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Morphogenesis in *Schizophyllum commune*. II. Effects of Monochromatic Light[1]

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Abstract. The photoinduction of fruiting bodies by light of defined wavelengths was studied in the fungus *Schizophyllum commune* Fr. Several properties of the induction were established. (1) The exposure-response relationship for induced fruiting was determined for light of 448 nm. (2) The Bunsen-Roscoe Law of Reciprocity was found to hold for the photoinduction of fruiting bodies for the interval 36 to 2000 sec with light of 448 nm. (3) Light of wavelengths from 320 nm to 525 nm induced fruiting bodies. Although the photoreceptor is unknown, it may be a flavin rather than a carotenoid, because light in the near ultraviolet (350 nm–400 nm) was inductive. (4) Neither red light (660 nm) nor far-red light (730 nm) induced fruiting bodies or affected the sensitivity of the fungus toward photoinduction by blue light (448 nm).

Light can significantly affect fungal growth, morphology, differentiation, and spore dispersal (4, 8, 11, 21, 22, 28). Carlile (4) divides fungal photoresponses into 2 categories: A) responses activated by visible light and probably by ultraviolet radiation and B) responses activated only by ultraviolet radiation.

Action spectra for photoresponses in group A have indicated that a common class of photoreceptor molecules may be involved: all responses in this group are initiated by light at the blue end of the visible spectrum and, in those cases investigated, activity was also elicited by radiation in the ultraviolet. The most thoroughly investigated photoresponse of this type is the phototropism of the sporangiophore of Phycomyces (5, 6, 29, 30). Other responses of this type have been studied in a variety of other fungi by Page (20), Madelin (18), Aschan-Aberg (2), Alasodura (1), Ingold and Nawaz (10), and Sargent and Briggs (26).

The most thorough studies of responses in group B have been done by Leach and his coworkers on the induction of sexual and asexual differentiation in ascomycetes and imperfect fungi (12, 13, 14, 15, 16, 17, 31). These fungi were affected by radiation with wavelengths between 230 and 380 nm; major peaks were located at 230 nm and 290 nm. No differentiation was ever induced by radiation with wavelengths longer than 380 nm.

Raper and Krongelb (25) and Perkins (24) described the induction by white light of fruiting bodies of the tetrapolar basidiomycete *Schizophyllum commune* Fr. The present paper describes several basic properties of that photoresponse as studied with light of defined wavelengths.

Materials and Methods

The detailed descriptions of the strains, media and culture methods, methods of inoculation, growth conditions, and scoring are given elsewhere (24). The work reported here was performed at Argonne National Laboratory, Argonne, Illinois.

Procedure. All experiments utilizing monochromatic light were performed with the established dikaryon and followed a strict schedule: Day 1: Start master cultures of the established dikaryon on minimal medium lacking thiamin. Day 6: Inoculate experimental cultures on complete plus yeast medium in small petri dishes (Falcon #1007). Day 11: Irradiate between 120 and 130 hr after inoculation. Day 14: Score.

Master cultures were grown in the dark in corrugated cardboard boxes lined with aluminum foil; 5 cultures were placed around an open dish that held 10 to 20 grams of soda-lime, 4 to 8 mesh. All cultures were maintained in a growth room at 22° ± 0.5°.

Radiation. "Monochromator boxes" of the following specifications were built by the shops at Argonne Laboratory. The boxes were wooden, measured 140 × 60 × 55 cm (inside dimensions), and their inner surfaces were painted nonglossy black. A 2 inch × 2 inch slide projector with a 500 watt quartz-iodide lamp was the source of radiation. The projector was affixed to the top of the

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box, and its beam passed through a hole in the top of the box. Interference filters were inserted in the slide holder to provide light of the appropriate wavelength. The filters were blocked to 800 nm or to 1.25 × their peak wavelengths and had half-band widths of between 4 and 5 % of their peak wavelengths.

Cultures to be irradiated were placed either directly on a shelf normal to the beam of light or on a rotating turntable (3 rpm) on the shelf. The turntable was covered with nonglossy black paper. The irradiance was varied by altering the distance between source and shelf or by changing the lamp current. Irradiances were measured with a compensated thermopile that had been calibrated against standard lamps. Thermopile voltages were determined by a high impedance electrometer. The temperature during irradiation varied between 26° and 30°. The cultures, however, were never kept at this somewhat elevated temperature for longer than 30 min.

