Investigations on the formation of coremia

I. Action of light on the formation of coremia in *Penicillium isariaeforme*

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Numerous investigations on the development and growth of fungi (Carlile 1965; Ingold 1962; Mohr 1961) have been carried out. Neither the chemical nature of the photoreceptor, however, nor the character of light initiating biochemical processes leading to changes in the growth rate and morphogenesis have been, so far, determined with sufficient accuracy. Thus, it seems necessary to examine the light reaction in a large number of species because an analysis of similarities and differences in reaction to light may contribute to elucidate the mechanism of photomorphosis.

Penicillium isariaeforme, which was isolated a few years ago (Stolk 1957) and has not yet been thoroughly examined, is a very photosensitive species. It forms coremia i.e. high coniform formations of mycelium on which sporulating conidiophores appear. The dependence of the structure of coremia on light conditions has already been established for *Penicillium claviforme* (Carlile 1961); this dependence is still more pronounced in *Penicillium isariaeforme*; light initiates the formation of coremia initials and their elongation. The growth of coremia is independent of sporulation, but in *P. claviforme* it ceases immediately after the „head” of the coremium (Carlile 1962) has been formed. In the above quoted paper Carlile mentions that morphogenesis of coremia in light does not always proceed in a typical way. He observed formation of high branched forms, their division etc. More detailed investigations on photomorphosis in *Penicillium isariaeforme* have been undertaken with the aim to investigate the action of white light on the growth of the mycelium and the production of dry mass. Series of observations on differentiation of coremia and morphology of the mycelium have been also carried out.

MATERIAL AND METHOD

The *Penicillium isariaeforme* strain used for investigations was obtained from the Centraalbureau voor Schimmelcultures in Baarn, Holland. Standard cultures were kept on agar with yeast extract. The following medium was applied for the culture of the mould: 50 g glucose, 1 g NH_4NO_3 , 1 g KH_2PO_4 , 1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0,003 g

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 0,001 g $\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$, 30 g agar for 1000 ml yeast extract; pH of the nutrient solution varied from 6,5—7,2.

30 ml of the medium were poured into Petri dishes of 10 cm diameter or into Erlenmeyer flasks 250 ml capacity and subsequently autoclaved for 1 hour at 1 atm. The medium was inoculated by use of a loop of a spore suspension. Cultures were kept in a light or a dark thermostat at $25 \pm 2^\circ\text{C}$. 3—9 fluorescent tubes, 25 W each, emitting white light constituted the light source. In order to obtain a higher light intensity for investigations on the action of light of various light intensities a suitable number of fluorescent tubes was applied. Blackened copper wire filters were applied to lower light intensities. A Zeiss luxmeter was used for measurements of the light intensity.

Table 1

Action of 3 light intensities on the mean growth rate of the coremial mycelium

Light intensity in lux	Mean growth rate in mm/24 hours coremial mycelium	Mean growth rate in mm/24 hours basal mycelium
0	—	3.82 ± 0.16 4.20 ± 0.12 3.90 ± 0.27
1	3.76 ± 0.75	4.48 ± 0.39
450—500	3.34 ± 0.53 3.80 ± 0.60	4.11 ± 0.15 4.70 ± 0.34 4.68 ± 0.19

During development daily observations were made on the morphological changes of the mycelia. The rate of growth of the mycelium was also measured every day in Ryan tubes (Ryan 1943). The daily increments of the mycelia were determined since the moment of formation of the „head” of the growing mycelia (usually 4 days following inoculation). Measurements were performed by means of a slide gauge with an accuracy to 0,1 mm. The height of coremia was measured after 14 days of growth in open dishes placed on the table of a microscope connected with a slide gauge. The microscope was focused on the tip of the coremium and the height was read on the slide gauge. The thickness of the agar layer and bottom layer of the glass dish were subsequently subtraced from this height. Each series of measurements comprised 6—10 dishes and on each dish 10 highest coremia were measured. 2—5 series were carried out for each light intensity.

