Investigations on the germination of spores of the fern *Matteucia struthiopteris* (L.) Tod.

Badania nad kiełkowaniem zarodników paproci Matteucia struthiopteris
(L.) Tod.

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INTRODUCTION

As early as 1828, fern prothallia were described by Bischoff. Prothallia cultures from spores, however, were grown much later by Leszczyć-Sumiński (1848). In his work Leszczyć-Sumiński gave the correct interpretation of a prothallium, described it and presented drawings of the successive developmental stages.

Germination of spores is the first developmental stage of a prothallium. In spores with a thin exosporium (*Polypodium vulgare*, *Athyrium*) just before germination begins, a slight greening of the content of the spore cell is observed. At the same time protoplasm, drops of fat and chloroplasts gather near the place of bursting of the exosporium (Karpowicz 1927). Each species is characterized by a different period of time elapsing between the moment of sowing and germination. *Matteucia struthiopteris*, *Polypodium vulgare* germinate after a very short time. *Asplenium* and *Polystichum* need a longer time to germinate.

There are two ways in which germination proceeds: in some species the first cell to appear is a cell of the filament, in other species the germinating spore forms first a rhizoid. The first cell of the filament is generally spheric or cylindrical and its divisions proceed at a very rapid rate. Rhizoids, in the number of 1—3, are formed as small colourless protuberances which elongate in a short time and are separated from the filament by a membrane. The first rhizoid appears generally on the side of the cell that is opposite to the direction of the growth of the filament, the next one close to the first. In early developmental stages of the prothallium, the rhizoids are colourless, some of them contain, however small chloroplasts.

The action of different factors on the germination of fern spores

made the object of numerous studies. Among the investigated factors are light (Heald 1898, Life 1907, Mohr 1956), temperature (Heald 1898, Conway 1949), concentration of the nutrient solution (Heald 1898), humidity and pH of the substratum (Conway 1949), radium and X rays (Rottman 1939).

The present work was undertaken with the aim to make detailed observations on the germination process of fern spores, and more specially, to examine the action exercised by several factors on the course of germination with special stress on the light as one of the most important factors.

MATERIAL AND METHOD

Preliminary observations on germination in light and darkness were carried out on spores of the following fern species (Szafer, Kulczyński, Pawłowski 1953): *Dryopteris filix-mas* (L.) Schott, *Pteridium aquilinum* (L.) Kuhn, *Asplenium ruta-muraria* L., *Asplenium trichomanes* L., *Polypodium vulgare* L., *Blechnum gibbum* Mett., *Polystichum falcatum*.

Sporophylls of these ferns collected in November 1959 from specimens growing in a glass house or in natural conditions in the Botanical Garden in Cracow provided the experimental material.

Most investigations were performed on *Matteucia struthiopteris* (L.) Tod., because of the great amount of spores produced by this species and their prompt and regular germination. Spores were collected by shaking the sporophylls over a white sheet of paper or by removing them from the sporangia with a preparation needle.

A suspension containing 10—20 mg of spores in 8 ml water was prepared for experiments on germination in light and darkness. For sowing 1 ml of this suspension was used for one Petri dish. Different sowing densities were obtained by weighing various quantities of spores and mixing them with 25 ml nutrient solution according to the following scheme:

Weight in mg of spores for 25 ml nutrient solution	50	20	10	5	2	1	0.1
Relative concentration	1	0.4	0.2	0.1	0.04	0.02	0.002

In all the other experiments 2 mg spores were sown per flask containing 25 ml nutrient solution.

The preliminary experiment (germination in light and darkness)

was carried out in Petri dishes 10 cm. diameter. Other experiments were performed in Erlenmayer flasks 100 ml capacity containing always 25 ml nutrient solution.

In order to find out the most suitable medium, tests of spore germination were performed on following media: Knop 1:50, Hoagland, Rhode, Rhode + peat extract.

The best growth was observed on Hoagland's nutrient solution (Bonner and Galston 1952) and this solution was used in all experiments. Cultures in Petri dishes were grown on Hoagland's solution solidified with agar (1,5%) — 25 ml per Petri dish; cultures in flasks — on Hoagland's solution 25 ml per flask.

