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Investigations on the action of light on the growth and development of *Penicillium claviforme* Bainier

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The section *Penicillium clavigerum* comprises three species: *Penicillium clavigerum, Penicillium claviforme,* and *Penicillium isariaeforme* (Raper and Thom 1949; Stolk and Meyer 1957). The development of coniform structures — the so called coremia — is characteristic of the species of this section. Sporulating conidiophores are formed either on the ends of the coremia (*P. clavigerum* and *P. claviforme*) or on the whole surface (*P. isariaeforme*). An interesting relation betwen differentiation and growth rate of the coremia on the one hand and the action of light on the other has also been observed within this section. *P. clavigerum* is not sensitive to the action of this stimulus (Carlile et al. 1962). During the development of *P. claviforme* there is a period during which the coremia are sensitive to light; this is manifested by an acceleration of growth and an ability to phototropic reaction (Carlile et al. 1961). In this species the photoreaction soon dies out and the formation of an apical sporulating layer ("the coremial head") is significating the completion of the photosensitive phase. *P. isariaeforme* shows a much more marked dependence on light conditions; coremia are formed only in light and sensitivity to this stimulus — expressed by an increased growth rate and a phototropic reaction — remains till the end of the development period (Carlile et al. 1962, Piskorz 1967).

Photobiological investigations made on species of the *P. clavigerum* section have shown (Piskorz 1967) that in *P. isariaeforme* a certain range of white light intensity determines before all the elongation of hyphae whereas other — comparatively high ranges — induce the formation of coremia; in middle ranges both these processes overlap.

Because of the specific structure of coremia in this species and its sensitivity to various external factors, the quantitative evaluation of the whole of photomorphogenetic phenomena proved, in the initial stage at least, extremely difficult. Differences in the coremial structures in *P. claviforme* presented a possibility of a more exact quantitative elaboration of photomorphosis in this section.

MATERIAL AND METHOD

The *Penicillium claviforme* Bainer clone as well as the clones of *A. clavatus* Desm., *A. giganteus* Wehm. and *P. clavigerum* Dem. were obtained from the Centraalbureau voor Schimmelcultures in Baarn, Holland. The author is specially indebted to dr A. Stolk from the Centraalbureau for making the last species available.

Details about culture conditions, standard cultures and nutrient solutions were given in a previous paper (P i s k o r z 1967).

The present paper summarises the results of a study on the effects of two physical factors — light and temperature — on the growth and development of *P. claviforme*.

L i g h t. In the light thermostat 8 fluorescent tubes, 25 W, emitting white light constituted the light source. The light intensity in the lower range was modified by means of blackened wire copper filters. Higher intensities were obtained by diminishing the distance between the illuminated dishes and the light source. A continuous illumination and temperature 25°C were applied. Light intensity was measured in lux by means of a Zeiss luxmeter. The culturing period was 7 days and the following intensities were applied: 0, 30, 130, 300, 900, 1800, 2300, 2500, 2700 lux.

T e m p e r a t u r e. A cooling box (Krakus 1600) with 8 light thermostats was adapted for investigations on the influence of temperature. The temperature of each thermostat could be maintened on a constant level within the range 5—25°C. The light intensity was 1100 lux. The culturing period varied; sporulation of the coremial head was the criterion indicating the completion of development.

The influence of light and temperature was estimated by measuring the height of coremia, their number per unit of the mycelial area, by making macroscopic and microscopic observations on the morphology of the coremia, and by determining dry weights of the coremia and mycelia.

T h e h e i g h t o f c o r e m i a. Two methods were initially used to measure the height of coremia. One of them, applied previously, consisted in measuring the difference of levels between the basal mycelium and the top of coremia; in the second case the length of a coremium was measured after it has been detached from the mycelium. This length was obtained by measuring separately the length of the head and foot and by taking the sum of these values. Considering that the results obtained by means of both methods were much the same and measurements of coremial parts were necessary to evaluate the changes in the structure of coremia preference was given to the second method. Difficulties encountered in the application of both these methods and the risk of desiccation of the coremia also decided about the choice of the method.

It should be stressed that a coremium is a well differentiated structural unit of the mycelium and detaching it from the basal mycelium does not increase the measurement errors. Measurements of the length were performed under a microscope equipped with an objective 5 × and an MB ocular 8 ×; the results were expressed in microns.

Macroscopic morphology. As it was already mentioned the coremium is differentiated into two parts: the basic one called "the coremial foot" and the part forming the base for sporulation, the so called "coremial head". The length of the foot and head, the breadth of the head and the foot at its base and just below the base of the head were determined. The results were used to describe the shape of coremia formed in various light intensities.

Microscopic morphology. Longitudinal sections of the coremium were made for each of the applied light intensities in the range 0—2300 lux. Preliminary fixations trials were performed by means of the Nawashin's (Mäckel 1928; Filutowicz and Kużdowicz 1951) fixative in normal and diluted concentrations with or without addition of Tween 20. Both modifications of the Nawashin's fixative with addition of a substance decreasing surface tension proved to be most efficient and were applied in further work. The coremia were dehydrated, embedded in paraffin, cut into 20 μ thick slides and stained with gentian violet. Measurements of the parts of coremia were performed by means of a 5 × objective and 15 × M eyepiece. For the observations and measurements of cell structures an 90 × immersion objective and an 15 × M eyepiece were used.

The number of cells was calculated on mycelial discs, 3,14 cm^2 in size, cut out in four places in one dish. The number of coremia was counted and the results expressed as the number of coremia per 1 cm^2 of the mycelium grown in given light and thermal conditions. These operations were preceded by an preliminary study on the effect of density of sowing on the number of formed coremia since a possibility existed that the number of coremia might depend on the sowing density. The basic suspension contained 20×10^6 spores (measurements in Thoma's chamber, Romiejs 1953). The effect of the following dilutions: 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-9} was tested. The sowing density proved to be without importance in this kind of investigations.

