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PHOTOMORPHOGENESIS IN PENICILLIUM ISARIIFORME III. THE ACTION SPECTRUM FOR PHOTOTROPIC CURVATURE OF THE COREMIA

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SUMMARY

The action spectrum for the phototropical curvature of the coremia of *P. isariiforme* was determined by means of unilateral illumination. The $I_{50}^{(\lambda)}$, that is the light intensity at wavelength λ at which 50% of the coremia react positively, was calculated from a series of experiments with the help of the probit analysis. The $I_{50}^{(\lambda)}$, the 95% confidence interval, and the relative quantum efficiency were calculated and a plot of the values gave the action spectrum. This spectrum is quite different from the action spectra for sporulation and initiation of coremia in the same fungus. However, with peaks at 425 nm, 450 nm and 476 nm, it resembles rather well the action spectra for other light-sensitive fungi. Therefore it might be assumed that in *P. isariiforme* the same photoreceptor as in other fungi is active. The great difference between the three action spectra remains to be explained. Possibly a screening pigment causes the distortion of the action spectra.

1. INTRODUCTION

For the light-sensitive fungus *Penicillium isariiforme* action spectra have been established for sporulation and for the initiation of coremia. The action spectra, with a single peak at 470–480 nm, are very much alike but they are strikingly different from those published for other light sensitive fungi. This might suggest that in *P. isariiforme* a quite different photoreceptor is present or that in this fungus one or more pigments absorb part of the visible light, thus giving a distortion of the action spectrum (BENNINK 1972a, b). In order to obtain more information about this we tried to find the action spectrum for a third light reaction in *P. isariiforme*. When the coremia have been initiated by means of light from above, we found a very strong positive phototropic curvature when the coremia are illuminated laterally. Only the white tips, nearly colourless in contrast to the deep yellow colour of the mycelium and coremia, show this light reaction.

2. MATERIAL AND METHODS

2.1. Culture conditions

Penicillium isariiforme Stolk & Meyer, strain 530, was obtained from the C.B.S. (Centraalbureau voor Schimmelcultures, Baarn, Netherlands). The fungus was cultured on the same medium as mentioned in a former study (BENNINK 1972a). Inoculation was done in a streak of about 4 cm on 50 ml solid medium in a petri

dish of 7 cm diameter. The dishes were placed without a cover in boxes, size 15 × 8 × 8 cm, made of black, the upper and front side of transparent plexiglass. These boxes were kept in an incubator at 25 °C (± 1 °C) illuminated with blue light from above for six hours daily. This light came from a series of fluorescent tubes, Philips 20 Watt no. 23, covered with blue plexiglass (Röhm & Haas, Darmstadt, no. 627). Under these conditions six-day-old cultures of the fungus show a row of coremia of about 10 mm high standing in a line along the streak of inoculation.

2.2. Illumination

Coremia formed during six days with blue light from above were illuminated during exactly three hours from aside with the 500 Watt Leitz Prado slide projector with interference filters as described earlier (BENNINK 1972a). These illuminations were carried out in a constant temperature darkroom at 25 °C (± 1 °C). Exactly 24 hours after the illumination the response of the coremia was established, only coremia of about 1.3 mm diameter being taken into consideration. The coremia were cut off and photographs were taken to permit comparison of the results of the experiments at any time.

Light measurements were performed in the same way as described earlier (BENNINK 1972a).

2.3. Standard for the estimation of the response to light

The response of the coremia varied between no change in direction of growth and a complete curvature of all the component hyphae into the direction of the light source, depending on light intensity and wavelength. A number of different responses is shown in *fig. 1*, from which we decided arbitrarily to indicate the reactions number one to five as negative and the reactions six and seven as positive. The next section shows that making a somewhat different choice between positive and negative reactions does not materially affect the ultimate result, i.e. the establishment of an action spectrum for the phototropic curvature of the coremia.

The percentage of positively reacting coremia in a given experiment was calculated and as a standard measure of light intensity at wavelength λ we chose $I_{50}^{(\lambda)}$, that is the light intensity at which 50 % of the coremia in a culture reacted positively.

2.4. Suppositions and model

From preliminary experiments it appeared that very probably there is a sigmoid relation between the percentage of positive reaction and the logarithm of light intensity (*fig. 2*).

In order to explain this sigmoid relation we started from the following suppositions:

1. Each coremium has for each λ a threshold of sensitivity d_λ, characterized by: the coremium reacts positively if and only if $d_\lambda < \log I$ (I = light intensity).
2. The thresholds of sensitivity of the coremia do not influence one another.

