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THE EFFECTS OF THE CULTURE MEDIUM AND LIGHT ON THE PIGMENT-SYNTHESIS AND SPORULATION IN ASPERGILLUS AND PENICILLIUM GENERA*

JUDIT HORVÁTH and I. SZALAI

Department of Plant Physiology, Attila József University, Szeged

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Summary

A study was made of the pigment-synthesis and sporulation of *Aspergillus repens, Penicillium notatum* and *Penicillium purpurogenum* on

1. Czapek-Dox,
2. Czapek-Dox supplemented with microelements,
3. potato agar, and
4. modified Czapek-Dox

culture media, illuminated with white, blue, green, orange-yellow, red and periodic light, or in the dark.

1. The experimental results showed that *Penicillium notatum* does not exhibit photosensitivity. The pigmentation and sporulation of *Penicillium purpurogenum* and *Aspergillus repens* are significantly inhibited by blue light, and to a lesser extent by green and orange-yellow light, while they are enhanced by red light and by darkness compared to the white light control.

2. The sporulation of *Penicillium notatum* and *Aspergillus repens* are delayed by blue light, accelerated by the dark, and unaffected by green, orange-yellow and red lights compared to the white control. There is no difference for *Penicillium purporogenum.*

3. In red illumination and in the dark the length of the conidiophores of *Aspergillus repens* decreases.

4. The vitality of the spores from the younger zones of the colony is greater than that of the spores in the older zones.

5. As regards the culture medium effects, Zn delays the pigmentation and sporulation in all three species, most strongly in *Penicillium purpurogenum,* and least in *Aspergillus repens.*

6. The zonation is inhibited by the presence of Cu in the culture medium, and enhanced by that of Mn. Zn apparently plays no role in the development of the zonation.

7. With regard to the nitrogen sources, the species studied utilize best the NH_4^+, less well the NO_3^-, and slowest of all the nitrogen of urea.

* Part and thesis from the diploma-paper of Miss MÁRIA TÓTH.

Introduction

It is generally characteristic of the entire vegetable kingdom that the reproductive organs are more richly pigmented than the vegetative organs. In the higher-order plants the varied display of colours of the flowers is provided by the anthocyanins, the various flavone derivatives and the carotenoids. Colonies of sporulating fungi are just as rich in colour, and differently pigmented from the mycelium. The question arises of whether there is a correlation between the individual stages of the sporulation, the colour contents of the pigments and their intensities. It has long been known that a whole series of pigments can be found in the various fungi. However, far less is known of the varied forms of the pigments or of their physiological roles than, for example, in the higher-order plants, in spite of the fact that many papers have been published on this theme (BESSEY, 1904; CARLILE, 1965; EBERHARD et al., 1961; NARASIMHACHARI, 1963). Even less in known of the connection between the appearance of the pigments and the sprorulation, which also justifies the study of these problems.

The strains of the genera *Aspergillus* and *Penicillium* selected for the experiments are noted for being rapidly and easily cultivated, for their abundant sporulation, and for their variably coloured pigments. On the basis of these three aspects, preliminary experiments on 10 *Penicillium* and 20 *Aspergillus* strains led to the selection of the following species:

Penicillium purpurogenum	(787)*
Penicillium notatum	(190)
Aspergillus repens	(–)

In accordance with the aims of the experiment, a study was made of:

a) The effects exerted on the sporulation and the pigmentation accompanying the sporulation by culture media differing essentially from each other in composition.

b) The effects exerted on the sporulation and pigmentation by the individual monochromatic light regions for strains cultivated on the same culture medium.

c) The intensity of the sporulation in both cases, i.e. as a function of the culture medium and the light. Our results were then compared with previous literature findings.