Dry weight of coremia and mycelia. Dry weights of coremia were determined by choosing 5 samples each with 10 coremia from the given experiment and drying them at 105°C. The dry weight of the mycelium was determined in cultures grown on nutrient solutions solidified with agar. In this case mycelial mats could be removed from agar without encountering the difficulties in remowing the *P. isariaeforme* mycelium.

Experiments were performed in three replications (only in exceptional

cases two) on 15—20 dishes or on 40 erlenmayer flasks. Mean values were calculated; in the majority of cases also the mean error according to the known formula.

Preliminary, relative and approximate determinations of the content of pigments in the coremia were performed by means of extraction with destilled water, ethanol, methanol, acetone and acetone + 5% glacial acetic acid. Methanol proved to be the best eluent. Light absorption of extracts was always determined for 565 mg fresh weight of coremia (the weight of coremia from a half of the dish) from cultures grown in darkness or cultures exposed to 700, 900 and 2300 lux. Coremia were immersed in methanol at 4°C for 24 hours, homogenized and the filtered extract was filled up to a constant volume 50 ml. A Hilger Uvispek spectrophotometer (with a glass prism) was used to measure the absorption of the solutions in the range 400—800 nm. Only mean values of extinction in the range 400—500 nm are presented in the graphs.

RESULTS

I. ACTION OF LIGHT ON THE GROWTH AND DEVELOPMENT OF COREMIA

The action of light on the growth and development of coremia of *Penicillium claviforme* is characterized by:

1. The height of fully developed coremia after sporulation of the head.
2. Macroscopic and microscopic structure of ripe coremia.
3. The number of formed coremia per unit of the mycelium area.
4. Dry weights of coremia and mycelia; all the measurements were determined for cultures exposed during growth to the following intensities of white light: 0, 30, 130, 300, 900, 1800, 2300, 2500 and 2700 lux.

I. 1. The height of coremia in dependence on light intensity

The results are presented in Fig. 1. and Tab. 1. They show that already very low light intensities (30 lux) suffice to induce a considerable elongation of the coremia; in light their height increases almost three times in comparison with darkness. Maximum height is attained in light of about 130 lux intensity and maintains on this level up till about 1800 lux; higher and increasing light intensities cause a gradual decrease of their height.

A comparison of the results obtained for *P. claviforme* with those for *A. giganteus* mut. *alba* (Zurzycka 1963) and *P. isariaeforme* (Piskorz 1967) shows a similar relationship between the elongation

of the conidiophores resp. coremia and the light intensity. Even very low light intensities stimulate the activity of the growth mechanism. The final values of elongation in optimum light conditions are variable and depend, most probably, on a number of factors, before all on genetic factors. For comparative studies it is necessary to introduce a magnitude characterizing the light growth reaction of the species in question in relation to other species which react to this stimulus in a similar way. This magnitude was called the elongation index. Under this term we understand the ratio of the height of the fructification attained in

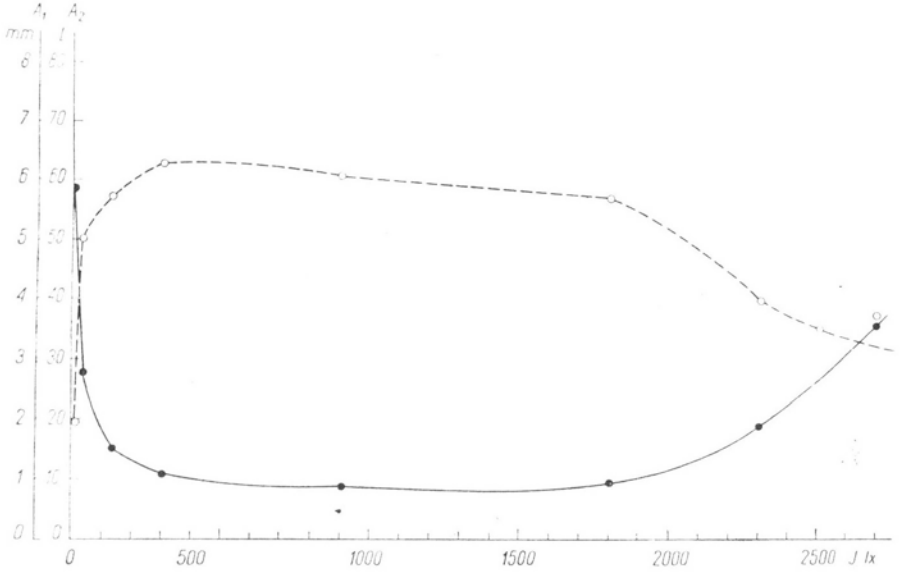

Fig. 1. Action of light intensity on the height and the number of coremia per 1 cm². Abscissae — light intensity in lux. Ordinates: A_1 — height in mm; A_2 — number of coremia. ● — number; ○ —height

Table 1

Influence of light intensity on the structure of *Penicillium claviforme* coremia

Light intensity in lx	Height of coremia in mm	Length of head in mm	Breadth of head in mm	Length of foot in mm	Breadth of foot in mm	
					Top	Base
0	1.93	0,77±0,02	0,77±0,01	1,16±0,04	0,27±0,01	0,58±0,03
30	5,08	1,73±0,03	1 08±0,03	3,15±0,08	0,50±0,07	1,16±0,03
130	5,74	1,89±0,05	1,21±0,04	3,85±0,07	0,60±0,03	1,33±0,04
300	6,26	2,23±0,05	1,12±0,04	4,04±0,10	0,64±0,02	1.27±0,07
900	6,09	2.23±0,07	1,02±0,03	3,87±0,08	0,57±0,03	1,48±0,06
1800	5,64	1,81±0,05	0,98±0,04	3,83±0,05	0,60±0,03	1,46±0,04
2300	3,97	1,25±0,10	0,95±0,03	2,72±0,06	0,60±0,03	1,06±0,04
2500	3,47	1,08±0.05	0,99±0,04	2,39±0,05	0,58±0,03	0,98±0,05
2700	3,68	1,56±0,05	0,94±0,03	2,12±0,09	0,40±0,02	1,10±0,06

optimum light conditions (350—450 lux) to the final height of the same fructifications formed in darkness. The elongation indexes of representatives of the section *P. clavigerum* and *A. clavatus* are recorded in Table 2. Within the section *A. clavatus* the elongation index of *A. clavatus* only is close to unity, the indexes of the two other species are much greater and rather approximate. Within the section *P. clavigerum*, on the other hand three organisms with increasing elongation

