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

II. Dependence of germination on the intensity of white light and the role of photosynthesis in this process

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INTRODUCTION

It appears from numerous investigations that light has a decisive influence on germination of spores of mosses and ferns. The decisive role of various photo-receptors (first of all — the phytochrome system) in the complex action of light has been recognized by Mohr and his coworkers.

The spores of *Funaria hygrometrica* do not germinate in darkness, and if, so at a very low percentage — in dependence on the population (Heitz 1942; Kofler 1959; Bauer and Mohr 1959), whereas, in light germination proceeds in a normal way. On the other hand, it is known from occasional observations that too high intensities cause a retardation or even a hold up of the germination process. The present paper aims to investigate the dependence of the germination process of spores of *Funaria hygrometrica* on the light of very low intensities limiting the germination process and of very high ones which inhibit this process. Application of white light of a constant spectrum makes an immediate comparison of intensities, active in both cases, possible and gives grounds for conclusions of ecological character. Germination processes have been considered with regard to changes of respiration and photosynthesis ability in the investigated material.

METHOD

Spore capsules were collected from fairly humid and sun exposed habitats. The material was stored and spores obtained and seeded according to the previously described method (Krupa 1964). Projecting incandescent lamps 250 W and 750 W were used as the light sources. The supplying voltage of the lamps was regulated in such a way as to obtain colour temperature 2850°K of the obtained radiation. This voltage was determined for every lamp by means of multiple comparisons to a standard lamp and accordingly selected on the grounds of the determined ratio of light energy emission in the range 450 nm and 670 nm. A glass Schott filter BG 26 — 6 mm thick with a 10 mm thick water layer was used to change the colour temperature of radiation. The spectrum of the obtained radiation applied

for all illuminations is presented in Fig. 1. The light was directed by means of a lens system into a light proof chamber in which the examined objects were placed. The selection of the optical system depended on the required radiation intensity. In case of low intensities the whole dish (50 mm in diameter) was illuminated. In high light intensities light was concentrated in such a way as to obtain a uniformly illuminated area of 20 mm or 10 mm diameter on the surface of the dish. Neutral filters from black coated bronze nets were used additionally in order to regulate the light intensity. The chamber was provided with a water jacket connected with the ultrathermostate. Experiments were carried out in temperature 29°C ($\pm 1^{\circ}\text{C}$). Light intensity was measured by means of a selene photo-element and expressed in lux. Spores were seeded out on a mineral medium solidified with 1% agar according

Fig. 1. Relative spectrum of radiation (axis Y) applied in experiments — curve 1; curve of the light source energy of colour temperature 2850°K — curve 2; transmission of the applied filters — curve 3

to the previously described method (Krupa 1964). When the illuminated surface was smaller than the diameter of the dish the sow surface was limited by a suitable mask. The formation of a filament of protonema at least $1/3$ of the diameter of the spore length (criterion of the II phase, Krupa 1964), was the basic criterion of germination. In some experiments the course of the I phase was also determined and bursting of the exosporium and green colouring of the spore content (Bauer and Mohr 1959) was the adopted criterion.

Measurements of respiration and photosynthesis were carried out by use of the microrespirometric method (Zurzycki 1955, Starzecki 1961). Photosynthesis was measured in two light intensities 2.5 klx and 10 klx. Determination of the number of spores in the test and the calculation method were given in the previous paper (Krupa 1964). Gas exchange was measured under temperature conditions 29°C .

RESULTS

The dependence of the percentage of germinated spores within 16, 24 and 48 hours on light intensity was presented in Fig. 2. Due to a wide range of light applied in experiments it was possible to determine three main ranges influencing the course of germination in various ways. Within the range of optimum intensities (6–50 klx) full germination (about 100% within 48 hours) was recorded and the

germination rate was not limited by light intensity. In lower light intensities (sub-optimum range) the light intensity checks either the rate of germination (0.8–6 klx) or the final germination percentage (0–0.8 klx). Finally, the supra-optimum range (80–120 klx) exerts a limiting influence on the rate and final percentage of germination. A rapid hold up of this process is observed specially in light above 100 klx.

Fig. 2. Percentage of spore germination (axis Y) in dependence on light intensity (axis X) after: 16, 24, 48 hours according to the II germination criterion

Fig. 3. Respiration intensity (curve 1) and photosynthesis intensity (curves 2 and 3) (axis Y) of spores exposed to light of various intensities (axis X) for 24 hours

Though there is no known intensity causing an absolute hold up of this process it may, however, be concluded from the obtained curves, that this intensity is about 120 klx. The results of measurements of respiration and photosynthesis of spores subjected to germination processes and illuminated with various light intensities for 24 hours were presented in Fig. 3. As it results from the diagram the respiration intensity of spores in the range of light intensity determined previously as an

optimum one (6–50 klx) is constant, though with a slight tendency for increase in the upper part of this range. When spores, on the other hand, are exposed to light of low intensity one may observe a decrease of respiration rate with a rapid drop between 70–100 klx. True photosynthesis shows a greater sensitivity to high light intensities as a considerable drop of the photosynthetic rate occurs already