Greatest difficulties were encountered in determining the dry weights. Comparatively most consistant results were obtained from cultures growing on fluid media; the fungi, however, developed poorly coremia, on the other hand, in spite of a typical structure corresponding to the applied light intensity, they were unusually numerous and delicate. Since it was not possible in this type of cultures to obtain pre-

cise data concerning the heights of coremia, the determination of the dry weights by means of this method was given up.

The mycelia were grown on agar plates and the first method which was tried to isolate the mycelia consisted in melting the agar by heating it to 70°C and in removing the agar by washing the mycelia with hot water. This method however does not exclude the possibility of washing away substances present in the mycelium or even fragments of the mycelium itself. Therefore in the present experiments the mycelia were removed from the agar by means of a sharp scalpel. In the preliminary series the standard error was established. Mycelia were dried in 105°C and weighted subsequently.

RESULTS

I. Action of light and darkness on the development of *P. isariaeforme*

P. isariaeforme growing in darkness forms a compact layer of mycelium (called further on the basal mycelium) on which short, woolly aerial hyphae grow. The mycelium is slightly yellow coloured and covered with sporulating conidiophores.

Fig. 1. *Penicillium isariaeforme* culture growing *a* — in darkness, *b* — in light (500 lux).

Coremia are never formed in darkness (Fig. 1*a*); in 500 lux light intensity *P. isariaeforme* develops coremia — the aerial hyphae elongate considerably, arrange parallel one to another forming coniform structures.

In the mycelia the formation of coremia is preceded by a differentiation of yellow pigmented parts, on the periphery of which in a certain distance coremia are formed. On fully developed coremia only a spore producing layer is formed. During development the coremium apex is free from any spores (Fig. 1*b*) and reacts vigorously to phototropic stimuli.

In light conditions the mycelium manifests a certain differentiation in growth; the growth of the basal mycelium seems to be independent of light, whereas, the growth of the coremial mycelium is initiated by light [and] the hyphae grow parallel to the direction of the incident light. There arises a question whether the rate of growth of the mycelium of both types depends on the light conditions of the

Fig. 2a. The action of light intensity on height of coremia, dry weight of mycelium, sporulation, formation of coremia in *Penicillium isariaeforme*.

Fig. 2b. Scheme illustrating the morphology of coremial mycelium in different light intensities.

culture. Table 1 shows the growth rates of the basal mycelium (Ryan et al. 1943) in darkness and light intensities 1 and 500 lux. The mean rate of growth of the coremial mycelium (growth was measured by method applied for measuring the final height of coremia) is also given in Table 1. The growth rates of the coremial and basal mycelium are identical within the limits of admissible errors and no distinct differences between the growth rates of the basal mycelium in light and darkness were observed.

With regard to different morphology of the mycelia of *P. isariaeforme* grown in light and dark conditions the dry weights of the mycelia were also compared. Results are presented in Table 2.

In spite of great differences in morphological structure no differences in the dry weights of the mycelia developing in darkness and 500 lux light intensity were established. Similarly no difference was observed in the dry weights of another photomorphogenic organism *Aspergillus giganteus* mut. *alba* (Zurzycka 1963) grown in light or darkness. In the case of *A. giganteus* the photosensitive conidiophores

Table 2

Dry weight of the *Penicillium isariaeforme* mycelium
after 12 days of culturing

Dry weight of mycelium in mg *	
light 500 lux	darkness
320 ± 11.1	297 ± 22.3
277 ± 10.2	266 ± 3.2

* The mycelium was separated from the agar surface with a sharp scalpel.

are utterly different regarding development and structure from the *P. isariaeforme* coremioforming hyphae; thus it seemed necessary to give some attention to the dependence of dry weights of the mycelia on the light intensity when investigating the action of light on elongation of coremia.