Table 2

Elongation index of species of the *Asp. clavatus* and P. *clavigerum* section (growth temperature 25°C±1°C; light intensity 350—450 lx)

Species	Height of conidiophores in mm		Elongation index
	Light	Darkness	
Section A. clavatus			
A. clavatus	1,4	1,0	1,4
A. giganteus	12,2	1,6	7,6
A. gigant. mut. alba	22,4	2,5	8,9*
Section P. clavigerum			
P. clavigerum	1,8	2,4	0,79
P. claviforme	6,3	1,9	3,3
P. isariaeforme	39,0	4,5	8,7**

*) Zurzycka, 1963
**) Piskorz, 1967

indexes are found; the value of the elongation index of *P. isariaeforme* is close to that observed for *A. giganteus* resp. *A. giganteus* mut. *alba*. In the range of high light intensities *P. isariaeforme* and *P. claviforme* behave in a different way. Light of about 2000—2700 lux almost completely inhibits growth in *P. isariaeforme*, and coremia attain the height of conidiophores formed in darkness; the behaviour of *P. claviforme* coremia grown in such conditions calls the phenomenon observed in *A. giganteus* mut. *alba*. High light intensities inhibit the growth of coremia but to a certain limit only. It may be thus supposed that either the growth system in *P. isariaeforme* is different from the photoreacting system in *P. claviforme* and *A. giganteus* mut. *alba*, or that additional systems reducing the sensitivity of the mould to higher light intesities are active in both latter organisms. The similar responses to the action of weak light shown by the fructification growth is an argument militating against the first supposition. The adoption of the second explanation requires the demonstration of the presence of a hypothetic pigment system protecting the organism against excessive radiation. A red pigmentation — more intensive in light — occurs in the coremial foot of *P. claviforme*. Extractions were made from mycelia exposed

during growth to light intensities of 0, 700, 900 and 2300 lux (for the details see methodical part). Absorption curves (Fig. 2) show that a group of pigments with an absorption spectrum very similar to that presented by the extract obtained by Schneiderhöhn (1945) for *Coprinus lagopus* is found in the coremial foot. Extinction values for wave lengths in the range 400—500 nm evidently depend on the light intensity applied during the development of the coremia; in the highest tested intensity

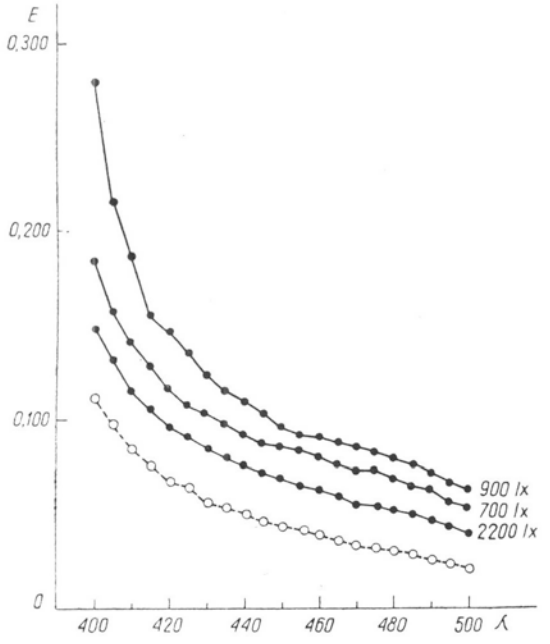

Fig. 2. *Penicillium claviforme*. extinction curve of pigments extraced from coremia, d = 1 cm
● — light; ○ — darkness

the content of pigments drops considerably. This drop may be caused by their decomposition by an excessive light intensity. Thus, theoretically one could assume the existance in *P. claviforme* of a system protecting the growth system; this problem, however, requires further investigations.

I.2. Macroscopic and microscopic structure of mature coremia

The dependence of the shape of coremia on the light intensity is schematically shown in Fig. 3. Schemes are based on data presented in Table 1.

As it has already been mentioned, coremia developing in light and darkness (Fig. 4). demonstrate a morphological differentiation into

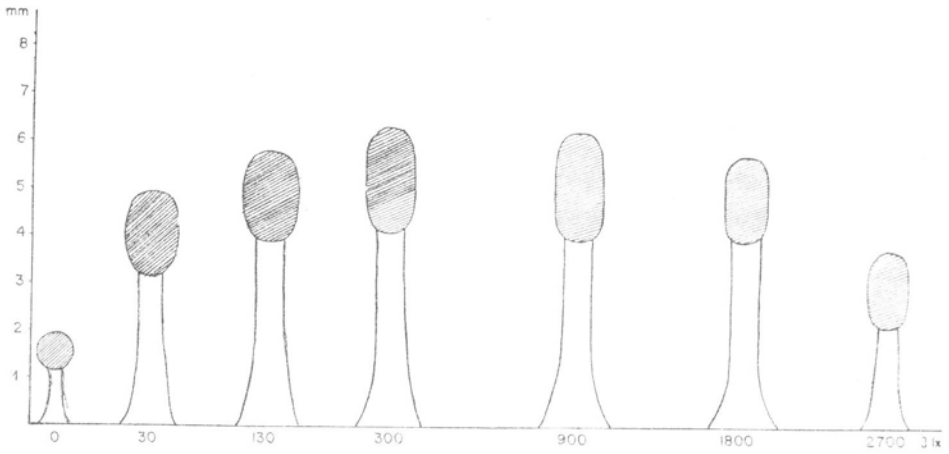

Fig. 3. Action of light intensity on the morphology of coremia (scheme).

a part deprived of spores — the foot — and a part bearing spores — the head. (Fig. 4a, 4b). Bidirectional changes due to the action of light are clearly visible in the scheme. A coremium from dark cultures has a short delicate foot with a round head at its end. The morphology of a coremium changes unter the action of increasing light intensity; its

