THE PHOTOTROPIC SENSITIVITY OF PHYCOMYCES AS RELATED TO WAVE-LENGTH

By E. S. CASTLE

(*From the Laboratory of General Physiology, Harvard University, Cambridge*)

(Accepted for publication, April 20, 1931)

I

The action of light on the photosensitive meristematic region of the elongating sporangiophore of *Phycomyces* leads to a temporary acceleration of growth (the "light-growth" response). If the sporangiophore receives unequal illumination on opposite sides, phototropic bending typically occurs, directed toward the more intense source of light. In this case bending is due to unequal growth on opposite sides of the meristem, not to a flexion of the sporangiophore as a whole. These two modes of response—acceleration of growth and bending—have been shown to be parallel indicators of the state of photic excitability of the sporangiophore (Castle, 1929–30).

It is of interest to study the relative efficiencies of different spectral regions in exciting the photic system of *Phycomyces*, since the stimulating efficiency of each wave-length may be taken to represent the *effective* absorption of light at that wave-length,—and thus, with certain necessary reservations, the absorption spectrum of the photosensitive material presumed to initiate the chain of events leading to response. A detailed justification of such a procedure has been given by Hecht (1920–21), although others have mapped out "biological absorption spectra" for animal and plant systems on the same assumptions.

The reservations which must be made, however, in such reasoning are these: first, it is clearly impossible by this method to dissociate from the absorption of the sensitive substance that of any secondary, screening pigment associated with it. This consideration is of particular importance in the case of plants, where accessory pigments are common. Thus the sporangiophores of *Phycomyces* contain pigment,

the amount of which seems to vary with the intensity of illumination under which the fungus has been grown. The nature of this pigment and its rôle in determining the phototropic behavior of *Phycomyces* will be considered elsewhere.

In the second place, unequal refraction of different wave-lengths of light within the organ in question may introduce complications. Curved, photosensitive plant structures of small dimensions function somewhat as lenses. For example, the clear, cylindrical sporangiophore of *Phycomyces* concentrates light on the side more remote from the source of illumination. While the exact light paths are hard to visualize (Blaauw, 1914; Oehlkers, 1926), it is obvious that the light must pass through several concentric layers having different refractive indices. It is possible in this case that the different wave-lengths do not reach the same effective place of absorption.

The mapping out of "biological absorption spectra" does give definite, useful information as to the photic sensitivity of the system in question, although the result may not be at once identified with the absorption spectrum of the underlying sensitive substance. In this paper are presented the results of a study of the sensitivity of the sporangiophores of *Phycomyces* to different spectral regions.

II

The method of experimentation depends on the fact that in a culture of *Phycomyces* placed between two equal sources of light opposed at 180°, the sensitive sporangiophores either (1) continue to grow upward at right angles to a line connecting the two sources (a state of continuous phototropic "indifference"), or (2) are oriented in approximately equal numbers toward each source—see Fig. 1. If the illuminations from the two sources are unequal, a relatively greater number of sporangiophores bend toward the more highly illuminated side.

This method of so-called "antagonistic" illumination has been used previously in work with the phototropic responses of sessile plants: by Massart (1888) to determine the discrimination of intensity by *Phycomyces nitens*; recently by Bergann (1930) to study the sensitivity of *Avena* coleoptiles to different spectral regions.

In the present experiments, the inclination of equal numbers of sporangiophores toward each source of light was found to be the most satisfactory end-point by which to judge equal phototropic effects.

The fact that the so-called "resultant law" does not hold rigorously for all individual sporangiophores may be due (1) to asymmetry of the sporangiophore, or (2) to initial chance orientation of individuals in the direction of each light, these sporangiophores then being held effectively oriented by one light alone. The latter is the most probable explanation of the "non-resultant" orientation of *Pilobolus* sporangiophores toward one or the other of two sources of light observed

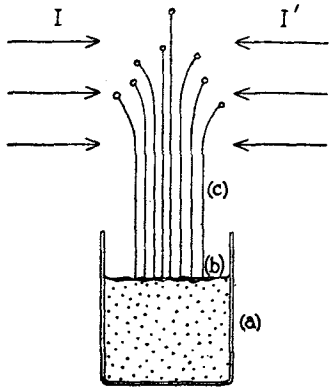

FIG. 1. Diagram of a culture of *Phycomyces* sporangiophores oriented between two equal sources of illumination ($I = I'$). Equal numbers of individuals are shown bent in each direction, and in the middle the characteristic "indifferent" class. In a culture as actually used, the sporangiophores are arranged in a row at right angles to the plane of the paper, to avoid shading one another. The symmetrical arrangement is illustrative only. (*a*), culture vessel and medium; (*b*), flat, vegetative mycelium; (*c*), sporangiophores oriented by two equal, opposed illuminations.

by Allen and Jolivette (1914). In this respect it is significant that the *young* sporangiophores of *Pilobolus* placed between two sources of light obey the "resultant law" (Pringsheim and Czurda, 1927). At this time the sporangium has not been formed, and the path of light in the sensitive meristem is relatively simple, in contrast to the complex optical situation following sporangium-formation (Van der Wey, 1929).

The general procedure was to equate the phototropic effects of different spectral regions by means of cultures of elongating, sensitive sporangiophores placed in beams of light opposed at 180°. The rela-

tive intensities of the two beams were then adjusted until equal numbers of sporangiophores bent toward each source of light. At this point of equal phototropic effect, the efficiency of each spectral region was taken as inversely proportional to its relative energy content.

