BOTANY

INFLUENCE OF DAYLIGHT ON THE FRUITING OF TWO ORANGE-YELLOW PIGMENTED MYXOMYCETE PLASMODIA

BY

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Introduction

In 1938 GRAY established that the yellow pigmented plasmodia of several myxomycetes: *Physarum polycephalum*, *Physarum tenerum*, *Didymium difforme* and *Fuligo septica*, are not able to fruit in absence of light. He obtained sporangia after treatment with diffuse daylight and with artificial light of different intensity.

This investigation deals again with the influence of diffuse daylight on the fruiting of two orange-yellow plasmodia: *Badhamia utricularis* (strain I) and *Physarum polycephalum*. We made use of the flowerpot-method of Miss SCHURE (1949), already mentioned briefly by SOBELS (1950): "Une culture associée de *Badhamia utricularis* (souche I) cultivée à la lumière du jour dans un pot de fleur, suivant la méthode de M.elle SCHURE, développe son plasmode dans les parties ombrées du récipient. Lorsque le plasmode veut fructifier, il se déplace pour former ses sporanges vers les parties plus éclairées."

SCHURE (1949) describes the following method to obtain sporangia of myxomycetes: "The culturing of the plasmodia to obtain fructifications succeeded best when red earthenware flowerpots were used as a basis for the plasmodium instead of a petri dish with agar. For this purpose flowerpots already used for cultivation of plants in the garden were scrubbed clean with a brush, the hole in the bottem was stopped and the pots were kept in water until the pores were entirely filled. The pots were kept moist by placing them in basins containing tapwater which were covered with a glass plate."

The above mentioned method has been elaborated for fructification of two-membered cultures and pure cultures of plasmodia.

Materials and method

New red earthenware flowerpots, about 8 cm high, were washed during one night with running tapwater. The hole in the bottom was stopped with a cork. The dried pots were wrapped up in paper and sterilized (1 hour at 140° C). In our first experiments we used 500 ml beakers of pyrex glass covered with a petri dish, but stray infections were then very frequent (fig. 1). In later experiments each flowerpot was placed in a high

petri dish fitted with a round piece of cellulose, which stabilized the pot and kept it moist. The dimensions of the petri dish were 8.5 cm high and 14.5 cm diameter (fig. 2).

The flower-pots were provided with half a teaspoon of oatmeal and 10 ml unwashed agar (buffered with phosphates on pH 6), or with a teaspoon of dry sterilized oatmeal. Sixty ml of tapwater or double dist. water or diluted phosphate buffer pH 6, (total phosphate M/75 in 1 liter) was poured into each petri dish. Lime containing tapwater was preferred, because these species of myxomycetes produce lime crystals on sporangia and capillitium.

The dishes, closed and wrapped in paper, tied over the top, were autoclaved (30 min. at 120° C).

This method is advantageous, because humidity in the earthenware pot decreases gradually toward the upper edge. The fruiting plasmodium will develop its sporangia wherever optimum moisture conditions for fruiting on the pot exist. Liquid on the bottom of the petri dish kept the pot moist.

The flowerpots were inoculated with a small part of a healthy plasmodium (about 1 cm^2) deposited near the bottom of the pot. The plasmodium spread quickly and moved toward the oatmeal-agar. The cultures were kept one week in darkness. The plasmodia grew abundantly, partly covering the flowerpot and in some cases spreading on the cellulose. The cultures were then placed in diffuse daylight, direct irradiation by the sun was avoided.

Growth and movement of the plasmodia could be watched through the cover of the petri dish, but soon the top became steamy. To prevent condensation a small thermo-element with a circular opening of 10 cm diameter was placed on each cover. Several elements were linked up and fitted with weak current. In this way each cover was heated and remained clear. The heating was so slight that it had no harmful influence on the growth of the plasmodium, but by using the thermo-elements we had to supply the petri dishes with more water (80 ml).

This method was also used with pure cultures; the flowerpots were provided with 10—15 ml unwashed agar, pH 6, and with a sterilized yeast suspension, deposited near the plasmodia.

For the media used, the way of preparing a sterilized yeast suspension and the growth in stock cultures of myxomycete plasmodia on oatmeal-agar we refer to COHEN (1939) and SOBELS (1950).

Experiments and results

Experiment 1, carried out with a wild culture of *B. utricularis* (strain I).

Six flowerpots were provided with oatmeal-agar and placed in double dist. water. They were inoculated on June 23, 1949 with a small piece of plasmodium. The cultures were kept in darkness 12 days; they developed into large plasmodia which spread on the flowerpots.

Then the cultures were brought into diffuse daylight entering from a window. Two of the 4 petri dishes were supplied with 20 ml non-diluted phosphate buffer pH 6. All the plasmodia showed the same reaction, they moved away from the daylight toward the shady side of the flowerpot (both inside and outside).