The reality of the problem raised is supported by a number of literature findings. Neurospora crassa conidium never contains carotinoid if it grows in the dark, but light promotes carotinoid synthesis in the mycelium (ZALOKAR, 1954).A number of literature data indicate that the photo-effect also depends on composition of the culture medium (CARLILE, 1965; MUNTANJOLA et al., 1968). For example, when *Penicillium clavigerum* is cultivated on malt agar it is insensitive to light, and grows with the same intensity in light or darkness; however, in 12-hour illumination daily on Czapek agar the rate of growth remains the same, whereas in 24-hour illumination it decreases. On the other hand, *Penicil-*

* The numbers in brackets denote the strain cultures maintained in the Viticultural and Oenological Research Institute.

lium claviforme requires light at the beginning of its development, until the mycelia have attained a length of some mm, but after this it becomes, apparently insensitive to the illumination (CARLILE, 1965). In contrast, *Penicillium isariforme* is sensitive to light throughout its entire life (CARLILE et al., 1962). These examples show that even species belonging to the same genus react in totally different ways to light, at times depending on their state of development, at others on the composition of the culture medium; thus, the sensitivity or insensitivity of the species to light can not be correlated with the kinship. It is unfortunate that extremely few literature data can be found with regard to the effects of light and culture medium on the sporulation. In the present paper new approaches are made and new relationships are sought, and hence there are few possibilities to compare our results with literature data.

Not only the pigmentation, but also the sporulation is affected by the nature of the light and the composition of the culture medium. The development, presence and absence of the fruit bodies, the spores and the conidia can be affected by light, while in addition their form and differentiation too depend on the illumination. *Aspergillus aureolatus* grows well in an appropriate nutrient medium in the dark, and the sporulation too is maximum, whereas in light the conidial formation is poorer (MUNTANJOLA et al., 1968; CARLILE, 1962). It must be mentioned, however, that the data of papers dealing with sporulation and light conditions are not only different, but in many cases contradictory, and thus it is at present not possible to give a uniform picture of this question. Several authors (FRIEDERICKSEN and ENGEL, 1960; LUKENS, 1963; MOHR, 1961) have stressed that sporulation is affected only by the short-wave region, the longer waves being ineffective.

The aim our studies is to attempt to decide between these contradictory data, and to provide extra information in this field of plant physiology, which in many respects is still unknown. It is assumed that the pigmentation is a fundamental factor, which influences not only the sporulation, but also the morphogenesis of the fungi.

Materials and Methods

1. Experimental objects

The species and strains used in the experiment were made available by the National Viticultural and Oenological Research Institute. They were maintained on potato agar culture medium in test-tubes, under a protective layer of paraffin oil, and always these accurately determined strains were used in the transoculations for multiplication.

2. Culture media

The following culture media were used to study the effects of the media:

a. Czapek—Dox solid culture medium, to which the sugar was added only before the final sterilization.

b. Czapek—Dox solid culture medium supplemented with microelements in the following four variations:

(1)	Basal nutrient medium (a) + $MnSO_4$	0.0025	g
(2)	Basal nutrient medium (a) + $ZnSO_4$	0.0025	g
(3)	Basal nutrient medium (a) + $CuSO_4 \cdot 5H_2O$	0.0025	g
(4)	Basel nutrient medium (a) + $MnSO_4$	0.001	g
	+ $ZnSO_4$	0.001	g
	+ $CuSO_4$	0.001	g

c. Potato agar culture medium (SZALAI and FRENYÓ, 1962).
d. Modified Czapek—Dox culture medium in three variations, containing 3 g $(NH_4)_2SO_4$, 3 g NH_4NO_3 or 3 g urea in place of $NaNO_3$ as source of nitrogen.

The culture media were freshly prepared, and after adjustment to the appropriate pH 30 ml of culture medium was poured into Petri dishes 10 cm in diameter.

After sterilization the culture media were stored for 3—4 days in thermostats at room temperature, and only those were used for inoculation which remained sterile. Under the usual sterile conditions, infection-free cultures were attained in 80% of the transoculations.

3. Cultural procedure (incubation)

The cultural conditions were varied according to the aims of the experiment. The cultures were placed in thermostats, kept in the dark, and developed at 26—28 °C (± 0.2 °C). In order to study the effect of light, the cultures were placed in a climatic chamber the temperature of which was 22—23 °C (± 1 °C).

The climatic chamber could be illuminated with light enriched in monochromatic light by the use of exchangeable white, red, orange-red, green and blue discharge tubes.

4. Study methods

The pigmentation and sporulation in the cultures were observed for 12 days following the transoculation.

Data recorded included the number, dimensions and development of the pigmentation of the concentric rings (zonation).

The development of the sporulation was followed in part with the naked eye, and in part by cytoscope, and was supplemented with microscopic measurements. For the exact performance of the measurements a squashed preparation was formed in the usual way (SÁRKÁNY and SZALAI, 1966), and the diameters of the spores, the lengths and diameters of the conidiophores and the diameters of the vesicles were measured with an ocular microscope.