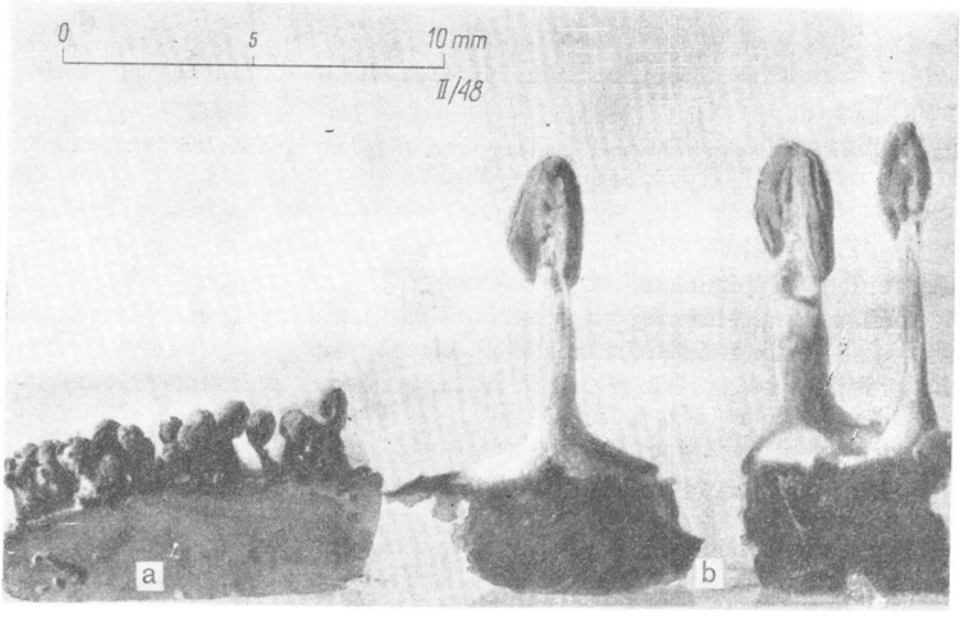

Fig. 4. *Penicillium claviforme;* coremia grown in darkness (*a*), and in light, 900 lux. (*b*).

foot elongates and becomes gradually thicker; the sporulating head is cylindrical in shape. This shape is maintained in a large range of light intensities. Highest intensities inhibit, to a great extent, the elongation of the foot but do not interfere with the formation of the spore producing layer. Similar phenomena are also observed in *P. isariaeforme* (P i s k o r z 1967) and in *A. giganteus* mut. *alba* Z u r z y c k a 1963 b). On the grounds of the coremial growth curve and the schemes the existence of such a high light intensity may be postulated in which — owing to a destructive effect of light on the growth system — the height of coremia will be identical with that of coremia grown in darkness, their shape, however, will be that corresponding to the „light" form. The breadth of coremial structures depends on light intensity at a much lower degree.

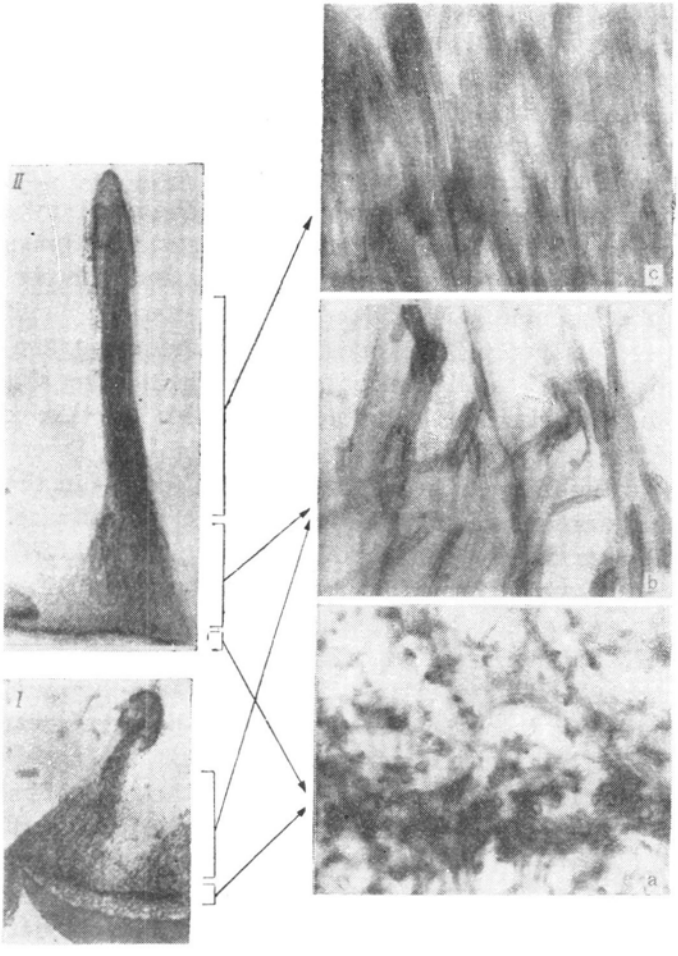

Fig. 5. Microscopic structure of a coremium of *Penicillium claviforme*

a) Basal mycelium; b) coremio-forming mycelium; c) elongation mycelium. Longitudinal section of a coremium grown in darkness, I and in light, II.

Table 3

Microscopic structure of the coremial foot, longitudinal section

Light intensity in lx	Basal mycelium in μ	Coremial mycelium in μ	Elongation mycelium in μ
0	75,9	852,8	172,6 (atypic)
30	83,2	956,8	1986,4
130	72,8	962,0	3064,9
300	72,8	1031,7	2376,4
900	70,7	1043,1	2646,8
1800	74,8	1021,2	2184,0
2300	71,8	903,7	874,6

Microscopic measurements added a series of interesting data to the macroscopic observations. It should be stressed that special attention was given to the part of a coremium which is able to elongate i.e. to the foot.