Cultures were kept in a dark and in a light thermostat; the intensity of light emitted by fluorescent tubes attained 2800—3000 lux. In both thermostats the temperature was about 26—28°C.

In experiments on the length of the light period the cultures were supplied daily with light for 1, 6, 12, 24 hours. Other experiments were performed in continuous light. In order to lower the light intensity neutral filters, made of photo-films, exposed to light for a suitable time were employed. Cultures were kept in boxes covered with filters and placed in a thermostat in which the light intensity was 3000 lux. Measurements of light intensity were performed by means of luxometer equipped with a photo-voltaic cell of Lange.

The counts and the observations were made 3—5 days following the moment of sowing. In experiments on germination in light and darkness, the cultures were examined every 5 days for a period of 40 days.

Microscopic observations were made on drops taken from the liquid nutrient solutions. Only the estimations of the percentage of spores germinated on agar media were performed directly on Petri dishes.

The bursting of the exosporium of the spore was adopted as the criterion of germination.

For microscopic observations objectives 5× and 10×, eyepiece — 15× were used. For more detailed observations an objective 40× was employed.

RESULTS

A. Germination in light and darkness

The older investigations on the influence of light on the germination of spores of ferns and mosses led to contradictory results. Borodin 1868 (after Heald 1898) discovered that light is a factor neces-

sary for germination; the bursting of the exosporium did not take place in darkness. Life (1907) on the other hand, found that light is not always necessary for germination. The ability of germination in darkness depends on the species and the growth conditions. It has been shown that out of 36 examined species 16 were able to germinate in darkness (Life and others).

In the course of the present work the germination of spores of *Matteucia struthiopteris* and 7 other species in light (3000 lux) and darkness was examined (Table 1).

The spores of *Matteucia struthiopteris* germinate in darkness relatively easily, though, in light the percentage of germinated spores is always much higher. Spores of *Polypodium vulgare*, *Pteridium aquilinum* and

Table 1

Influence of light (L) and darkness (D) on germination percentage of fern spores

Species	Number of days after sowing					
	5	10	15	20	25	40
1. <i>Matteucia struthiopteris</i>	L 80 D 5	90 15	91 15.5	91 30.5	91 43	
2. <i>Polypodium vulgare</i>	L 85 D —	94 2	94.5 2.5	94 3	94 17	
3. <i>Polystichum falcatum</i>	L 73 D —	77 —	91.5 —	94 —	94 1.5	94 5
4. <i>Pteridium aquilinum</i>	L 58 D 3	87 14	87.5 17	23		
5. <i>Dryopteris filix-mas</i>	L 3 D —	28 —	44 —	46 —	51.5 —	
6. <i>Blechnum gibbum</i>	L 12 D —	27 15	43 15	50 16.5	62 17	26
7. <i>Asplenium trichomanes</i>	L 12 D —	88.5 —	92 —	96 —	96 —	96 —
8. <i>Asplenium ruta-muraria</i>	L 8 D —	64.5 —	72.5 —	76 —	80 —	—

For species 1—4 a suspension containing 10 mg. spores in 8 ml. water was prepared. For the other species — 20 mg spores in 8 ml. water. In both cases 1 ml. of this suspension was sown into a Petri dish.

Blechnum gibbum behave in a similar way. In the experimental conditions adopted in this work three species: *Dryopteris filix-mas*, *Asplenium trichomanes* and *Asplenium ruta-muraria* did not germinate in darkness; *Polystichum falcatum* germinated in darkness with a great delay.

B. Density of sowing

Spores were sown in various sowing densities on agar plates and were allowed to germinate in light and darkness (Table 1). Spores from high sowing densities germinated later in comparison to spores from low sowing densities. Unfortunately, however, the adopted method, viz. the germination on solid nutrient media, does not permit to express the results quantitatively. For this reason similar experiments with

Fig. 1. Influence of spore sowing density on their germination. x-axis — relative spore sowing density, y-axis — percentage of germination: a — after 3 days, b — after 12 days

various sowing densities were performed on liquid nutrient solutions. The results for *Matteucia struthiopteris* are shown in fig. 1. It can be seen that in the first period the sowing density influences to certain extent the germination rate. After 3 days, when the majority of spores had already germinated, a certain optimal sowing density (0.04) becomes discernible. Too high and too low sowing densities cause a delay in germination. These differences, however, cease to be visible after a time (12 days).