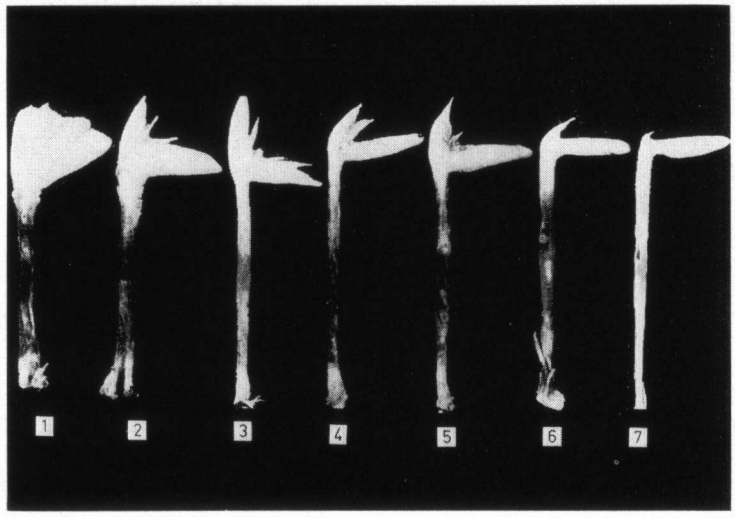

Fig. 1. Examples of different reactions of coremia upon illumination.
no. 1–5 negative reaction.
no. 6 and 7 positive reaction (see text).

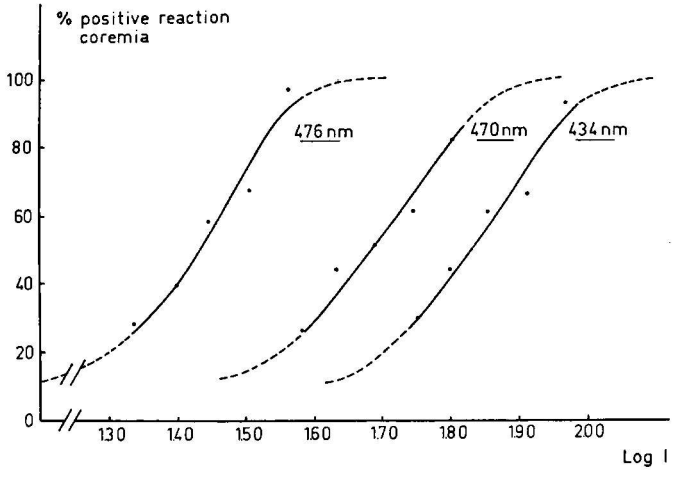

Fig. 2. Sigmoid relation between percentage of positive reaction and logarithm of light intensity

3. At radom choice of a coremium from a culture the concerned d_λ is a realisation of the random variable d_λ, which is supposed to have a normal distribution with unknown mean μ_λ and unknown variance σ_λ^2.
4. The observations are considered to be independent. This leads us to the model for $r_\lambda(I)$, the reaction of a coremium to an intensity of light of wavelength λ:

$$r_\lambda(I) = \begin{matrix} 1 \text{ if } \log I - \mu_\lambda + u_\lambda > 0 \\ 0 \text{ if } \log I - \mu_\lambda + u_\lambda \leqslant 0 \end{matrix}$$

in which u_λ is $N(0,\sigma_\lambda^2)$ distributed and 1 means positive reaction and 0 means negative reaction. Under these suppositions the theoretical response curve of the percentage positively reacting coremia against the logarithm of light intensity is known: It is the cumulative distribution function of the normal distribution with mean μ_λ and variance σ_λ^2 (FINNEY 1952). μ_λ equals the logarithm of light intensity giving 50% positive reaction at wavelength λ, i.e. $\mu_\lambda = \text{Log } I_{50}^{(\lambda)}$. So it was our goal to estimate μ_λ and to use the values obtained to establish the action spectrum.

2.5. Experimental set-up

To estimate μ_λ for each wavelength we carried out the following experiments: At each wavelength we chose five light intensities of increasing values in such a way that it might be expected that the 50% reaction level was situated somewhere between the lowest and the highest light intensity. At each light intensity the percentage of positively reacting coremia was determined from four experiments with about 50 coremia. In these experiments interference filters transmitting at 419 – 426 – 434 – 446 – 449 – 459 – 466 – 470 – 476 – 481 – 491 and 510 nm were used.