II. Action of light intensity on growth and development of *Penicillium isariaeforme*

Investigations on the action of light intensity on formation of coremia in *P. isariaeforme* is the subject of the second part of the present work. The following light intensities were applied for the illumination of the cultures: 0, 1, 50, 150, 500, 900, 1200, 2600 lux. The results are summarized in table 3 and fig. 2.

The growth rate of coremia, their final height and the dry weights of the mycelia were measured and the morphology of coremia and sporulation degree were described.

As it has been mentioned previously moulds cultured in darkness produce conidiophores more or less uniformly on the whole surface of the mycelium whereas they fail to form coremia. The average height of conidiophores is 4,7 mm. Sporulation processes begin in approximately 8 days old cultures and are less intensive than in optimum light conditions.

Light of 1 lux intensity causes formation of high aerial hyphae of 23,5 mm average height; the growth rate is very high and the increments for 24 hours attain sometimes 12,5 mm. As a rule in this light intensity cultures fail to fructify. In higher light intensities 50 lux and more — aerial hyphae, which partly associate into still untypically developed coremia, are also formed in the initial phase. In this light intensity coremia attain maximum height, 31,5 mm, and their maximum increment for 24 hours is 5,0 mm.

Table 3

Light intensity in lux	Dry weight of the mycelium in mg	Height of coremia or aerial mycelium in mm	Sporulation	Morphology	Time of growth of coremia or aerial mycelium days
0	310.2	4.5	+++	low coremial mycelium	—
1	221.3	23.5	—	high loose aerial hyphae	7
30	—	31.4	++	high loose aerial hyphae	
50	385.0	31.5	+++	formation of few coremia	9
500	424.6	39.0	++++++	atypical coremia with apical brush	11
900	382.0	25.4	++++++	typical coremia conidial apex without brush	8
1200		16.4	++++	typical coremia conidial apex without brush	5
2600	189.0	3.9	++	very few typical coremia	2

Sporulation is very intensive and takes place all over the surface of the coremium. Maximum height of coremia is attained in 500 lux and coremia are typically developed; there is however a delicate brush on the top part of the coremium formed of loose hyphae. Maximum increment for 24 hours is 6,0 mm; final height of coremia 39,0 mm; abundant and uniform sporulation takes place on the whole surface of the dish. Doubling the light intensity (800—900 lux) causes a certain decrease of the growth rate (3,50 mm for 24 hours) and the final height of coremia (25,4 mm). The apex is also built in a different way. There are no more free hyphae which accrete to one another but a firmly accreted conus. Sporulation is also less intensive than in the previous light intensity (500 lux). The increase of light intensity causes a gradual decrease of the coremium length (1200 lux — 16,4 mm).

The most marked inhibition of the growth rate of the coremia was observed for 2600 lux; the coremia attain only 3,9 mm height; this means that in this case coremia are even lower than conidiophores in darkness. Coremia, though very small, are in this light intensity developed in a roughly typical way. Mycelia, however, present a morbid appearance. This permits to conclude that from this light intensity starts its destructive action.

Mean dry weight values of the mycelia developing in various light intensities are presented in table 3. The dry weights of the mycelia, which are considerable in dark-

ness, decrease when the mould is cultured in very low light intensities. The dry weight increases concomitantly with light intensity, and exceeds even values obtained for dark cultures. The highest intensity inhibits again the formation of the dry mass of the mycelium.

DISCUSSION

Penicillium isariaeforme shows a pronounced spatial differentiation of its structure under the action of light. The final morphological structure (Fig. 1 and 2) is the result of the cooperation of two factors: elongation and differentiation (sporulation) and both these processes are governed by different light intensities. Even a very low light intensity (1 lux) causes the appearance of elongation centres on the basal mycelium. On the other hand the growth of this basal mycelium is more or less uniform for a large range of light intensities. An aerial mycelium is rapidly formed around these centers.

