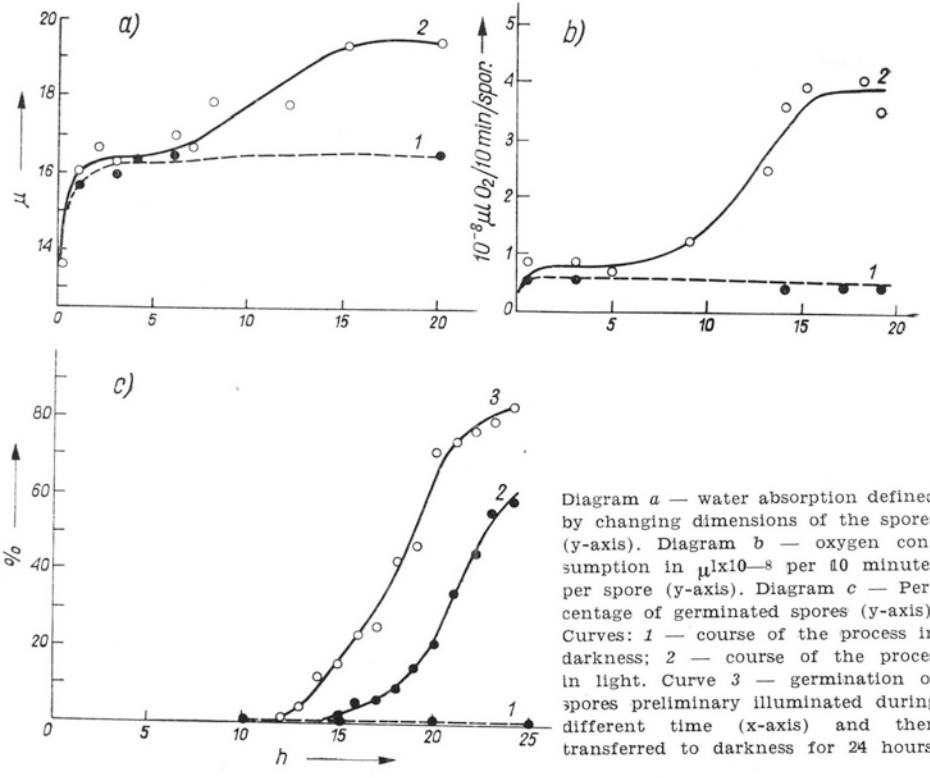

Diagram *a* — water absorption defined by changing dimensions of the spores (y-axis). Diagram *b* — oxygen consumption in μlx10—8 per 10 minutes per spore (y-axis). Diagram *c* — Percentage of germinated spores (y-axis). Curves: *1* — course of the process in darkness; *2* — course of the proces in light. Curve *3* — germination of spores preliminary illuminated during different time (x-axis) and then transferred to darkness for 24 hours.

Fig. 7. Water absorption, respiration and germination of spores in light and in darkness.

scope lamp. Since these conditions differ markedly from those employed in the remaining experiments, the observations were repeated with statistical measurements of germinating spores on agar plates under the conditions described in the section on Methods. The results are presented in Fig. 7*a*. When the germinating spores were illuminated, three phases were distinct, differing in respect to rate of water assimilation. A phase

Table 2

Changes in the dimensions of the spores in the course of germination

Type of spores	Dimensions in μ	Volume in μ	Water content percent of dry mass
Air-dried	16,52 × 13,52	1580	18,2
Stationary phase	diam. 16,50	2350	45
Germinating	diam. 19,41	3820	66,4

of very rapid swelling lasts 20—30 minutes, followed by a stationary phase lasting about 8 hours; about 8—16 hours after the spores were transferred to the medium a phase of secondary rapid water absorption sets in. When the spores germinate in darkness, only the first and second phases were observed, not followed by the third phase of increased water absorption. The stationary phase (phase II), which under illumination ends after 8 hours, in darkness continues until the end of the experiment. Assuming that the changes in the volume of the spores are due only to water absorption, they may be regarded as a rough indication of the water content of the spores. However, such an estimation is encumbered with considerable error due to simplification of the shape of the spores, assumed to be ellipsoidal in the air-dried condition and spherical after swelling. The results of such an estimation are presented in Table 2.