The second optical system used was the Argonne Biological Spectrograph (19). Irradiance was varied by changing the slit width and by placing a nylon mesh over the exit tunnel of the arc source. All irradiances were determined with calibrated thermopiles. Amplified output voltages were traced on a strip-chart recorder that was calibrated with a standard voltage imput. The temperature during irradiation was 22° ± 1°.

Cultures to be irradiated were placed horizontally at the focal curve. The horizontal beam of light was reflected downward with front-surfaced mirrors through the lid of the petri dish onto the fungus; the mirrors were adjusted so that the reflected light was normal to the agar surface. The arrangement of replicate culture dishes on the focal curve was such that determinations of wavelength were accurate to ± 6 nm (in 1 experiment, however, accuracy was ± 12 nm).

Except where specifically mentioned, all irradiation was given through the lid of the petri dish; the thermopile was placed so as to measure the irradiance at the surface of the agar. The absorbance of the lids was corrected for when necessary. Manipulations during exposures were carried out under "safe" lights with peak emission at 560 nm and 1/100 band width of 30 nm (27).

Results

Exposure-Response Relationships. Preliminary experiments indicated that light of 400, 448, and 500 nm was effective in inducing fruiting bodies. The relation between response and exposure at 448 nm was then determined. All exposures were 16 and two-third min (1000 sec). Both an interference filter (448 nm) and the spectrograph were utilized. Light from the spectrograph was 450 ± 12 nm. All treatments of less than 10^{17} quanta cm^{-2} were given in a monochromator box, and treatments greater than 10^{17} quanta cm^{-2} were made in either a monochromator box or with the spectrograph. Certain exposure-response points for treatments of more than 10^{17} quanta cm^{-2} were pooled; for data that were pooled, however, the differences in energy between the largest and smallest exposures were never more than 17.5 % of the largest dose. In all experiments, 40 to 80 cultures were irradiated at each intensity.

The shape of exposure-response curve was similar to that found with white light (24): there is a range of intensity over which response is roughly linear, and there is a range beyond which response shows no further increase (Fig. 1). Statistical significance of the differences was shown by a one-way analysis of variance ($P<0.01$).

Experiments to Establish Reciprocity. A series of experiments was designed to determine whether the Bunsen-Roscoe Law applies to this photoresponse—if the product of intensity and time is constant, then the response will be constant (3). In each experiment, a series of the same 7 time-intensity combinations was each given to 2 sets of 20 cultures. Each combination delivered to the mycelia a total exposure of 1.05 ± 0.01 × 10^{17} quanta cm^{-2} at 448 nm. All irradiation was performed between 120 and 130 hr after inoculation. Two estimates of the response—each based on a set of 20 cultures—were obtained at each time-intensity combination. Reciprocity (*i.e.* equal response) was found to hold, at this quantum level, for exposure times between 36 and 2000 sec (see table I for a typical experiment).

Response as a Function of Exposure Time. Previous exposure-response curves were obtained by holding exposure time constant and varying the irradiance. Experiments were performed in which this relationship was reversed: irradiance was held constant, but the exposure time was varied. In each

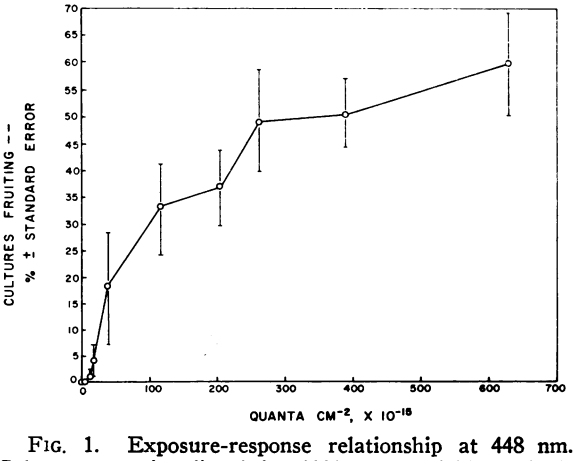

FIG. 1. Exposure-response relationship at 448 nm. Cultures were irradiated for 1000 sec to yield the indicated incident radiant energies. Each point is the mean of up to 7 experiments, and the standard error for each mean is indicated by a vertical bar.

Table I. *Reciprocity of Response at 448 nm*

All cultures were irradiated 120 to 130 hr after inoculation. Each percentage is based on 20 cultures.