Experimental results and observations

One aim of the work was to determine the changes induced in the sporulation of the fungi by the composition of the culture medium and the nature of the light. Attention was paid to:

a) the beginning and course of the pigmentation of the mycelium;
b) the time and course of the sporulation;
c) the structure of the zones formed during the sporulation.

The pigmentation of *Penicillium purpurogenum* on Czapek-Dox agar in various monochromatic lights

1. In white light the pigmentation of the mycelium begins visibly on the lower side of the culture medium on the fifth day, and proceeds elliptically outwards. The mycelial mass is wine-red in colour, and the pigment diffusing out into the culture medium is a vivid purple. The sporulation begins in the centre of the colony on the eighth day and proceeds outwards in rings, and on the tenth day the entire colony is sporulating (Table 1). The zonation (Fig. 1) is not definite (it is indistinct), and proceeding autwards fromt the centre: dark grey (0.6 cm) → greenish-grey (1.5 cm → light brown (0.5 cm) greyish-brown (0.6 cm).

2. In blue light the pigmentation in the mycelium begins on the sixth day, but the characteristic purple colour develops more slowly than in white light. Only an extremely small amount of pigment diffuses out of the mycelium into the culture medium, and therefore this is a pale pink colour. The sporulation in blue light begins only on the tenth day (Table 1). The sporulation is accom-

panied by zonation (Fig. 1). Seven zones develop: dark grey (0.2 cm) → brownish-grey (0.3 cm) → black (0.3 cm) → brownish-grey (0.4 cm) → dark grey (0.6 cm) → brownish-grey (0.3 cm) → light grey (0.3 cm).

3. In green light the pigmentation in the mycelium begins on the fifth day, with a vivid purple colour. The diffusion out of the pigment into the culture medium is less significant than in white light, but stronger than in the culture grown in blue light. The sporulation begins on the ninth day (Table 1). Ring formation occurs (Fig. 2), and four zones develop: dark grey (0.6 cm) → brownish-black (0.7 cm) → grey (0.8 cm) → greenish-grey (0.9 cm).

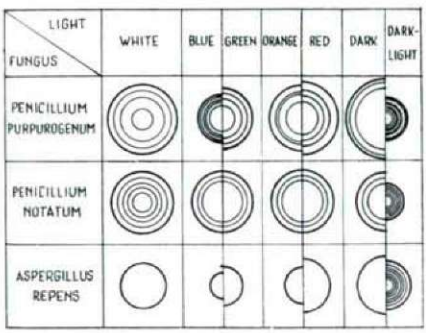

Figure 1. Effect of light on the formation of the sporulation rings

4. In orange-red light the pigmentation in the mycelium begins on the fifth day with a wine-red colour, which diffuses out abundantly into the culture medium. The sporulation begins on the ninth day, the zonation is insignificant and the colour differences and zone boundaries are not marked (Table 1). There are four zones (Fig. 1): dark grey (0.5 cm) → grey (0.8 cm) → dark grey (0.9 cm) → grey (0.8 cm).

Table 1. Zonation, pigmentation and sporulation of *Penicillium purpurogenum* in different monochromatic lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	4	5	8
Blue	7	6	10
Green	4	5	9
Orange	4	5	9
Red	4	5	8
Dark	3	5	7
Periodic	12	6	9

5. In red light the pigmentation of the mycelium begins on the fifth day, and pigments diffuse out into the culture medium. The sporulation begins on the eighth day, i.e. earlier than in cultures illuminated with other monochromatic lights (Table 1). The zonation during the sporulation is weak (Fig 2).

Four zones develop: greenish-grey (0.7 cm) → brownish-grey (0.6 cm) → dark grey (0.9 cm) → brownish-grey (0.9 cm).

6. The pigmentation in the mycelium of a colony grown in the dark begins on the fifth day, and diffuses out very intensively into the culture medium. The sporulation begins on the seventh day, i.e. the earliest of all (Table 1). Three very weakly developed zones are formed, with indistinct boundaries (Fig. 1): brownish-grey (3.1 cm) → light brown (0.2 cm) → brownish-grey (0.3 cm).