C. Light intensity

Neutral filters, lowering suitably the basic light intensity (3000 lux) were applied in experiments on the influence of the light intensity on spore germination. The results are presented in fig. 2.

They permit to establish that the light intensity assuring a rapid

Fig. 2. Influence of light intensity on spore germination. x-axis — light intensity in lux, y-axis — percentage of germination: a — after 5 days, b — after 12 days, c — after 24 days

germination of fern spores exceeds 300 lux. In very low light intensities germination is inhibited. It may be supposed that a shortage in light supply in the first period may be a factor limiting germination.

D. The length of the daily light period

In experiments on the influence of the length of the light period the germinating spores were exposed daily to 1, 6, 12 and 24 hours of light. In all experiments its intensity was 3000 lux. For the purpose

Fig. 3. Influence of length of the daily light period on spore germination. x-axis — daily light period in hours, y-axis — percentage of germination: a — after 5 days, b — after 12 days

of comparison, one of the culture flasks was kept in complete darkness. The results are shown in fig. 3. A shortage of the time of illumination depresses the percentage of germination. Two factors can be made

Fig. 4. Influence of length of the daily light period on spore germination. The spores received the same amount of light. x-axis as in fig. 3, y-axis — percentage of germination: a — after 5 days, b — after 12 days, c — after 24 days

responsible for this effect, viz. the light intensity and the length of the light period. In this connection the problem arises, which of these two factors is the more important. To settle this problem further experiments were performed in which the spores were exposed to equal light intensities for 1, 6, 12 and 24 hours per day.

The results (fig. 4) suggest that the response shown by the spores is similar to the photoperiodical reaction of higher plants. To induce germination not only a certain amount of light energy is necessary but also illumination of a longer duration. A too short illumination of spores (1—6 hours per the day), even with light of high intensity (3000 lux) is a factor limiting the rate of germination. Spores illuminated with light of the same intensity during 24 hours, however, germinate normally. Insignificant differences in the germination rates were observed when the daily light supply was reduced from 6 to 12 hours.

E. Light and polarity of spore germination

Light also influences the polarity of germinating spores. When light is applied from above in the majority of species (Mohr 1956b) a protuberance appears in the spore on the side turned away from light.

This protuberance — a future rhizoid — is separated from the rest of the cell content by a transversal wall, and is almost free from chloroplasts. The first cell of the filament which contains chloroplasts elongates in the direction opposite to the rhizoid — towards light. The experiments on germination in different light intensities and in darkness have shown that the light intensity influences decisively the sequence of the development of the rhizoid and the first cell of the filament and the direction of their growth. A change of the light intensity causes disturbances in the normal polarity described by Mohr (1956b).

In darkness and in low light intensity in all the germinating spores of *Matteucia struthiopteris* the first cell to appear is a filament cell; the later outgrowing rhizoids are comparatively scarce and short. In light intensity about 3000 lux, about 12% of spores begin to germinate by forming strongly elongated rhizoids.

In suitable light intensities (60—600 lux) disturbances in polarity are observed. Most probably they are connected with the change of the type of germination (fig. 5).

Normally the germinating spores develop simultaneously the first cell of the filament and the rhizoid, sometimes the filament cell precedes a little the appearance of the rhizoid. In some cases a complete change of the polarity of the rhizoid is observed. The rhizoid, as well as the filament, are directed towards light and contain many chloroplasts. Its diameter attains the dimensions of the filament. The highest percentage (11%) of germinating spores with disturbances in polarity was observed for light intensity 300 lux.

A similar developmental anomaly was described by Wettstein (1953) for mosses. In very high light intensities their spores begin to germinate by forming photonegative rhizoids. Initially the rhizoids

Fig. 5. Scheme of the polarity of germinating spore: a — 0—20 lux, b — 60—600 lux, c — 3000 lux

are narrower than the later outgrowing filament and have no plastids. If the spores begin to germinate by developing the filament, the later outgrowing rhizoids will be as broad as the filament. Gradually they become more and more narrow; concomitantly the size of plastids and

the content of chlorophyll decrease. According to Fitting 1950 (after Wettstein 1953) it may be assumed that in the case of mosses an inversion of polarity takes place with the result that the filament develops into a rhizoid.