By means of the probit analysis, a method developed by GADDUM (1933) and BLISS (1934 a, b) and amply described by FINNEY (1952), the maximum likelihood estimation and a 95% confidence interval for μ_λ were determined. In this method the observed percentages of positively reacting coremia are transformed to probits.

According to FINNEY (1952) the probit of an observation is defined as the normal equivalent deviate (N.E.D.) of the concerned percentage increased with 5.

The transformation into probits causes the theoretical response curve to become a straight line. From the transformed data by means of a weighed regression the estimator of μ_λ and the 95% confidence interval is determined. It was also possible to test whether the model fitted the observations.

3. RESULTS

When six-day-old cultures of the fungus were illuminated unilaterally during three hours with light of low intensity immediately after the last light period of six hours we did not obtain a positive reaction. However, when the same illumination was given at the end of an 18 hours dark period the coremia showed a positive reaction. This kind of experiment showed that light sensitivity of the

coremia increases during the dark period. From experiments with dark periods of varying duration it appeared that light sensitivity of the coremia was highest after a dark period of 30 hours and that from 30 to 40 hours dark period no change in sensitivity could be observed. Experiments with light of different wavelengths confirmed these observations, leading us to the conclusion that it was permitted to compare the results of the different experiments when a dark period from 30 to 40 hours was given.

In a number of experiments we varied the duration of the unilateral illumination with monochromatic light from one to nine hours. From these experiments it appeared that a duration of three to six hours was most efficient for obtaining a positive reaction of the coremia. For convenience we decided to use exactly three hours of illumination in all further experiments.

The experiments were carried out with the interference filters from 419 to 491 nm at light intensities from 20 to 95 erg. cm^{-2}. sec^{-1}. At wavelength 510 nm a light intensity of 190 erg. cm^{-2}. sec^{-1} was not sufficient to give a 50% positive reaction.

At this intensity only a 5% positive reaction was obtained. At wavelengths of 520 nm and higher it was not possible to get a positive reaction, not even at light intensities of more than 250 erg. cm^{-2}. $sec.^{-1}$.

The results of the experiments are given in *table 1.*

The maximum likelihood estimation of μ_λ and the 95% confidence interval were computed on the Electrologica X8 computer of the Mathematical Centre in Amsterdam. The χ^2 tests calculated for each wavelength showed no disagreement between observations and model so that the assumed model was not rejected.

The $I_{50}^{(\lambda)}$ and the 95% confidence interval for each wavelength is given in *table 2.* In this table the relative quantum efficiency is calculated according to the following formula:

$$1 \text{ erg.cm}^{-2}\text{.sec}^{-1} = \frac{\lambda}{1987} \times 10^{11} \text{ quanta.cm}^{-2}\text{.sec}^{-1}.$$

A plot of these values versus wavelengths yields the action spectrum for light induced positive curvature of the coremia of *P. isariiforme* (*fig. 3*).

4. DISCUSSION

The action spectrum for phototropic curvature with maxima at 450 nm, 470–480 nm, and a rather low maximum at 425 nm resembles much more the action spectra established for other light-sensitive fungi e.g. *Phycomyces blakesleeanus* (DELBRÜCK & SHROPSHIRE 1960, CURRY & GRUEN 1959), *Trichoderma viride* (KUMAGAI & ODA 1969, GRESSEL & HARTMANN 1968), *Penicillium claviforme* (FARAH SALMAN 1971). There are, admittedly, slight differences, but in spite of these we may assume that in *P. isariiforme* we are concerned with the same photoreceptor as in other light sensitive fungi. Till now the identity of the photoreceptor is quite unknown and the action spectrum found for phototropism in *P. isariiforme* did not give any further information.

Table 1. Results of the illumination experiments for phototropic curvature of the coremia of *P. isariiforme* at the wavelengths from 419 to 510 nm.