7. In periodic illumination the pigmentation of the mycelium begins on the sixth day, and resembles the phenomena seen in white light. The sporulation begins on the ninth day (Table 1), the zonation is intense (Fig. 1), and there are 12 zones: brown (0.2 cm) → grey (0.2 cm) → brown (0.2 cm) → light brown (0.2 cm) → greyish-brown (0.2 cm) → light brown (0.2 cm) → brown (0.2 cm) → greenish-grey (0.2 cm) → dark grey (0.2 cm) → greenish-grey (0.2 cm) → light brown (0.2 cm) → grey (0.2 cm).

Pigmentation in *Penicillium purpurogenum* in constant white light on culture media of various compositions

1. On a Czapek-Dox agar culture medium containing Mn the pigmentation of the mycelium is already significant on the fifth day; the colour is wine-red, but only the orange-yellow colour-component diffuses out into the culture medium. The sporulation begins on the eight day. The ring formation is very

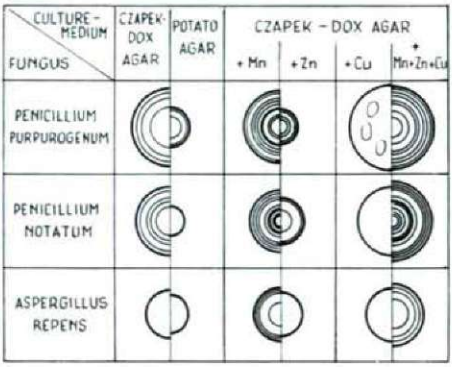

Figure 2. Effect of composition of culture media on the formation of the sporulation rings

sharp (Table 2), with nine zones (Fig. 2): grey (0.2 cm) → greenish-grey (0.3 cm) → brown (0.4 cm) → greenish-grey (0.3 cm) brown (0.4 cm) → grey (0.3 cm) → brown (0.4 cm) → light brown (0.2 cm) → brown (0.5 cm).

2. On a Czapek-Dox agar culture medium containing Zn a vivid purple pigmentation of the mycelium appears on the seventh day, and a purple colour also diffuses out into the culture medium. The sporulation can be observed only on the tenth day (Table 2). Five sharply separated zones can be distinguished in the sporulation (Fig. 2): grey (0.2 cm) → light brown (0.3 cm) → brownish-grey (0.5 cm) → pink (0.2 cm) → grey (0.6 cm).

3. On a Czapek-Dox agar culture medium containing Cu the pigmentation in the mycelium appears on the fifth day. A very strong purple colour diffuses

out into the culture medium. The sproulation appears on the eight day; rings are not formed (Table 2), but a colour difference develops on the sporulation surface, and violet-brown spots appear in the brownish-grey conidial field (Fig. 2).

4. On a Czapek-Dox agar culture medium containing Zn, Mn and Cu the pigmentation appeared on the fifth day, and the sporulation on the eighth day. The sporulation exhibits a strong zonation, but the strong zonation effect of the culture medium containing only Mn is not attained. There are seven rings (Fig. 2): greyish-brown (0.3 cm) → brown (0.3 cm) → grey (0.4 cm) → brown (0.5 cm) → brownish-violet (0.5 cm) → grey (0.6 cm) → brownish-grey (0.6 cm).

5. On potato agar culture media the yellowish-red pigmentation of the mycelium can be observed on the sixth day (Table 2), the culture medium becoming pale red. Three zones develop: greenish-grey (1.0 cm) → ligth brown (0.2 cm) → greenish-grey (0.2 cm).

Table 2. Zonation, pigmentation and sporulation of *Penicillium purpurogenum* on various culture media containing microelements

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (K)	4	5	8
Czapek + Mn	9	5	8
Czapek + Zn	5	7	10
Czapek + Cu	2	5	8
Czapek + Mn + Zn + Cu	7	5	8
Potato agar	3	6	9

The pigmentation of *Penicillium notatum* on Czapek-Dox agar in various monochromatic lights

1. In white light the pigmentation of the mycelium can be observed on the third day; a pale yellow pigment is synthetized, which diffuses into the culture medium. The sporulation begins on the fifth day (Table 3). Five rings develop in the sporulation (Fig. 1): yellow (1.2 cm) → brownish-grey (0.3 cm) → light brown (0.3 cm) → dark brown (0.5 cm) → greyish-yellow (0.6 cm).