The above described complete inversion of the polarity of rhizoids, which manifest a positive phototropism and attain the diameter of the filament and its content of chloroplasts, was observed for the first time in germinating spores of *Matteucia struthiopteris*.

DISCUSSION

Some *Pteridophytes* are able to germinate in complete darkness. This ability depends on the species and the growth conditions. According to Life (1907) spores with a higher chlorophyll content show a greater ability of germination in darkness. E.g. spores of *Equisetum* which possess great quantities of chlorophyll show a high germination percentage both in light and in darkness. This is also true in respect of fern spores. Spores of *Matteucia struthiopteris*, which contain comparatively great quantities of chlorophyll, show in darkness a relatively high germination percentage (Tab. 1).

Laage 1906 (after Life 1907) states that many species of ferns are able to germinate in darkness if cultivated on sugar and peptone solutions. The same result was obtained with spores of mosses. Thus, most probably light initiates in germinating spores processes connected with the metabolism of sugars (Herald 1898).

It results from our investigations that only light of a sufficiently high intensity (about 3000 lux) can initiate germination of fern spores. These results corroborate Life's statement (1907) that, in low light intensities, spores of *Alsophila pruinata* are able to develop only to the stage consisting in the bursting of the exosporium. Further stages of germination were completely inhibited. It results from Herald's (1898) work that a dose of light supplied only once, is not sufficient to initiate the germination of spores. Experiments of this author demonstrated that spores which did not germinate in cultures kept in darkness for 12 hours and were then exposed to light for 14 hours, did not germinate any more when taken back into darkness again, in spite of a great content of chlorophyll. A similar phenomenon was established for mosses.

Similar effects were also observed with spores of *Matteucia struthiopteris*. To initiate germination, an illumination of longer duration was necessary. The highest percentage of germination was found after a 5 days long exposition to continuous light.

Similar results were obtained also by Mohr (1956a) for spores of *Dryopteris filix-mas* (L.) Schott.

Light intensity is the main factor determining the polarity of germinating ferns spores. Suitable light intensities can cause disturbances in spore germination resulting in an inversion of the polarity of the rhizoid. In this case the rhizoid attains the diameter of the filament and its chloroplast content. This phenomenon described for the spores of *Matteucia struthiopteris* in this work, has not been as yet established for fern spores.

Mohr (1956b) has observed that the rhizoids grown in blue light of low intensity were strongly elongated and broad with many chloroplasts, but nevertheless were always narrower than the filaments.

A similar disturbance of polarity that was described in this work for fern spores was observed by Wettstein (1953) for spores of mosses.

SUMMARY

1. A study was made on the action of several external factors on the germination of spores of the fern *Matteucia struthiopteris* (L.) Tod. and some other species of ferns.

2. The ability of fern spores to germinate in darkness depends on the species and on the growth conditions. Among the eight investigated species, three do not germinate in darkness, in the others the germination percentage in darkness is much lower than in light.

3. The sowing density of spores is a factor determining to a certain extent the first stage of germination. There is an optimal sowing density assuring the highest percentage of spore germination. Lack of sufficient light supply is a factor inhibiting the germination of spores in too dense sowings.

4. A certain minimum of light intensity is necessary for initiating germination. In too low light intensities a distinct and irreversible inhibition of germination takes place.

5. Illumination of longer duration is necessary to initiate germination. Light of high intensity supplied for a short time does not stimulate germination.

6. Light intensity is a factor determining the polarity of germinating spores. The majority of fern species begins to germinate in light by forming rhizoids. Suitably chosen light intensities induce distinct disturbances in the polarity of germination of the spores of *Matteucia struthiopteris*. The growth of the rhizoid and the basic cell of the filament are simultaneous; the rhizoid presents a similarity of shape with the first cell of the filament and manifests a positive phototropism. A phenomenon of this type was observed for ferns for the first time.

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