Fruiting occurred in the cultures buffered on pH 6 after 5 days in daylight, one day later in the dishes with double dist. water. A day before sporangia appeared, the plasmodia changed their reaction toward daylight; they moved to the light exposed sides of the flowerpots, contracted and started fruiting. In most cases the sporangia appeared in a well defined zone just on the border of shade and light in a sickle shaped figure. Sometimes we observed sporangia on the moist cellulose.

At first the sporangia were yellow similar to the orange yellow pigment of the plasmodium. A few hours later the colour darkened into light brown and finally into brown-black. One or two days later mature sporangia were often covered with a white layer of lime crystals. The two plasmodia kept in darkness showed no sign of fruiting.

Experiment 2, carried out with a wild culture of *B. utricularis* (strain I).

This experiment showed similar results as experiment one. The plasmodia were inoculated on May 24, 1949 in flowerpots provided with oatmeal-agar, this time placed in pyrex beakers. The cultures were kept 6 days in darkness and then moved in daylight. After a rather long time sporangia appeared.

Fig. 1 shows the dark sporangia arranged in a sickle shaped figure just on the border of shade and light in the flowerpot. The plasmodium fruited after being in daylight 19 days. The arrow shows the direction of the light.

Experiment 3, carried out with a two-membered culture of *B. utricularis* (strain I), with *Rhodotorula minuta.*

Eight flowerpots were provided with oatmeal-agar and placed in lime containing tapwater. They were inoculated on November 24, 1949 and kept in darkness for 8 days. The plasmodia developed abundantly and were then placed in daylight.

Two cultures fruited in 5 weeks, sporangia appeared on the illuminated sides of the flowerpots. One plasmodium changed into a sclerotium and 3 cultures died. The 2 cultures kept in darkness died without fruiting.

Experiment 4, carried out with a pure culture of *B. utricularis* (strain I) feeding upon agar supplied with a sterilized suspension of *Torulopsis laurentii.*

Six flowerpots were provided with 15 ml unwashed agar pH 6, and placed in 60 ml double dist. water. They were inoculated on July 12, 1949. The plasmodia were supplied with a sterilized suspension of *T. laurentii*, during the first month weekly and then every fortnight.

The first week the cultures were kept in darkness, then they were placed in diffuse daylight. All cultures were affected by an excess of moisture and appeared poor in comparision with two-membered cultures. After 3 months one culture produced a few dark sporangia of normal shape. The spores when inoculated on unwashed agar, developed into a small orange-yellow plasmodium. The remaining 6 cultures died without fruiting.

Experiment 5, carried out with a two-membered culture of *P. polycephalum* with *Saccharomyces cerevisiae* var. *ellipsoideus*. This culture was obtained through the kindness of Dr W. SEIFRIZ, Philadelphia.

Eleven flowerpots were provided with dry sterilized oatmeal, each placed in 80 ml double dist. water and inoculated on July 27, 1950. After one week in darkness the cultures had developed into abundant growing plasmodia, covering the inside and the outside of the pots.

After 7, 8, 9 and 10 days cultures, two at a time, were transferred into daylight at one o'clock. In each case the cultures fruited about 20 hours later. Next morning we could observe the formation of yellow sporangia scattered all over the flowerpots, showing no preference for light or shady side, similar to the spreading of the network of the plasmodial veins. The maturing sporangia darkened slowly and about 7 hours later their colour became brown-black. After 2—3 days of drying some of the sporangia were covered with a white layer of lime crystals. The 3 cultures kept in darkness 16 days did not show any sign of fruiting.

Experiment 6, carried out with a two-membered culture of *P. polycephalum* with *S. cerevisiae* var. *ellipsoideus*.

Six flowerpots were supplied with dry sterilized oatmeal, each placed in 80 ml double dist. water and inoculated on January 9, 1950. After one week in darkness the plasmodia showed good development and were transferred into daylight. In each case fruiting occurred in a few days; sporangia were scattered over the flowerpots on the light as well as on the shady side and on the moist cellulose.

2 cultures fruited after 1 day in the light,

3 cultures fruited after 2 days in the light,

1 culture fruited after 3 days in the light.

Fig. 2 shows one of the cultures which fruited after 2 days in the daylight. Note that some of the mature sporangia are covered with lime crystals.

Experiment 7, carried out with a two-membered culture of *P. polycephalum* with *S. cerevisiae* var. *ellipsoideus*.

Two erlemeyer flasks of 100 ml, the bottom covered with one teaspoon dry oatmeal and about 15 ml tapwater, were autoclaved (30 min. at 120° C). They were inoculated on July 23, 1950. After 16 days in darkness the plasmodia spread upon the glass wall with a dense network of orange-yellow veins.

One culture was transferred into daylight and within 24 hours fruiting occurred. The plasmodium had changed into a dark network, speckled with brownish-black sporangia. The second culture kept in darkness, remained vegetative.