2. In monochromatic light the orange-yellow pigmentation of the mycelium begins in every case on the third day; a lemon-yellow pigment migrates out of this into the nutrient medium. The sporulation begins on the fifth day, with a yellowish-grey colour, and separates into three zones (Fig. 1). The greyish-yellow conidial field (3.2–3.5 cm) can be found at the centre, followed by a light brown ring (0.3 cm), and finally by a brownish-grey zone (0.8 cm) (Table 3).

3. In the dark the pigmentation of the mycelium is already very intense on the third day, and a very concentrated orange-yellow material diffuses out of the mycelium inti the culture medium. The sporulation appears on the fifth day, and three zones develop (Fig. 1), the colours of which are the same as

those of the cultures illuminated with monochromatic light. At the centre there is a greyish-yellow zone 3.2–3.4 cm in diameter, surrounded by a 0.3 cm thick light brown ring, with outside this a 0.8 cm thick brownish-grey ring (Table 3).

Table 3. Zonation, pigmentation and sporulation of *Penicillium notatum* in various lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	5	3	5
Blue	3	3	5
Green	3	3	5
Orange	3	3	5
Red	3	3	5
Dark	3	3	5
Periodic	8	4	6

4. In periodic illumination the pigmentation begins on the fourth day, and a lemon-yellow pigment diffuses out of the orange-yellow mycelial mass into the culture medium (Table 3). The sporulation begins on the sixth day, and well separated zones develop in the conidial field. There are 8 zones (Fig. 1): light brown (1.0 cm) → brown (0.2 cm) → dark brown (0.2 cm) → greyish-brown (0.4 cm) → light brown (0.2 cm) → bluish-grey (0.3 cm).

Pigmentation and sporulation of *Penicillium notatum* in constant white light on culture media of various compositions

1. On Czapek agar culture medium containing Mn the pigmentation can be well observed on the third day; this is orange-yellow, and a lemon-yellow material diffuses out of it into the culture medium. The sporulation begins on the fifth day (Table 4), and 11 zones develop (Fig. 2): light brown (0.2 cm) → orange-yellow (0.3 cm) → greyish-yellow (0.2 cm) → brownish-yellow (0.2 cm) → light brown (0.2 cm) → brown (0.3 cm) → greyish-yellow (0.2 cm) → light brown (0.2 cm) → brown (0.4 cm) → greyish-yellow (0.3 cm) → grey (0.3 cm).

2. On Czapek agar containing Zn the pigment appears in the mycelium on the fourth day, and an orange-yellow pigment diffuses out into the culture medium. The sporulation begins on the sixth day (Table 4), and three zones develop in the conidial field (Fig. 2): greyish-yellow (1.6 cm) → light brown (0.3 cm) →bluish grey (0.4 cm).

3. On a Czapek agar culture medium containing Cu the orange-yellow pigmentation of the mycelium can be observed on the third day, and this diffuses out into the culture medium. The sporulation appears on the fifth day (Table 4). There is no zonation in the conidial field (Fig. 2), the entire colony being a homogeneous yellowish-grey.

4. On a Czapek-Dox agar culture medium containing Cu, Mn and Zn the pigmentation begins in the mycelium on the third day. The colony is orange-yellow, but the pigment diffusing out into the culture medium is light yellow.

5. On potato agar culture medium in white light the pigmentation in the mycelium begins only on the fifth day, and is organe-yellow in colour. The sporulation too is later, appearing only on the seventh day (Table 4). The centre of the colony is greyish-yellow (1.3 cm), followed by a 0.4 cm wide light brown ring, with finally a 0.3 cm grey zone (Fig. 2).

Table 4. Zonation, pigmentation and sporulation of *Penicillium notatum* on various culture media

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (control)	5	3	5
Czapek + Mn	11	3	5
Czapek + Zn	3	4	6
Czapek + Cu	—	3	5
Czapek + Mn + Zn + Cu	11	3	5
Potato agar	3	5	7

Pigmentation and sporulation of *Aspergillus repens* on Czapek-Dox agar in various monochromatic lights

1. In white light the mycelium produces yellowish-green pigments, and a light green pigment diffuses out into the culture medium. Pigmentation can be observed on the fifth day, and sporulation on the seventh day (Table 5). The conidial field is a uniform green and there is no zonation (Fig. 1).