wavelength (in nm)	intensity (erg. $cm^{-2}.sec^{-1}$.)	total number of coremia considered	positive reaction (%)	probit
419	55	57	23	4.26
419	63	47	49	4.97
419	72	35	60	5.25
419	82	39	51	5.02
419	93	43	72	5.58
426	43	40	38	4.69
426	50	45	29	4.45
426	57	51	55	5.13
426	65	43	49	4.97
426	74	49	65	5.39
434	56	63	30	4.48
434	63	36	44	4.85
434	72	23	61	5.28
434	82	53	66	5.41
434	93	41	93	6.48
446	30	43	35	4.61
446	34	43	47	4.92
446	38	26	54	5.10
446	44	51	47	4.92
446	50	39	74	5.64
449	21	34	15	3.96
449	24	27	30	4.48
449	27	53	40	4.75
449	31	48	73	5.61
449	36	51	69	5.50
459	31	48	38	4.69
459	35	46	54	5.10
459	40	46	65	5.39
459	46	35	69	5.50
459	52	35	71	5.55
466	25	39	33	4.56
466	29	38	39	4.72
466	33	40	53	5.08
466	37	55	58	5.20
466	43	51	71	5.55
470	38	46	26	4.36
470	43	36	44	4.85
470	49	41	51	5.03
470	56	38	61	5.28
470	64	39	82	5.92
476	22	57	28	4.42
476	25	41	39	4.72
476	29	43	70	5.52
476	32	42	67	5.44
476	37	33	97	6.88
481	30	20	20	4.16
481	35	39	64	5.36
481	39	62	63	5.33

Table 1. (continued)

481	45	51	65	5.39
481	51	38	82	5.92
491	56	49	51	5.03
491	64	43	58	5.20
491	72	51	45	4.87
491	82	42	67	5.44
491	94	51	78	5.77
510	190	20	5	–

Table 2. The $I_{50}^{(\lambda)}$ and 95% confidence interval, together with the relative quantum efficiency from the filters transmitting 419 to 510 nm.

wavelength (in nm)	$I_{50}^{(\lambda)}$ (erg.-$cm^{-2}.sec^{-1}$.)	95% confidence interval ($erg.cm^{-2}.sec.^{-1}$)	relative quantum efficiency	confidence interval
419	71.0	65.0–78.0	0.42	0.38–0.46
426	59.5	53.0–70.5	0.49	0.42–0.56
434	66.5	62.0–70.5	0.43	0.41–0.46
446	38.0	32.5–44.0	0.74	0.64–0.86
449	28.5	27.0–30.5	0.97	0.92–1.03
459	34.5	27.5–38.0	0.79	0.72–0.99
466	32.5	29.0–36.0	0.82	0.75–0.92
470	48.0	44.5–51.5	0.56	0.52–0.60
476	26.5	25.0–27.5	1.00	0.95–1.06
481	34.5	28.5–37.5	0.76	0.69–0.92
491	59.5	38.0–68.0	0.42	0.37–0.66
510	$\gg$190		$\ll$0.13	

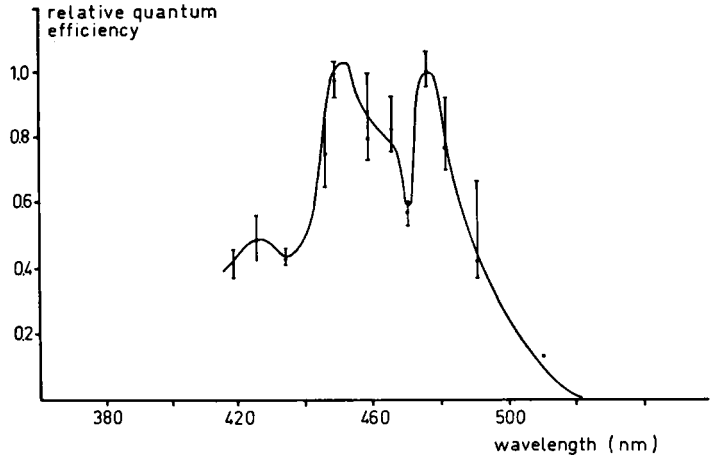

Fig. 3. Action spectrum for light induced phototropic curvature of coremia of *P. isariiforme*.

The action spectrum for the phototropic curvature of the coremia of *P. isariiforme* differs strongly from the action spectra determined for sporulation and for initiation of the coremia of the same fungus (BENNINK 1972a, b). This may be due to the presence of two or more different photoreceptors, as was also suggested by BERGMAN et al. (1969) in their study on *Phycomyces*. In *P. isariiforme* we cannot decide about the presence of one or more photoreceptors. On the other hand we have to bear in mind the possibility of pigments other than the photoreceptor absorbing in the same region as the latter and in this way causing a distortion of the action spectrum.

P. isariiforme shows a very bright yellow colour when grown in the dark. Absorption characteristics of partially purified extracts from the mycelium gave us the indication that a kind of screening function might be responsible. Therefore it seems to be of interest to check what kind of pigment causes this colour.

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