Irradiance	Exposure time	Exposure	Cultures fruiting	
Quanta cm⁻² sec⁻¹	*Sec*	*Quanta cm⁻²*	%	
2.90×10^{15}	36	1.04×10^{17}	95	75
7.91×10^{14}	133	1.05×10^{17}	90	90
3.69×10^{14}	286	1.06×10^{17}	75	75
2.11×10^{14}	500	1.05×10^{17}	95	75
1.21×10^{14}	870	1.05×10^{17}	95	65
1.05×10^{14}	1000	1.05×10^{17}	95	80
5.27×10^{13}	2000	1.05×10^{17}	85	70

Before analysis, the proportions were transformed by $x = \sin^{-1}\sqrt{p}$, where p is the proportion of cultures fruiting and x is the variable treated in the analysis of variance.

Analysis of variance

Item	DF	SS	MS	F	P
Between treatments	6	0.0695	0.01158	2.70	>0.05
Within treatments	7	0.2190	0.03129		
Total	13	0.2885			

experiment, 40 cultures were illuminated for 5, 36, 133, 286, 500, 870, 1000, or 2000 sec. Exposures were made in a monochromator box with a 448 nm interference filter; irradiance was held at 1.05×10^{14} quanta sec^{-1} cm^{-2}. All irradiation was performed between 120 and 130 hr after inoculation. The results of the experiments were similar: the responses to exposures of less than 500 sec were roughly linear with exposure time; responses to exposures greater than 500 sec were approximately equal (Fig. 2 details 1 experiment).

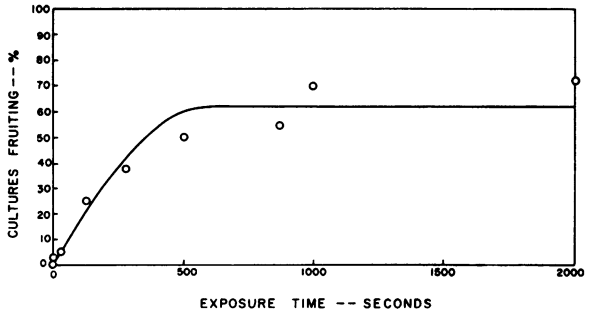

FIG. 2. Response as a function of exposure time. Cultures grown 120 to 130 hr in the dark were irradiated with 1.05×10^{14} quanta sec^{-1} cm^{-2} for the times indicated. The wavelength was 448 nm. Responses at 500, 870, 1000, and 2000 sec were homogenous. ($\chi^2_3 = 6.226$, $0.10<P<0.20$) with a mean of 61.9 %. Accordingly, 61.9 % is used as a mean response in the "plateau region."

Spectral Sensitivity. A series of 6 experiments was performed with the spectrograph to determine the spectral sensitivity of photoinduction. The experimental details and raw data are tabulated in Perkins (23). Almost all exposures were at a level in which response should be approximately linear with dose, if one assumes that the shape of the exposure-response curve at 448 nm (Fig. 1) applies approximately at other wavelengths. Data presented elsewhere (23) suggest the assumption is justified. In 1 experiment, very high exposures (probably above saturation) were given at 510, 525, and 540 nm to determine if any response could be elicited by light of these wavelengths.

Within each experiment, the relative effectiveness of light at each wavelength was normalized internally to the effect at 475 nm. The mean normalized value for each wavelength was then calculated and plotted as a function of wavelength in Fig. 3: light of every wavelength tested between 320 nm and 525 nm was photoinductively active, and no light of wavelength longer than 525 nm was photoinductive.

Effect of Red and Far-red Light on Sensitivity to Blue Light. The importance of red and far-red light for photomorphogenesis in green plants (7) and reports of effects of red light on fungi (4, 9, 22) prompted experiments to determine whether red or far-red light had any detectable effect on the sensitivity of the fungus to blue light. Monochromator boxes and 448 nm, 660 nm, and 730 nm interference filters isolated blue, red, and far-red light, respectively. In each experiment, 30 cultures were subjected to each of 3 treatments. The elapsed time between irradiation with red or far-red light and blue light varied from 5 min to 35 min. The irradiation of all 90 cultures in each experiment required 2 hr. The results of a typical experiment (table II) showed that prior exposure to neither red nor far-red light affected the sensitivity of the fungus to blue light.

Discussion

The exposure-response curve shown in Fig. 1 is the aggregate of numerous experiments. Although it was clear that the differences were statistically

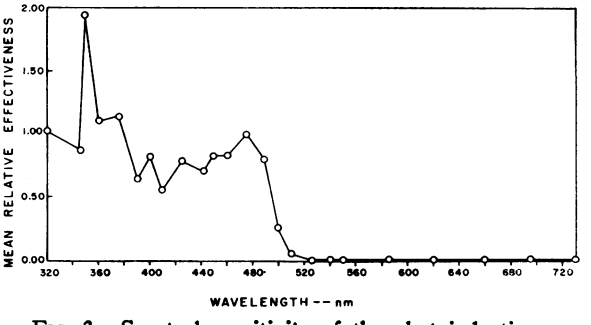

FIG. 3. Spectral sensitivity of the photoinduction.