2. In blue light the pigmentation in the mycelium begins on the sixth day; this is yellowish-green and diffuses out into the culture medium. The sporulation begins only on the ninth day (Table 5). There is no zonation (Fig. 1), and the conidial field is a vivid green colour.

Table 5. Zonation, pigmentation and sporulation of *Aspergillus repens* in various lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	—	5	7
Blue	—	6	9
Green	—	5	8
Orange	—	5	7
Red	—	5	7
Dark	—	4	6
Periodic	7	6	8

3. In green light the mycelium is yellowish-green, and the pigmentation begins on the fifth day. A light green pigment diffuses out into the culture medium. The sporulation can be observed beginning from the eighth day (Table 5), the conidial field is green, and there is no zonation (Fig. 1).

4. In orange-red light the colour of the mycelium is yellowish-green; the pigmentation begins on the fifth day (Table 5). A light green pigment diffuses out into the culture medium. The sporulation begins on the seventh day; there is no zonation (Fig. 1), and the conidial field is green.

5. In red light the mycelium is yellowish-green and a light green pigment diffuses out into the culture medium. The pigmentation of the mycelium begins on the fifth day, and the sporulation on the seventh day (Table 5). The spore mass is green; there is no zonation (Fig. 1).

6. In the dark the mycelium is yellowish-green, the pigmentation beginning on the fourth day (Table 5). Sporulation can be observed on the sixth day. The colour of the spores is dark green; there is no zonation (Fig. 1).

7. In periodic illumination a yellowish-green pigment is synthetized in the mycelium, and diffuses out into the culture medium. The pigmentation can be observed on the sixth day, and the sporulation on the eighth day (Table 5). Zonation occurs during the sporulation, and seven zones develop (Fig. 1): dark green (0.2 cm) → light green (0.3 cm) → yellowish-green (0.3 cm) → dark green (0.4 cm) → light green (0.2 cm) → yellowish green (0.2 cm) → dark green (0.2 cm).

Pigmentation and sporulation of *Aspergillus repens* in constant white light on culture media of various compositions

1. On Czapek agar culture medium containing Mn the mycelium is brownish-yellowish-green. The pigmentation begins on the fifth day. Pigment does not diffuse out of the mycelium into the nutrient medium. The sporulation can be observed from the seventh day (Table 4), and during the sporulation there is zonation (Fig. 2): brownish-green (0.3 cm) → yellowis-green (0.8 cm) → green (0.4 cm) brownish-green (0.4 cm), i.e. four different zones.

Table 6. Zonation, pigmentation and sporulation of *Aspergillus repens* on different culture media

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (control)	—	5	7
Czapek + Mn	4	5	7
Czapek + Zn	—	5	7
Czapek + Cu	—	5	7
Czapek + Mn + Zn + Cu	4	5	7
Potato agar	—	6	8

2. On Czapek-Dox agar culture medium containing Zn the colour of the mycelium is brownish-yellowish-green. The pigmentation begins on the fifth day, and an orange-yellowish-green pigment diffuses out into the culture medium. The sporulation can be observed from the seventh day (Table 6); it is greyish-green in colour, and there is no zonation (Fig. 3).

3. On Czapek agar culture medium containing Cu the mycelium in white light is brownish-green. The pigmentation begins on the fifth day. A brownish-

green pigment diffuses out into the culture medium, but it is of a lighter shade than that of the mycelium. The sporulation can be observed on the seventh day (Table 6); its colour is green, and no zonation can be distinguished (Fig. 3).

4. On Czapek agar culture medium containing Cu, Zn and Mn the pigmentation of the mycelium begins on the fifth day. A brownish-yellowish-green colour develops, which does not diffuse out into the culture medium. The sporulation can be observed from the seventh day (Table 6); zonation is weak, and the boundaries of the zones are indistinct (Fig. 2): brownish-green (0.3 cm) → yellowish green (0.9 cm) → green (0.4 cm) → brownish-green (0.5 cm).

5. On potato agar culture medium the colour of the mycelium is brownish-green. Synthetization of the pigments begins on the sixth day. A light yellowish-green pigment diffuses out into the culture medium. The sporulation appears only on the eighth day, and the spore mass has a brownish-green colour (Table 6).