Respiration

The microrespirometric measurements were carried out with amounts of the material (spores) selected to give total oxygen consumption in the respirometer chamber of the order of 10^{-3} μl/10 mins. The mean oxygen consumption per spore is shown in Table 3. In Fg. 7b the data contained

Table 3

Oxygen consumption by spores growing in light and in drakness in 10^{-8} μl/10 mins/spore

Time in hours	1/2	1	3	5	9	13	14	15	17	18	19
in light	0.92	–	0.78	0.66	1.23	2.44	3.54	3.93	–	4.01	3.41
in darkness	–	0.47	0.57	–	–	–	0.38	–	0.46	–	0.44

in Table 3 are represented graphically. To a certain extent, the changes in oxygen consumption during germination are analogous to the simultaneously occurring changes in the water content of the spores. During the period between ½ and 8—10 hours oxygen consumption by spores in darkness remains at a steady level amounting to 0.47×10^{-8} µl/mins. When the germinating spores were illuminated, during the phase of secondarily increased water absorption, i.e. after 8—15 hours on the medium, oxygen consumption was greatly increased (6—8 fold). Although oxygen consumption by air-dried spores was not investigated, it is presumably less than during the stationary phase.

Course of germination in time

The studied spores of *Funaria hygrometrica* fail to germinate in darkness, whereas spores continuously illuminated show 1% germination after 15 hours. The percentage of germinated spores then increases, reaching 60% after 24 hours, and 95% after 48 hours. Illumination with light impulses lasting 3 hours of the spores germinating in darkness at different intervals after sowing them on the medium was not sufficient to induce germination when the spores were left in darkness after the impulse. Under these conditions the exosporium bursts, but the process halts at this stage in spite of observation during a further 48 hours in darkness. In the last variant of the experiments the germinating spores were illuminated and then transferred after various intervals of time to darkness. The percentage of germinated spores was determined 24 hours after transfer to darkness. The results are illustrated by the upper curve in Fig. 7c. The shape of the curve is similar to that of the curve obtained during continuous illumination of the germinating spores. Germination of 1% of spores in darkness requires illumination of the spores for at least 12 hours continuously. Transfer of the cultures from light to darkness after the process of germination has already set in (15—24 hours after inoculating the medium) increases the percentage of germinated spores only upto a certain point (by 20 to 40%), indicating that only those spores in which the processes preceding germination were sufficiently advanced during illumination are capable of completing germination in darkness.

DISCUSSION

In spite of extensive studies on germination of spores of mosses and ferns, the physiological processes at this stage of the life of the cells are not sufficiently elucidated. One of the reasons for the discrepancies, respectively noncomparability of the results of different studies is the lack of uniform criteria of germination. The morphologic changes during