Fig. 4. Course of germination in light of low intensities: 2, 32, 130, 300 lx. Axis X — time in hours, axis Y — percentage of spores which completed the I ○ or II ● phase of germination (see text).

in the range 40–70 klx. In light of 100 klx the intensity of this process drops practically to zero. Measurements of gas exchange performed in light of 2, 5 and 10 klx (Fig. 3) show that the saturation point of photosynthesis lies very high (above 2, 5 klx). At 10 klx intensity the gas exchange of spores shows only a slight predominance of photosynthesis over respiration. This predominance, which is quite pronounced in the range of low and medium light intensities (0, 8–40 klx), decreases considerably in higher light intensities and in the range above 70 klx photosynthetic production becomes negligible. A comparison of both these processes i.e. course of germination (Fig. 2.) and respiration and photosynthetic ability (Fig. 3) indicates

that there is a certain but not quite strict correlation between them. In the range in which photosynthetic ability is highest (6—40 klx) germination proceeds at optimum rate. A considerable inhibition of photosynthetic ability (40—70 klx) is reflected in a lower rate of germination; as long, however, as respiration is not held up the final percentage of germinated spores indicates full germination.

Fig. 5. Protonema filaments produced in optimum conditions (6 klx) — *a*, and in intensive light (96—114 klx) — *b* and *c*

A great decrease of germination is concomitant with a reduction of the respiration process resulting from a destruction of the respiratory system. In the range of low light intensity (0,8—6 klx) (Fig. 2). there is apparently no correlation between the lower rate of germination and the photosynthetic abilities of the spores. It should be stressed, however, that measurements of gas exchange (Fig. 3) in the range above 10 klx do not indicate to the occurrence of photosynthesis during germination but to potential possibilities. As a matter of fact, in the range of low light intensities, the photosynthetic process is limited not by the efficiency of the photosynthetic apparatus (as it was the case in high light intensities) but by the prevailing light intensities. Thus the reduced rate of germination may reflect the low rate of photosynthesis. In order to determine the processes occurring in low light intensity more precisely observations of the course of germination were carried out in light of 2, 32, 130, 300 lx and changes of the percentage of spores which had completed the I and II phase of germination were recorded in the course of time. It results from the summarized in fig 4 that germination does not occur in darkness. Very low light intensities, however (30—100 lx) are already capable to induce the I phase of germination at a considerably high percentage (40—60%); whereas to induce the II phase of germination light of higher intensity — about 300 lux — is required.

These results suggest that requirements for light conditions are not equal for individual phases of germination; it may be concluded on the grounds of low light intensities inducing the I phase that in this phase the role of photosynthesis seems to be negligible. Light intensity capable to induce a full (though proceeding at a lower rate) germination process equals about 200 lux. The action of high light intensities is not only visible in the reduction of germination rate and percentage of germinated spores but also influences a series of intracellular processes. Microscopic observations show that a hold up of the growth of the protonema after it has attained about 60 μ length proves to be a common phenomenon in the range of 90–114 klx. It is most probably connected with a deficiency of building compounds (see photosynthesis). A hold up of chloroplast divisions (comp. Fig. 5a and b) is also observed. As far as the number of chloroplasts increases greatly in optimum light conditions and chloroplasts move to the growing chloronema cell which becomes intensively green so in higher light intensities division of chloroplasts does not take place. Chloroplasts remain in the sporal part of the cell (see Fig. 5b) or get occasionally discoloured and desintegrated (see Fig. 5c). Extremely high light intensities often cause considerable vacuolisation of the protoplasme of the protonema cell.

DISCUSSION

Intensive investigations carried out recently by Mohr and al. led to the classification of the photobiological systems active in germination of fern spores (review, see Mohr 1963). The usual method applied in these investigations consists in determination of the influence of monochromatic light on germination. In the present investigations the dependence of the germination process on the intensity of white light was examined. This method gives less accurate data concerning the role of individual photobiological systems but it permits to a certain extent to: a) verify the conclusions drawn from investigations with monochromatic light, b) to attain high intensities of radiation and, thus, to examine the influence of a very wide range of light intensities, c) it constitutes the basis for conclusions of ecological nature. In darkness the germination of *Funaria hygrometrica* spores is completely held up or if it takes place so at a very low percentage — in dependence on the plants used for experiments (Heitz 1942; Kofler 1957; Bauer and Mohr 1959). The material used for the present investigations did not germinate at all.