Morphogenetic effects of the nitrogen sources

Since it was observed during our experiments that the presence of the different microelements in the culture medium frequently gave rise to essential colour modifications, the question arose of whether a similar role is also played by the macroelements forming the culture medium. By variation of the N-sources, an attempt was made to answer this question. Literature data were found that the fungi do not utilize nitrogen bound in different forms to the same extent (UBRIZSY and VÖRÖS, 1968), but no indication was given as to the effects of these on pigmentation and sporulation. Cultures were therefore grown on various culture medium combinations in which the $NaNO_3$ of the Czapek culture medium was replaced by $(NH_4)_2SO_4$, NH_4NO_3 and urea.

Our assumption was not completely groundless, for as can be seen from Table 7 the individual N-sources have different effects from the points of view of pigmentation and sporulation.

Table 7. Effects of various N-sources on the beginning of pigmentation and sporulation

N-source	Day on which phenomenon begins					
	Pigmentation			Sporulation		
	Asp. rep.	P. notat.	P. purpur.	Asp. rep.	P. notat.	P. purpun.
$NaNO_3$ control	6	5	6	8	6	8
$(NH_4)_2SO_4$	3	4	5	6	5	7
NH_4NO_3	4	4	5	6	5	7
Urea	12	11	10	13	13	13

Vitality of the spores taken from different zones, on the basis of the intensity of germination

Although neither the composition of the culture media nor the different types of light inhibited the sporulation completely, but merely delayed it, this does not mean that spores of the same vitality developed under all the study

conditions. In order to provide an answer to the above question, test germinations were carried out on every culture, and in a few special cases spores taken from zones of different colours and different ages at the same time were germinated and their growths followed.

In the first case the cover was removed from a sterile culture in a Petri dish, the culture was transferred to a sterile new culture medium, and the spores made to fall through by tapping the walls of the dish.

The results of the study confirmed that germinative spores developed on every culture medium, and in every variation of monochromatic light.

In the second study it was hoped to clarify which part of the colony is the one from which the germinative spores originate. The separate examination of the zones led to the following results:

1. Three rings developed on a colony of *Penicillium notatum* grown in the dark. The spores from all three rings germinated, but the rates of germination were different; the parameters involved were the time of appearance of the colony, and the rate of increase of the colony diameter (Table 8).

Table 8

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	0.3	1.2	2.0
zone 2	—	0.2	0.6	1.6	2.4
zone 3	—	0.2	0.7	1.7	2.5

2. There is no zonation on a culture of *Penicillium purpurogenum* grown on a Czapek-Dox culture medium supplemented with Cu, but there are violet-brown spots in the brownish-grey conidial field. Spores were taken from both sites onto Czapek-Dox culture medium, with the results shown in Table 9).

Table 9

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
violet-brown	—	—	—	—	0.2
brownish-green	—	0.1	0.6	1.5	2.2

3. A culture of *Penicillium purpurogenum* on Czapek-Dox culture medium supplemented with Zn grows very weakly, but 5 zones develop during sporulation. The germinations of the spores originating from the different zones are given in Table 10.

4. The sporulation of *Aspergillus repens* exhibits zonation only in certain cases, for example on Czapek-Dox culture medium supplemented with Mn, where 4 zones develop. The germinations of the spores originating from the individual zones are shown in Table 11.

Table 10

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	—	0.2	0.7
zone 2	—	—	0.2	0.6	1.2
zone 3	—	—	0.2	0.7	1.2
zone 4	—	—	—	—	0.2
zone 5	—	0.4	1.2	1.2	2.0

Table 11.

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	0.2	0.6	1.2
zone 2	—	—	0.2	0.7	1.4
zone 3	—	—	0.4	0.9	1.6
zone 4	—	0.1	0.5	1.0	1.5

Microscopic study of the morphogetic changes

If the external factors are altered, not only the pigmentation and zonation but other changes too can occur in the morphogenesis; these latter are not visible with the naked eye, and accordingly the colonies were subjected to a thorough microscopic analysis which led to a number of positive results. Although significant differences were not found in the diameters of the conidiospores, the thicknesses of the hyphae, the metulae and the sterigmae, and in the sizes of the vesicles, the lengths of the conidiophores of *Aspergillus repens* varied in a characteristic way depending on the nature of the light. In the dark and in the long wavelength region, conidiophores generally 250 μ in length developed, whereas in white light and in the other monochromatic lights the length was 350–400 μ. For the other species examined, merely differences depending on the specific characteristics were observed, and the change of the external factors had no effect.