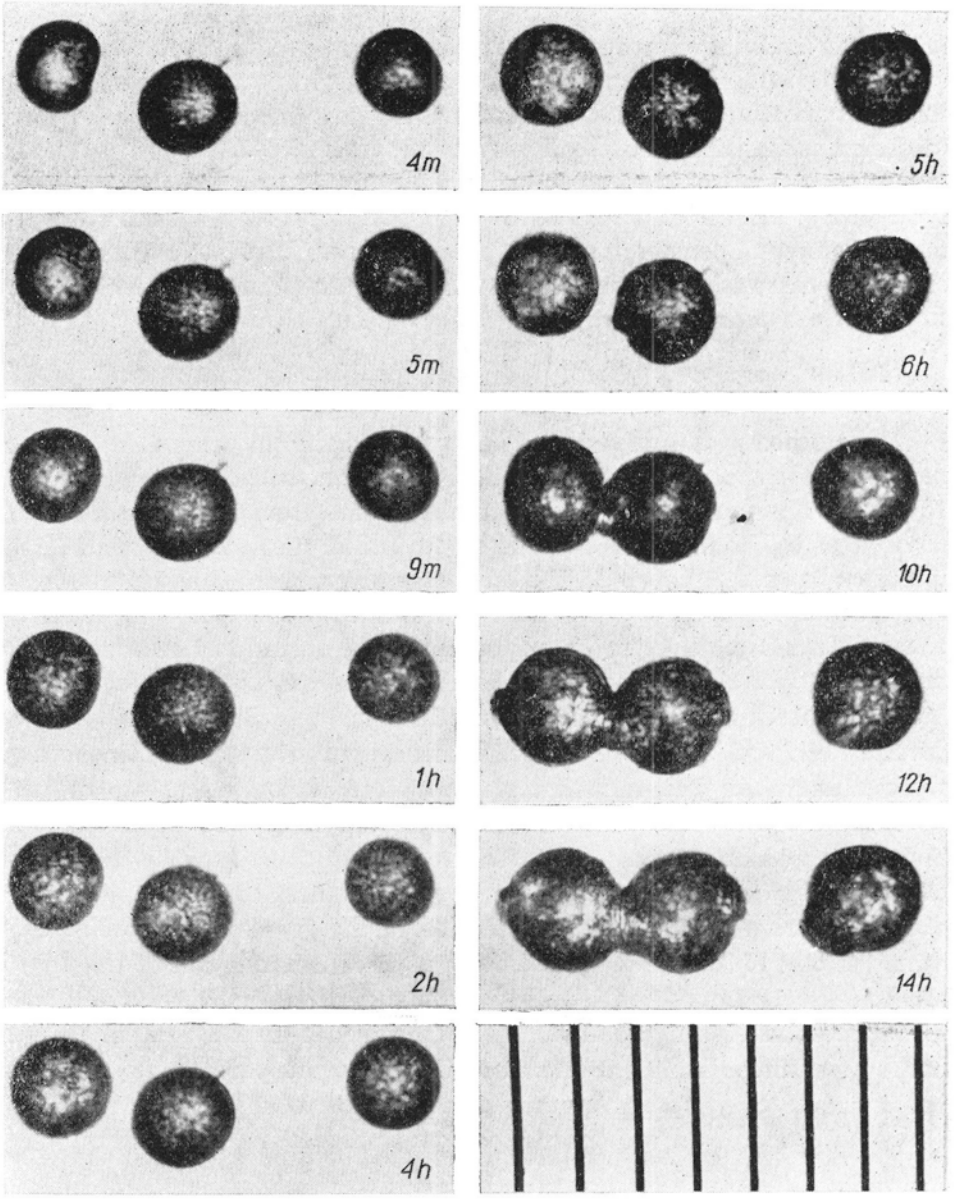

Plate 1. Changes in the shape and dimensions of the spores during germination.

germination which can be observed microscopically may be classified into the following series:

1. Air-dried spores.
2. Absorption of water resulting in increased dimensions and volume of the spores.
3. Greening of the spore content and bursting of th exosporium.
4. Distension of the cell wall, indicating beginning growth of the protonema or rhizoid.
5. Further growth of the protonema or rhizoid.

Most authors who define the criteria of germination strictly, regard spores with green content and burst exosporium as already germinated (Listowski 1927, Kofler 1959, Mohr 1959). However, this criterion of germination is not acceptable without reservations. Apart from the fact that when the exosporium is very thick and untransparent its bursting may be overlooked, according to Orth (1937) the exosporium may burst merely as the result of swelling of the spore unrelated to physiological processes. Our findings (p. 187) show that under specified conditions the cell content may assume a green color and the exosporium may burst without leading to further stages of germination. These observations induced us to regard the beginning of the fourth phase as the criterion of germination (see Methods). Our observations of the effect of external factors on germination agree, in principle, with those of other investigators. As is known, spores may germinate in pure water; newertheless, the chemical composition of the medium is not an indifferent factor, as shown by the results of comparison of different media. Since the osmotic activity as well as pH were very similar in all three cases and each of the media contained all the elements considered necessary to the life of plants, the differences in germination which were observed must be ascribed to different ionic composition of each medium. Data published by Heitz (1942) suggest a favorable effect of the K_2HPO_4 to KH_2PO_4 ratio in the medium of Pringsheim. The present experiments, which were carried out in a broad range of pH, showed considerable tolerance of the spores to acidity of the medium, although germination was optimal at neutral or slightly alkaline reaction, which is in agreement with the observations of Heitz. In contrast to spores of *Funaria*, those of *Dryopteris*, for instance, germinate optimally at pH 5.4 (Mohr 1956). The optimal temperature for germination, inaccurately stated to be 16—25° in earlier studies (Servettaz 1913, Lesage 1918), was established by Kofler (1959) to be 25—29°. Our findings, although employing somewhat different criteria, are in strict agreement with those of Kofler.

There have been only few studies on the efect of moisture on germination. Lesage (1918) claimed that spores germinate only in actual contact with liquid water, air humidity being insufficient for germination.

Solidification of media (1—1.5%, as commonly employed), is not an obstacle to assimilation of water for germination. The results of our preliminary experiments show that the water content of the medium and air humidity required for germination are less than is usually believed, and that even in spite of absence of condensation water on the surface of the agar medium germination occurs, although it is retarded.