Coremia in light and darkness begin to develop in the basal mycelium. This is a layer of thick walled interlaced cells forming an elastic mat. The structure of the basal mycelium does not show any dependence on light conditions of the culture. In some parts of the basal mycelium aerial hyphae grow out of it and form a hemispheric structure (macroscopically the lower part of the foot) in which the tightly and disorderly arranged mycelial hyphae become loose and take a characteristic claviform shape. The height of this part of the mycelium — the elongation part — is almost independent of the light conditions (Table 3) being slightly higher in light than in darkness. Negligible differences are also observed in the shape of the cells which form this layer (Table 4) Essential changes appear only in the highest part of the mycelial foot — in the elongation mycelium. In darkness this part is developed in an untypical way: it is thin, its cells are loosely and chaotically distributed and the number of layers of cells which form this part does not exceed 4—5. Although no difficulties were encountered in determining the size of the cells, the estimation of their number, on a given length was practically impossible as the hyphae overlap each other. Thus, the number of cells in the elongation layer was calculated by dividing the mean length of the whole layer by the mean length of the cells.

Light induces very pronounced changes in the elongation mycelium. Already low light intensities suffice to stimulate efficiently the development of this part; microscopic changes occuring in it are the cause of macroscopic elongation of the whole coremium. Contrary to expectation the elongation of individual cell is insignificant, though not negligible; the diameter of the cylindrical cells increases as well. It follows from the calculation of the mean volume of the cells forming the elongation layer and grown in various light intensities that this factor, within the

Table 4

Mean dimensions of the cells of the coremio-forming and the elongation mycelium

Light intensity in lx	Coremio-forming mycelium in μ			Elongation mycelium in μ		Volume of the cells of the elong. myc. in μ	Ratio of the length of the elongation mycelium and the length of cells = number of cells of the elongation mycelium
	Breadth upper part	Breadth lower part	Length	Length	Breadth		
0	7,6	4,9	38,5	38,6	3,3	329,6	4,5
30	7,8	3,9	40,9	56,3	4,8	1017,9	35,3
130	7,4	4,2	41,4	68,8	4,6	1142,8	44,5
300	8,1	4,8	42,7	60,1	4,6	998,3	39,5
900	8,7	5,2	41,8	53,4	4,6	888,6	49,6
1800	8,4	5,1	40,3	42,5	4,5	883,9	51,4
2300	7,8	4,8	37,5	38,3	4,4	581,8	22,8

range of optimum intensities (to 900 lux), causes an approximatively threefold increase of the cell volume compared with dark conditions.

The magnitude, however, which undergoes the most essential changes is the number of cells. This number increases almost tenfold. This process seems to be less sensitive to light than the processes which lead to an increase of the cell size (Table 4).

In light the cell growth in the elongation layer seems to be the result of an increase of the cell number and size. In this connection the question arises how these phenomena are reflected in the increase of dry weight of the coremia.

I.3. Dry weight of coremia and mycelia

Table 5 presents the dependance of the dry weight of coremia on the light intensity. Concomitantly with the growth of coremia and the development of the elongation layer the dry weights of these structures proved to increase. Thus, changes induced by light are not only connected with changes in water content but also, and before all, with an increase of the amounts of metabolic products. Hence arises a question: whether the increase of coremial dry weight is the consequence of an activation of the metabolism in situ (in the coremia), or the result of a migration of metabolites from the basal mycelium to the coremia. In the latter case light would be reduced to the role of a directing agent. In the first case an increase of dry weight of the whole mycelium developed in light compared with that grown in darkness should be expected. In the second case the dry weight should be independent of the light conditions. It results from the determinations of dry weights of mycelia grown on liquid and solid media (Table 6) that the second alternative is the more probable i.e. the basic metabolism does not undergo any changes. Changes

in the number of coremia developing in various light conditions support this alternative considering that the heavier the coremia the fewer in number are they formed by the mycelium.

Table 5

Dry weight of coremia of *P. claviforme* in dependeance on the light intensity

Light intensity in lx	Dry weight of 10 coremia in mg
0	2,95±0,11
30	3,92±0,11
130	7,80±0,12
300	7,95±0,90
900	8,42±1,10
1800	6,95±0,01
2300	3,54±0,22
2500	3,06±0,11

Results obtained for *P. isariaeforme*, however, can not be neglected. An increase of dry weight of the mycelium was established for light intensities causing optimum elongation (Piskorz 1967). Let us recall that in *Penicillium isariaeforme* the elongation layer is almost 4 cm thick and forms the whole coremium. In *P. claviforme* this layer is small in size and the coremia are not numerous — and therefore differences in

Table 6

P. claviforme, dry weight of 7 days old mycelia

Temperature	Nutrient medium	Dry weight of the mycelium in mg	
		Light 900 lx	Darkness
25°C	solid	408,3±15,5	411,2±5,8
25°C	liquid	443,1±6,9	449,5±9,2
20°C	solid	437,6±15,3	445,3±14,4

dry weight — because of the great weight of the basic mycelium — are to slight to become detectable by means of the dry weight measurements; similarly such differences could not be detected in *A. giganteus* and *A. giganteus* mut. *alba* (Zurzycka 1955, 1963).

I.4. Action of light intensity on the number of coremia formed on a unit of mycelial area

The results from this series of experiments are plotted in Fig. 6. and 7. They suggest that light intensity influences not only the height and shape of coremia but also their number. The highest number of