Discussion

The experiments led to the finding that the pigmentation and sporulation of the studied fungi are physiological processes which can be affected by the light and the nutrient. However, the effects do not appear uniformly for the individual species.

Photo-effects

The pigmentation and sporulation of the colonies depend on the nature of the light. Independently of the nature of the light, the pigmentation of the mycelium in *Penicillium notatum* is followed 2 days later by the sporula-

tion; in *Penicillium purpurogenum* and *Aspergillus repens* there is a 3–4 day interval between the pigmentation of the mycelium and the sporulation, which is the shortest in the dark, and the longest in blue light.

In many respects our results agree with the literature data. RAPER et al (1953) observed that the development of the reproductive organs in *Aspergillus orenatus* is inhibited by the light, but only blue light of short wavelength is effective.

The nature of the photoreceptors is still unknown. They were earlier considered to be carotinoids, and more recently as riboflavin (NARASIMHACHARI, 1963), and there is a fairly general conception that in the most light-sensitive fungi this same pigment acts as the photoreceptor of the sporulation (LUKENS, 1963).

The formation of the sporulation rings can also be affected by photoeffects. With the exception of periodic illumination, *Aspergillus repens* does not exhibit zonation, but here too the periodic variation of the temperature must be reckoned with as the inducing cause (Fig. 1). In the dark *Penicillium notatum* forms 3 rings, similarly as in monochromatic illumination, while the sporulation zonation is promoted by white light. The large number of rings formed in the periodic illumination can be attributed in part to the temperature changes. In *Penicillium purpurogenum* the zonation was enhanced by blue light, in contrast with the other two species, where the blue light was ineffective in this respect (Fig. 1).

The morphogenetic effect of blue light is marked in the development of the length of the *Aspergillus repens* conidiophore. While these are short in the dark or in red light, they attain a much greater length in blue light. Similar observations have been made by JONSON and HALPIN (1952). Photomorphogenesis was not observed in the other two species.

Culture medium effects

In addition to the light, the composition of the culture medium also plays a role in the generative development of the fungi.

Zn inhibits the pigmentation and the beginning of the sporulation in all three species. The shift in time amounted to 3–4 days. The most striking microelement effect was exhibited in the number of zones, in that the presence of Mn enhanced the zonation, whereas the presence of Cu decreased it. As regards the microelement effect, however, specific differences too were revealed, because *Aspergillus repens*, for example, in contrast to the other two species, forms sporulation rings only in the presence of Mn (Fig. 2). This phenomenon can be classified among the qualitative differences as opposed to the quantitative effects of the microelements, such as the fact that, on Czapek-Dox agar culture medium in the presence of Mn, *Penicillium notatum* forms twice as many zones as in the absence of Mn (Fig. 2). Similarly, in the case of Penicillium purpurogenum the addition of Mn enhances the formation of the rings, and in our experiments their number rose from 4 to 9. This effect of Mn requires further study, for the number of rings roughly doubled for the same colony diameter (Fig. 2).

The experiments in which the N-sources in the culture medium were varied led to the result that all three species utilize NH_4^+ most quickly, if the beginning of the colony formation is taken as the basis of the measure of utilization.

The second place is occupied by NO_3^-, and the third by the urea–N. The utilization of the three N-sources outlined above is confirmed by the beginning of pigmentation and sporulation too. Our findings are in contrast with those of UBRIZSY and VÖRÖS (1968), according to whom the N of NO_3^- is preferentially utilized by the fungi, although we agree with the finding that the utilization of NH_4^+ is a process requiring more energy than that for NO_3^-.

Literature data confirm that, even though to a restricted extent, the fungi utilize organic N–sources too (aminoacids, amides, peptides, proteins and nucleic acids (UBRIZSY and VÖRÖS, 1968).

We observed a new phenomenon in the relation of the zonation and sporulation: differences appear in the germination of the spores and in the intensity of their growth; the spores originating from the peripheral zones exhibit a greater vitality than the spores in the older zones.

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Addresses of the authors:

JUDIT HORVÁTH

Prof. Dr. I. SZALAI

Department of Plant Physiology

A. J. University

H — 6701 Szeged P. O. Box 428,

Hungary