The role of light in germination of *Funaria* spores has been repeatedly confirmed. The studies of Heitz (1942), and especially those of Kofler, (1959) demonstrated that the race of *Funaria hygrometrica* and the conditions of development of the spores are important factors determining the manner in which the spores react to light. The material used in our experiments was derived from a single uniform population; moreover, the attempt was made to obtain always a representative average sample of the spores for each experiment (see Methods) with the purpose of eliminating possible differences of reactivity to light of spores from different capsules. In no case was germination of the spores in darkness observed. The discrepancy between our findings and those of Heitz and Kofler may be due not only to racial differences, but also to different criteria of germination. Information on the physiology of germination can be obtained only by comparing the various processes accompanying germination, especially changes in their intensity in the course of germination and mutual correlations. The fundamental part of this study was an attempt to make such a comparison concerning the water balance, respiration and the effect of light on these processes. Air-dried spores when transferred to an environment of suitable moisture begin to absorb water very intensively, presumably by physical processes based on swelling of cell colloids and osmotic equilibrium with the environment. This is indicated by the appearance of a vacuole toward the end of the aforementioned phase. The process takes place in light as well as in darkness. After osmotic equilibrium is achieved (usually after 20—30 minutes under suitable conditions), a phase of stabilization sets in, marked by only minimal or no water absorption and maintenance of metabolic processes on a steady level. Further progress of germination requires light. In darkness the stationary phase continues without change. On the other hand, under illumination after 8—10 hours a second phase of water absorption sets in, accompanied by greatly enhanced respiration. A certain analogy with the increasing dimensions of the spores described in this paper may be found in the observations of Kofler (1959, Fig. 4). The relation to metabolism and requirement of light in the second phase indicate that it is not based on purely physical processes. A similar biphasic course of water absorption was described by Czosnowski (1962) in embryos of lupine. According to Mohr (1959) the action of light on germination of *Funaria* spores is governed

by the phytochrome system. The author assumes existence in the spores of a factor initiating germination, the effect of which is manifested when a threshold level is reached. Enhanced or diminished activity of this factor is associated with the phytochrome system and is regulated by the antagonistic action of near and far red. Our experimental results appear to indicate that the action of light through the phytochrome system exerts a fundamenal effect on metabolic activity. On the other hand, Kofler (1959) found that sugars stimulate germination in darknes, an effect that is similar, under certain circumstances, to that of light. A trophic effect of light must therefore also be considered, especially in experiments on mineral media. Studies on the influence of intesity of light and its spectral range are needed to elucidate the role of photosynthesis in the action of light on germination.

SUMMARY

1. The influence of various external factors, such as composition of the medium, pH, moisture and temperature on the germination of *Funaria hygrometrica* spores was studied.

2. Absorption of water during germination was found to occur in two stages: the first brief stage based on physical phenomena, and a second stage which requires light and is associated with metabolic processes.

3. Intensity of respiration also shows biphasic changes. Increased respiration preceding germination occurs only in light.

4. Germination of the spores requires continuous illumination with light of 800 lx intensity during at least 12 hours. Transferring the cultures to darkness after germination has already begun causes only a temporary increase in the percentage of germinated spores.

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Badania nad fizjologią kiełkowania zarodników Funaria hygrometrica. Cz. I. Wpływ światła na kiełkowanie na tle gospodarki wodnej i procesów oddechowych

Streszczenie

1. Zbadano zależność przebiegu kiełkowania zarodników *Funaria hygrometrica* od kilku czynników zewnętrznych jak: skład podłoża, pH, wilgotności, temperatury.

2. Stwierdzono, że proces pobierania wody w okresie kiełkowania odbywa się w dwu etapach: pierwszy przebiega w krótkim czasie i jest związany z procesami fizycznymi, drugi odbywa się tylko w świetle i związany jest z przemianą materii.

3. Natężenie oddychania zasadniczo wykazuje również dwuetapowe zmiany. Wzrost natężenia oddychania poprzedzający wykiełkowanie zachodzi tylko w świetle.

4. Do wykiełkowania zarodników konieczne jest ich oświetlenie światłem ciągłym o natężeniu 800 lx przez co najmniej 12 godz. Zaciemnienie kultury podczas rozpoczętego już kiełkowania powoduje tylko częściowe zwiększenie % wykiełkowanych zarodników.