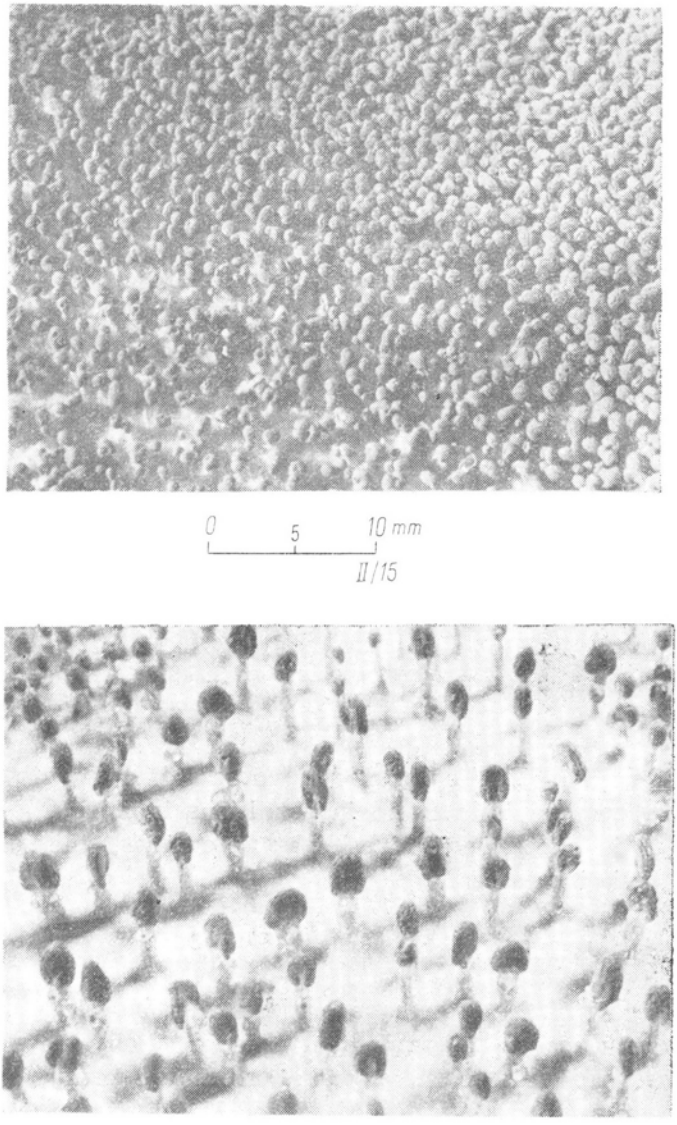

Fig. 6. Number of coremia per unit area formed in darkness (above), and in light, 900 lux (below).

coremia was found in mycelia developing in darkness (59/cm²). In light intensity 30 lux this number drops to 28/cm². An increase of light intensity causes a further drop of the number of formed coremia and in the optimum range for elongation this number varies from 12 to 8/cm². A second increment of the number of coremia up to 36/cm² is caused by a further increase of light intensity (2300—2700).

It results from a comparison of the curves in Fig. 1 and 6. that there is a close correlation between the elongation of coremia and their

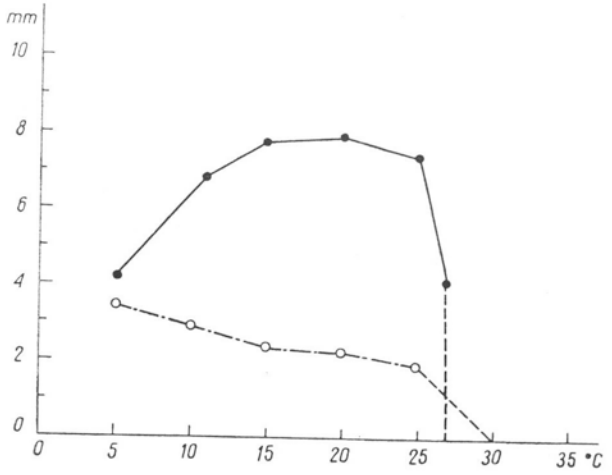

Fig. 7. Action of temperature on the coremial height of *P. claviforme*
○ — darkness; ● — light

number per 1 cm². In light intensities favourable for growth processes the number of coremia is low and in conditions inhibiting growth, i.e. in darkness or in excessively high light intensities, the number of developed coremia increases.

II. ACTION OF TEMPERATURE ON FORMATION OF COREMIA IN *P. CLAVIFORME*

The second part of the present paper deals with investigations on the action of temperature on the growth of coremia, their dry weight and number formed per unit of the mycelial area. The experiments were performed on cultures grown in the dark and in light intensity 1100 lux respectively. The following temperatures were applied: 5, 10—11, 15, 20, 25, 27 and 30°C. The results are shown in Table 7 and Fig. 7 and 8.

II.1. The growth of coremia in various temperatures

The growth of coremia in various temperatures is presented in Fig. 7. The height of coremia increases slightly with the decrease of temperature of the culture. Highest coremia were found in 5°C. This fact, however,

Table 7

Influence of temperature on growth and development of coremia of *Penicillium claviforme*

Temperature	Number of coremia per cm²		Height of coremia in mm		Length of head in mm		Length of foot in mm		Dry weight of 10 coremia in mg	
	Light	Darkness	Light	Darkness	Light	Darkness	Light	Darkness	Light	Darkness
5°C	16,9±0,80	6,8±1,1	4,20	3,46	1,93±0,04	1,20±0,04	2,26±0,03	2,26±0,04	5,6±0,4	4,6±0,0
10°C	22,8±0,7	32,9±1,4	6,85	2,97	3,31±0,02	1,33±0,04	3,54±0,05	1,64±0,03	4,5±0,4	2,5±0,2
15°C	27,5±0,8	48,1±3,4	7,78	2,30	3,72±0,02	0,89±0,03	4,06±0,02	1,41±0,03	4,0±0,1	2,2±0,2
20°C	14,1±1,0	96,5±8,3	7,94	2,25	2,82±0,05	0,90±0,02	5,12±0,02	1,33±0,03	7,6±0,5	1,5±0,1
25°C	7,0±0,5	58,7±2,7	7,40	1,93	2,70±0,03	0,77±0,02	4,70±0,06	1,16±0,02	7,0±1,8	3,0±0,4
27°C	5,9±0,3	—	4,09	—	1,16±0,03	—	2,93±0,04	—	2,9±0,6	—
29°C	0,0	—	0,0	—	0,0	—	0,0	—	0,0	—
30°C	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

can be attributed to abnormal growth considering that this temperature is the factor limiting the development of *P. claviforme*. In light cultures in the temperature range 11—25°C the elongation of coremia is almost independent of this factor. In extreme temperatures 5° and 27°C, a marked drop of the height of coremia is observed. This is most probably connected not only with an inactivation of the growth system but also with a worse development of the whole mould. A thermal inactivation of the growth system seems to take place in temperatures above 27°C. This is suggested by the fact that the coremial initials do not grow into normal coremia. A decrease of temperature stimulates the growth; this indicates that the thermal inactivation is at least partly reversible.