In low light intensities a high elongation rate is observed (maximum 24 hours increment attains even 12,5 mm) though the mean value for the period of 16 days is considerably lower. This apparent discrepancy is caused by the limited period of elongation, as this process comes to an arrest in cultures 10 days old. It may be that the energy requirement for elongation processes in this light intensity is so high that it finds its reflection in changes of the general metabolism of the mould. This change also could explain the decrease of dry weights of the mycelia grown in low light intensities. A balance between metabolism and elongation processes is soon established and both these processes increase proportionally to the light intensity up to 500 lux. In this range of light intensities the maximum rate of growth is about 6 mm for 24 hours and the mould continues to grow till about the 14 th day of culturing. Sporulating coremia are found on the whole surface of the dish. This range of light intensities (1—500 lux) is the range with the predominance of elongation processes; beyond this range, for higher light intensities the elongation processes are gradually inhibited and arrested at 2600 lux.

In the range 400—900 lux an other process — differentiation of coremia and fructification — takes place (Fig. 2). It seems that within a large range this process is independent of the intensity of the stimulus.

In *P. isariaeforme* we come across at least two different photobiological processes overlapping each other and separable only in low light intensities (Fig. 2) by an inhibition of differentiating processes, or in very high ones by a complete arrest of growth.

Investigations on the action of light intensity on morphological processes in fungi were carried out mostly for a few fixed light intensities. Elongation in low light intensities was described by Borris (1934 a and b) for *Coprinus lagopus*. He called it „etiolation”. A similar phenomen was observed also in gigant conidiophores of *Aspergillus giganteus* mut. *alba* (Zurzycka 1963). In this mould a shifting of balance from sporulation and differentiation in favour of etiolation is also observed in low light intensities. On the other hand some data reported in li-

terature suggest that the formation of reproductive organs occurs only on the condition that appropriate light intensity for the given species has been applied. (Borris 1934 a and b for *Coprinus lagopus*; Plunkett 1956, 1953 for *Polyporus brunnalis*, and Koch 1958 for *Polystictus ostreatus*).

A similar phenomenon was observed by Rakoczy (1962) who investigated the fructification of myxomycetes.

As it has already been mentioned differentiation processes of the mycelium take place probably around certain centres in which morphological changes occur leading to an acceleration of growth and subsequently to the formation of coremia. This process is, on the other hand, conditioned by the ability of the mycelium to a parallel arrangement. This process seems to be more sensitive to the action of external factors, especially light. Besides, this process seems to be reversible at least up to a certain differentiation stage (i.e. to the moment of spores formation). Therefore it will be the subject of further more detailed investigations.

SUMMARY

1. A study was made on the action of light intensity on the dry weight of the mycelia of *Penicillium isariaeforme*. The dry weight increases with the light intensity so long as this intensity does not exceed a certain limit (about 900 lux). Higher light intensities have an inhibiting action on the production of dry mass.

2. Already very low light intensities are sufficient to start the elongation of coremia; the elongation is inhibited by light intensities exceeding about 900 lux.

3. Sporulation and the complete formation of ceremia is conditioned by light intensities exceeding 500 lux. The sporulation process is less sensitive to the inhibiting action of high light intensity than the elongation process.

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Badania nad wytwarzaniem koremiów

*I. Wpływ światła na tworzenie koremiów u *Penicillium isariaeforme**

Streszczenie

1. Zbadano wpływ intensywności światła na suchą masę grzybni *P. isariaeforme* i stwierdzono, że wzrasta ona do pewnej intensywności światła. Światło 2600 luksów wpływa hamująco na produkcję suchej masy.
2. Elongacja koremiów rozpoczyna się w bardzo słabym natężeniu światła; powyżej 900 luksów proces wydłużania się koremiów ulega szybkiemu zahamowaniu.
3. Proces zarodnikowania i pełnego wykształcenia koremiów jest uwarunkowany intensywnością światła powyżej 500 luksów. Podlega on w znacznie mniejszym stopniu niż elongacja hamującemu działaniu wysokich intensywności tego czynnika.