The I phase of germination is induced by white light of very low intensities of the order 10 lux (the percentage depends on intensity) but the II phase takes place only in higher light intensities (above 200 lux). It corroborates Bauer and Mohr's finding (1959) that occurrence of the I phase depends on a low — energetic photo — biological system (Mohr 1963) the phytochrome system. The correlation between photosynthesis and the germination process is not so simple as it appears from comparison in Fig. 2 and 3 of the present paper. In a certain range of intensity (10–50 klx) a coincidence of the optimum course of germination and the maximum photosynthetic ability of the cell is observed. In a lower range the germi-

nation rate is reduced, though, full photosynthetic ability is preserved. In higher intensities (50–90 klx) a very pronounced destruction of the photosynthetic apparatus is connected with a slight decrease of the germination process. The apparent lack of correlation between germination and photosynthetic ability in low light intensity (below 6 klx) has already been explained in the preceding chapter. In this range the really prevailing intensity of photosynthesis — dependent on the light intensity may be a factor limiting germination in spite of full photosynthetic ability of the spores as it results from gas exchange measurements performed in strong light. The above mentioned drop of photosynthetic ability occurs in light of high intensity, (above 50–80 klx) when the respiration ability is fully preserved. This result is in agreement with numerous findings (Steeman Nielsen 1949 and 1952; Biebl 1954; Zurzycki 1955, 1961, 1964) who established that too high light intensities cause, first of all, an inactivation of the photosynthetic apparatus but initially do not affect the respiration system which is only destructed by application of higher doses of radiation. In spite of photosynthesis being considerably checked it has been established that spores exposed to light of e.g. 100 klx for 24 hours germinate in an almost normal way, though, at a lower rate; in this range of light the germination ability seems to be correlated with respiration rather than with photosynthesis. On these grounds a hypothesis may be drawn that the dependence of germination on photosynthesis is rather qualitative and that photosynthetic intensities constituting only a small part of the maximum potential capacity of the cell in this range suffice to maintain germination. The optimum germination not limited in its course by light intensity occurs only in this range in which the effective course of photosynthesis is maximal. The decrease of photosynthesis because of the destruction of the photosynthetic apparatus or because of low light intensity) is connected with a decrease of germination rate. A very low photosynthetic activity, however, suffices for the spores to germinate at a considerably high percentage after a sufficiently long period of time. With regard to this conception photosynthesis as a process of producing organic compounds — does not play a decisive role, anyway in the first stage of germination; it is, however, indispensable to induce some metabolic processes. This hypothesis is supported by a low effective photosynthetic productivity even in optimum conditions (compare Fig. 3) as well as by the fact that fern spores are capable to germinate in darkness without the effect of increase of fresh weight (Mohr and Ohlenroth 1962). Cytological changes taking place in the chloronema cell developed in high light intensity occur only in intensities which considerably inhibit germination (practically an almost complete reduction of photosynthesis and a very great decrease of respiration). This observations are in agreement with numerous data indicating to the presence of a photooxidative chlorophyll decomposition only after a complete hold up of photosynthesis (Montfort 1937; Kandler and Schotz 1956; Zurzycki 1957 and others). In these conditions the hold up of the growth of the protonema should be rather explained by destruction of the cell and not by exhausting of building compounds.

SUMMARY

1. The germination course of spores of *Funaria hygrometrica* has been examined in a wide range of light intensities of a constant spectral composition. Three main ranges influencing germination in various ways have been established.

2. The course of germination is correlated, to a certain extent, with the activity of respiratory and photosynthetic processes. A great decrease of the intensity of photosynthesis causes a decrease of germination rate but only a hold up of respiration causes a drop of the percentage of germinated spores.

3. Two phases depending on light intensity have been distinguished in the germination process. To induce the first phase a very low light intensity (10—100 lux) is necessary; to induce the second phase spores must be exposed to light of at least 200 lux.

4. High light intensities cause destructive intracellular changes with, among others, desintegration and discolouration of chloroplasts and vacuolisation of the protoplasm as their outer signs.

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Badania nad fizjologią kiełkowania zarodników Funaria hygrometrica

Cz. II. Zależność kiełkowania od natężenia światła białego i rola fotosyntezy w tym procesie

Streszczenie

1. Zbadano przebieg kiełkowania zarodników *Funaria hygrometrica* w szerokim (2 lux — 114 klx) zakresie intensywności światła o stałym składzie spektralnym wyznaczając trzy zasadnicze zakresy wpływające w różny sposób na kiełkowanie. Pierwszy zakres suboptymalny (0,8—6 klx) ogranicza tempo kiełkowania jak i ostateczny % wykiełkowanych zarodników. W zakresie intensywności optymalnych (6—50 klx) następuje pełne kiełkowanie i tempo kiełkowania nie jest ograniczane intensywnością światła. Zakres supra-optymalny (80—120 klx) działa hamująco zarówno na tempo, jak i ostateczny % kiełkowania, a szczególnie powyżej 100 klx obserwuje się gwałtowne zahamowanie tego procesu.

2. Przebieg kiełkowania wykazuje pewną korelację z aktywnością procesów oddechowych i fotosyntetycznych. Silne obniżenie intensywności fotosyntezy powoduje zwolnienie tempa kiełkowania a dopiero zahamowanie oddychania obniża znacznie % wykiełkowanych zarodników.

3. Wyróżniono w procesie kiełkowania dwie fazy, których przebieg zależny jest od natężenia światła. Dla zaistnienia fazy I potrzebne jest bardzo małe natężenie światła (30—100 lx), zaś dla wywołania fazy II konieczne jest oświetlenie zarodników światłem o intensywności co najmniej 200 lx.

4. Wysokie natężenia światła powodują destrukcyjne zmiany wewnątrzkomórkowe objawiające się m.in. dezintegracją i odbarwieniem chloroplastów, wakuolizacją plazmy.