II.2. Macroscopic structure of coremia and their dry weight

Data concerning the macroscopic structure of coremia are based exclusively on measurements of the lengths of the coremial head and foot. It results form Table 7 that in darkness the lengths of the head and foot slightly increase concomitantly with the drop of temperature. This increase, however, is so small that no greater importance can be ascribed to it. As far as the head is concerned, a tendency to an increase of the spore-forming area is observed concomitantly with a decrease of the growth temperature both in light and in darkness.

On the grounds of measurements of coremial dry weights it may be only stated that optimum dry weights occur in temperatures which are optimum for the elongation of coremia. In darkness the dry weight slightly changes (the extreme temperature of 5°C excepted); in light it shows a course more or less analogous to that of the coremial elongation. Q_{10} (from 11°C on) equals about 1,8.

II.3. Action of temperature on the number of coremia

The number of coremia formed both in light and darkness depends on temperature in which cultures developed. The optimum temperature for the number of coremia formed in darkness is 20°C (Fig. 8). In other temperatures rapid drop of the number of coremia is observed; it is more pronounced in higher temperatures than in lower ones. A complete inhibition of the growth of mycelium is caused by temperature 30°C; spores germinate but are unable to form a compact mycelial mat. A drop of temperature induces immediately a normal development of the mycelium and coremia. In light the number of developed coremia is considerably lower than in darkness at corresponding temperatures. The maximum is also shifted towards lower temperatures and occurs already at 15°C. Above and below this temperature a drop of the number of

coremia is recorded; this drop being considerably smaller in lower temperatures. It results from the graphs in Fig. 8, that light „mitigates" to a certain extent the effect of temperature. This is specially pronounced in extreme temperatures. At 5°C the mycelium develops faster and the number of formed coremia is higher than in darkness. At 30°C the formation of islets of a delicate basal mycelium with slightly marked

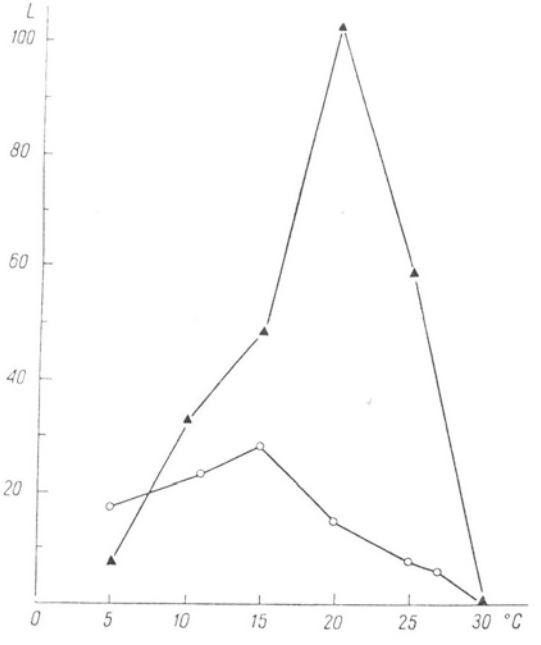

Fig. 8. Action of temperature on the number of coremia per 1 cm². △ — darkness; ○ — light

coremial initials which never grow into normal coremia is observed. As in dark cultures the normal growth is inhibited but not injured in an irreversible way. In this case, too, coremia start normal development after the culture has been transferred to lower temperature. It follows from these facts that the thermal limits of growth and development of *P. claviforme* are rather narrow: 5°—28°C, the optimum thermal range being 15—20°C.

DISCUSSION

The development of *P. claviforme* is markedly influenced by light conditions and temperature. Light acts in two ways: it is the factor controlling the number of developing coremia and their final height.

The number of coremia is already determined in a very young mycelium by processes leading to the formation of coremial initials, whereas growth is determined by an increase in size and number of cells forming the so called elongation layer of the coremium.

Metabolic processes leading to the differentation of a certain number of coremia, determined by light and temperature conditions, occur, without doubt, in the basal mycelium. Despite many efforts we were not successful in finding morphological features distinguishing the hyphae with the initials in the basal mycelium. The only, microscopically discernible, feature is the formation of a kind of centers of more densely interwoven hyphae among the other hyphae in the basal mycelium. Thus far, no data concerning biochemical processes leading to the formation of light induced initials of fructification are available. A morphological criterion of differentiation of trophocysts was established only for *Pilobolus* (Page 1956, Ingold 1962). In this way the action of light on this process could be studied disregarding the further development of sporangiophores. From the action spectrum and investigations on inhibitors a conclusion can be drawn that in *Pilobolus* riboflavine is the photoreceptor responsible for the formation of trophocysts. Nothing is known, however, on biochemical processes initiated by this photoreceptor in the cells of fungi (Ingold 1952).

Morphological changes shown by the cells of the elongation layer — such as the increase of the size and the number of its cells and the concomitant shifting of the fructification time of coremia exposed to light — present many similarities with the changes observed with the cells of *Blastocladiella emersonii* (Cantino and Horenstein 1957, Cantino and Turian 1959). This fact can be the starting point for further researches on biochemical processes controlling this mechanism, provided that in *P. claviforme* it is confined to the elongation layer exclusively. Changes in dry weight of coremia of *P. claviforme* and specially *P. isariaeforme* can be interpreted as consequences resulting from changes in respiratory processes similar to changes occuring in *Blastocladiella* which also lead to an increase of dry weight in light. It will be interesting to carry out some comparative investigations within the species of the *P. clavigerum* section considering that *P. clavigerum* does not react to light, *P. claviforme* shows elongation changes only within a certain layer of the mycelium (the elongation layer), whereas in *P. isariaeforme* the whole coremium is able to elongate; as a matter of fact, this coremium is formed by one layer only, viz. the elongation layer.