Fig. 3 shows the sporangia of the above mentioned culture. Only very rarely sporangia of *B. utricularis* have been obtained in this way.

Discussion of the results

The orange-yellow plasmodia of *B. utricularis* (strain I) and of *P. polycephalum* form sporangia in diffuse daylight only. The two species react, however, quite differently with respect to daylight.

B. utricularis moves away from the light, toward the shady side of the flowerpot. Just before fruiting the reaction of the plasmodium with regard to daylight changes and it moves to the illuminated side of the pot, (exp. 1—4 and fig. 1). The plasmodium shows a contraction, followed by the formation of sporangia.

The time between the moment of the transfer of the flowerpots into daylight and the fruiting of *B. utricularis* is fairly long and varied. In summer (May, June) it takes from 19—5 days, (exp. 2, fig. 1 and exp. 1), in winter (November) it takes up to 35 days, (exp. 3). In the pure culture which fruited (exp. 4) the time was abnormally long.

Fruiting of *P. polycephalum* occurs almost immediately after exposure to daylight. There is no question of movement of the plasmodium to the shady side of the flowerpot, sporangia appear anywhere on the pot. Apparently the light-intensity is of importance, as is shown by variation in time between transfer to daylight and fruiting. This is shown clearly in the case of *P. polycephalum*. In winter (January) it takes from 1—3 days (exp. 6, fig. 2), in summer (July) it takes about 20 hours (exp. 5).

GRAY (1938) has already established for *P. polycephalum* a correlation between the light intensity and the rhythm of fruiting. With more light fruiting proceeds earlier; this is in agreement with our own results. In July the days are longer, the light intensity is higher and fruiting proceeds faster than in January.

The statement of GRAY (1938): "that myxomycetes, like other organisms, have the power of changing their phototropic responses", agrees with our own observations on *B. utricularis* (strain I). In 1949 GRAY established that the fruiting of yellow plasmodia is more frequent in light than in darkness, whereas light is without any influence on the fruiting of colourless plasmodia. GRAY points out the direct connection between the yellow pigment and the influence of light on the fruiting process, or as expressed in another way, the yellow pigment of the plasmodium is the receptive element to light.

The proper nature of the yellow pigment is hardly known and several authors do not agree in their conceptions (SOBELS (1950)). We can ask what happens in the plasmodium of *B. utricularis* just before fruiting

and also whether the change in phototropic responses has its reflection upon the light sensitivity of the pigment?

Another problem of great importance, independent from the above mentioned questions, is the origin of the process of slow darkening in the maturing sporangia. SOBELS (1950) suggests that the lethal reddening of the dying plasmodium caused by an oxidation under influence of the enzyme tyrosinase and the darkening of the maturing sporangia, are based on analogous processes.

The shape of the sporangia of *P. polycephalum* varies with the humidity of the medium. On the flowerpot the sporangia have distinct stalks. When fruiting takes place on the very wet cellulose, the stalks are much shorter or wanting. The plasmodia are sometimes combined into plasmodiocarp-like clusters. For *B. utricularis* we did not notice the exact shape of the sporangia, often they were close together, only sometimes separate (fig. 1).

These observations agree with COHEN (1942). He studied the shape of the sporangia with regard to the humidity of the medium and observed that with decreasing moisture sporangia develop separately with stalks. With increasing moisture they show transitions to sessile forms and plasmodiocarp-like forms. Perhaps the flowerpot method opens new possibilities for work in this direction.

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Summary

The method of Miss SCHURE in cultivating plasmodia on flowerpots, has been worked out for two-membered and pure cultures.

Light is necessary for the fruiting of two orange-yellow pigmented plasmodia: *Badhamia utricularis* (strain I) and *Physarum polycephalum*. They react however differently upon daylight.

In agreement with GRAY we assume that the yellow pigment of the plasmodium is the receptive agent to light.

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Legend

Fig. 1. A wild culture of *Badhamia utricularis* (strain I) on oatmeal-agar inoculated on May 24, 1949, was kept 6 days in darkness and then moved into diffuse daylight. The plasmodium fruited after being in daylight 19 days. The dark sporangia are close together arranged in a sickle shaped figure just on the border of shade and light in the flowerpot. The arrow shows the direction of the light.

Fig. 2. A two-membered culture of *Physarum polycephalum* with *Saccharomyces cerevisiae* var. *ellipsoideus* inoculated on January 9, 1950, was kept in darkness one week and then brought into diffuse daylight. The plasmodium fruited two days later, sporangia are scattered over the flowerpot on the light as well as on the shady side. Some of the mature sporangia are covered with lime crystals.

Fig. 3. A two-membered culture of *Physarum polycephalum* with *Saccharomyces cerevisiae* var. *ellipsoideus* inoculated on July 23, 1950. The culture was kept in darkness 16 days and then transferred into daylight. The yellow plasmodium fruited within 24 hours and changed into a dark network speckled with brownish-black sporangia.

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