Table II. *Test to Determine if Pre-exposure to Red orFar-red Light Alters Sensitivity of Fungus to Blue Light*
All cultures were irradiated 120 to 130 hours after inoculation

Treatment	Wavelength	Irradiance	Exposure time	Exposure	Cultures fruiting
	nm	*quanta sec*$^{-1}$ *cm*$^{-2}$	*sec*	*quanta cm*$^{-2}$	
1	660	2.0×10^{14}	500	1.0×10^{17}	17/30
	448	1.2×10^{14}	1000	1.2×10^{17}	
2	730	2.0×10^{14}	500	1.0×10^{17}	18/30
	448	1.2×10^{14}	1000	1.2×10^{17}	
3	448	1.2×10^{14}	1000	1.2×10^{17}	18/30

significant, the standard errors revealed a high degree of variation in responses between experiments. Because of this marked variation from experiment to experiment, subsequent studies were designed so that all treatments were given at a single time. With this procedure, it was possible to repeat experiments and obtain qualitatively similar results. Repeated experiments frequently gave photoresponses which differed quantitatively, but the qualitative pattern of response was always identical. The cause of the variation is unknown.

The fact that reciprocity holds has implications for the metabolic events underlying photodifferentiation. Presumably, the photoresponse is initiated when an activated photoreceptor molecule interacts with other metabolic intermediates to start a series of reactions that result in differentiation. The applicability of the Bunsen-Roscoe Law means that the length of time over which this interaction takes place is not important to the final outcome. In other words, the effect of this interaction has "durability." In the case of *Schizophyllum,* the effect of the interaction is "durable" between, at least, 36 sec and 2000 sec.

Because reciprocity holds in *Schizophyllum,* the response should be dependent upon the total radiation delivered, and exposure-response curves derived from varied exposure times at constant intensity and from varied intensities with a constant exposure time should be similar. This was indeed the case [compare Fig. 1 and 2 of this paper and Fig. 1 of Perkins (24)]. In both cases, there was an energy range over which response was roughly a linear function of total radiation and a range beyond which increased energy had little further effect upon the response.

Three points should be noted about the studies on spectral sensitivity and on the lack of effects of red and far-red light:

(1) No response was ever obtained from radiation of wavelength longer than 525 nm, but radiation at every wavelength tested from 320 nm to 525 nm was photoinductive. Neither red light (660 nm) nor far-red light (730 nm) was photoinductive.

(2) Because an exposure-response relationship was not determined at each wavelength tested, the positions and relative heights of the "peaks" in Fig. 3 cannot be taken too seriously; Fig. 3 should instead be regarded as a curve identifying an active area of the spectrum rather than as a detailed action spectrum.

(3) There was no indication that either red or far-red light had any effect on the sensitivity of the fungus to blue light (table II). Phytochrome thus does not appear to be present in *Schizophyllum.* These findings are in agreement with the majority of studies with other fungi (4).

A major concern of any photobiological investigation is the identity of the photoreceptor molecule. Although Fig. 3 is not necessarily a reflection of the absorption spectrum of the photoreceptor, we can conclude that the photoreceptor absorbs between 320 nm and about 500 nm. Differentiation elicited by light of wavelengths longer than 380 nm clearly excludes *Schizophyllum* from the group of fungi affected only by ultraviolet radiation. The promotion of growth leading to aggregated cells by both blue and ultraviolet radiation is evidence that the photoresponse in *S. commune* is of type A.

The many studies on the effect of blue light on fungi and green plants have generated a controversy over the identity of possible receptor molecules. The 2 most likely candidates always suggested are carotenes and flavins, but no resolution of the problem has yet been offered. Carlile (4) reviewed information relevant to fungal photoresponses and presented arguments supporting flavins as the photoreceptor; Thimann and Curry (29, 30) reviewed much of the same data but favored carotenes as the receptor.

The present study offers little to settle this controversy because the action spectrum (Fig. 3) does not show reliable fine structure. The activity elicited by light of wavelengths shorter than 400 nm, however, is more compatible with a flavin receptor, because carotenes have little or no absorption in the ultraviolet (4).

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