It remains to consider the relation between the processes occurring in the basal mycelium and the elongation of the coremia. Two hypotheses may be advanced. According to the first the primary photobiological process consists in changes occurring in the basal mycelium, the

elongation being a secondary process resulting from the fact that the whole metabolism of the mould is switched to a "light" type. The other alternative is that two parallel biochemical processes are induced by light. It is not possible at the present moment to solve this problem. The first hypothesis is supported by the following facts: 1. no differences are recorded between the total dry weights of mycelia from dark and light conditions; 2. for a given light intensity, there is a close correlation between the number of coremia and their final height: the higher the coremia the lesser is their number per unit area. These facts suggest that a constant level of metabolites is maintained in the mycelium; these metabolites are used either to elongate the coremia, or to increase their number. If, however, the mould is first allowed to grow in darkness and to form the correspoding number of coremial initials and later exposed to light, then the coremia elongate and attain more or less the height which is specific for the applied light intensity. The hypothesis of two photobiological processes occurring in parallel is supported by the fact noted by Carlile (1951) that the removal of the head of a differentiated coremium induces new elongation processes in the foot.

It follows from our research that the temperature which is most favourable for the development of the mycelium and specially of the coremia of *P. claviforme* is shifted towards lower values. Temperature is, besides light, a factor controlling the number of developing coremia. The effect of this factor is partly attenuated by light. Temperature alone, however, without the cooperation of light, is not able to induce the growth of the elongation layer, and the final heights attained by the coremia seem to be, almost, independent of the temperature of growth. This is another argument speaking for the existence of two separate photobiological processes in *P. claviforme*. Extreme temperatures, specially supraoptimal ones inhibit the growth processes but do not — probably — injure irreversibly the growth mechanism.

The obtained results do not permit to give precise answer to the question how the sporulation mechanism is influenced by light and temperature. In some fungi, specially in parasitic ones, (Cruickhank 1962, Leach 1962) light in the visible spectral range is a factor inhibiting sporulation. If the number of coremia formed per unit of the mycelial area is the measure of the sporulation intensity in *P. claviforme* then it can be stated that light causes a considerable decrease of sporulation. The decrease of the number of coremia is always associated, however, with an increase of the sporulation area. The irregularity of this surface does not permit a numerical evaluation of the increase of its area.

The light type of coremia of *P. claviforme*, as in *P. isariaeforme* (Piskorz 1967) is maintained even in the highest intensities of light. In contrast to the results obtained in our study on *P. isariaeforme* we

could not determine exactly the lower limit of light intensity below which the formation of light coremia is prevented. Even the lowest intensity applied in this study (30 lux) induced the appearance of the light type of coremia.

SUMMARY

The paper summarizes the results of a study on the action of light, in the intensity range 0—2700 lux, on the development and growth of *P. claviforme*.

1. The effect of light intensity in the range 0—2700 lux on the development and growth of *P. claviforme* has been examined. It has been established that in light the mould develops coremia characterized by a high foot and an elongated head; the coremia grown in darkness are shorter and ended by a globose head.

2. Microscopic observations on the morphology of a coremium showed that light induces the differentiation of an elongation layer — the elongation mycelium — from a scarce and untypically developed mycelium. In the optimum light intensity range the cells of this layer increase in size (2—3 times) in comparison with darkness and their number in one layer increases about 10 times. The basal and coremia forming mycelia are either insensitive to action of light or at a small degree.

3. The maximum height of the coremia is attained within the range 100—1800 lux. Above and below these limits gradual growth inhitibion is observed.

4. There is a inverse and close relationship between the number of coremia formed per unit area and their height. The better the elongation conditions the smaller the number of coremia.

5. Dry weights of mycelia grown in darkness and light do not show any significant differences; changes in the dry weights of the coremia, however, are parallel to changes in their height.

6. Growth and development of coremia in a given light intensity depend also on the temperature of development. Optimum thermal conditions for the development of *P. claviforme* are between 15—20°C.

7. Temperature cannot replace light in the elongation process of coremia; the height of coremia in the range 15—25°C is independent of the growth temperature. The relationship between the number of coremia and temperature is similar for mycelia grown in light and darkness; however, in the temperature range 15—20°C considerably fewer coremia are formed in light than in darkness.

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Badania nad wpływem światła na rozwój i wzrost Penicillium claviforme Bainier

Streszczenie

1. Zbadano wpływ intensywności światła w zakresie 0—2700 luksów na rozwój i wzrost *Penicillium claviforme*. Stwierdzono, że na świetle wytwarza się typ o wysokiej nóżce i wydłużonej główce, w ciemności koremia są krótkie z okrągłą główką.

2. Morfologia mikroskopowa koremium wykazała, że z nieznacznej i nietypowej grzybni tzw. elongacyjnej wytwarza się na świetle warstwa elongacyjna, której komórki w optymalnym zakresie intensywności powiększają swe rozmiary i objętość (2—3x), a także zwiększa się ich liczba w piętrze (10x) w porównaniu z ciemnością. Grzybnia podstawowa i koremiotwórcza nie podlega lub podlega w nieznacznym stopniu działaniu światła.

3. Optymalna wysokość koremiów występuje w zakresie 100—1800 luksów. Powyżej i poniżej tej wartości następuje stopniowe zahamowanie wzrostu.

4. Istnieje ścisły związek pomiędzy liczbą koremiów a ich wysokością. W optymalnych warunkach elongacji liczba koremiów jest najmniejsza.

5. Całkowite suche masy grzybni hodowanej na świetle i w ciemności nie wykazują zasadniczych różnic, natomiast sucha masa koremiów zmienia się równolegle ze zmianami ich wysokości.

6. Wzrost i rozwój koremiów w danej intensywności światła zależy również od temperatury hodowli. Optymalne warunki termiczne dla rozwoju *Penicillium claviforme* przypadają na temperatury 15°—20°C.

7. Temperatura nie może zastąpić światła w procesach elongacji koremiów; wysokość koremiów w zakresie 15—25° jest niezależna od temperatury hodowli. Zależność liczby koremiów na świetle i w ciemności od temperatury ma podobny charakter, aczkolwiek na świetle w temperaturze 15—20° tworzy się ich znacznie mniej.