Opposed beams of light of equal intensity and identical spectral composition were obtained from a single source by mirrors placed at two points of an isosceles

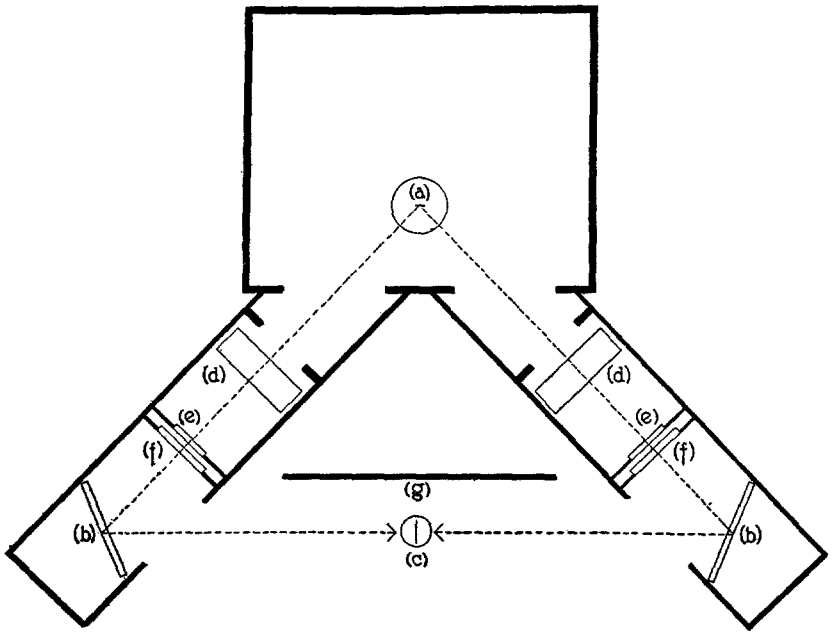

FIG. 2. Plan of apparatus with covers removed from lamp housing and beam housings (heavy lines). Dotted lines represent the light-paths coming from the 400 watt projection bulb (*a*), passing various filters (*d*), (*e*), (*f*), reflected by the mirrors (*b*) and falling on the culture of *Phycomyces* (*c*) from opposite sides. (*d*) cells containing $CuCl_2$ solution; (*e*), neutral intensity filters; (*f*), Wratten "monochromatic" filters; (*g*), screen to shield cultures on the optical bench from stray heat radiation from lamp housing.

triangle (see Fig. 2). At the middle of the third side of this triangle the opposed illuminations were equal; at other points the intensities were unequal, each being found to vary inversely with the square of its distance from the lamp, which therefore approximated a point source. The relative intensities falling on opposite sides of a culture could thus be varied by changing the position of the culture

between the mirrors, or by the interposition of neutral intensity filters (Fig. 2, *e*) in the beams before reflection. For this latter purpose Eastman neutral filters were used. Special photometric calibration of these filters is essential, since the manufacturer's transmission values were found to deviate by as much as 10 per cent from the true values.

In order to secure relatively monochromatic light of high intensity, a 400 watt, 115 volt, plane-filament projection lamp was used as the source of illumination, in conjunction with Wratten "monochromatic" filters interposed in the two lateral beams (Fig. 2, *f*). This method of obtaining fairly monochromatic light has been

TABLE I

Relative spectral energy distribution of 400 watt, 115 volt projection lamp, and percentage transmission of glass cell containing a layer 3.3 cm. thick of 0.065 molar $CuCl_2 \cdot 2H_2O$.

Wave-length	Relative energy of source, color temp. 3000°K.	Transmission of $CuCl_2$ screen	Wave-length	Relative energy of source, color temp. 3000°K.	Transmission of $CuCl_2$ screen
(*mμ*)		(*per cent*)	(*mμ*)		(*per cent*)
350	0.062	—	490	0.614	89.5
360	0.084	—	500	0.673	88.8
370	0.105	—	510	0.738	88.0
380	0.130	—	520	0.804	87.0
390	0.157	—	530	0.870	85.8
400	0.187	66.0	540	0.934	84.0
410	0.223	72.5	550	1.000	82.3
420	0.262	76.0	560	1.068	79.9
430	0.301	82.5	570	1.145	76.0
440	0.344	85.5	580	1.203	71.5
450	0.392	87.3	590	1.268	66.5
460	0.443	88.7	600	1.334	60.0
470	0.495	89.5	610	1.400	53.8
480	0.554	89.8	620	1.465	47.5

described in detail by Hecht (1920–21), Bachmann (1929), and others. Infra-red radiation was screened from each beam by a glass cell containing a solution 3.3 cm. thick of 0.065 molar $CuCl_2 \cdot 2H_2O$. The percentage transmission factors of this filter were determined spectrophotometrically throughout the visible spectrum and used in the subsequent computations.

The relative energy content of each "monochromatic" beam was then calculated as follows: (1) the energy of the source at each wave-length was taken from a calibration of a similar 400 watt, 115 volt projection lamp having a color temperature of 3000°K, the relative energies being calculated by the use of Wien's equation (Table I) (see Forsythe, 1919 Report of Standards Committee on Py-

rometry, 1920). (2) The energy of the lamp at each 10 mμ interval was then multiplied in turn by the transmission factor for that wave-length of each of the filters interposed in the beam of light, *viz.*: the transmission factors of the copper chloride screen (Table I), of the neutral intensity filter (if used), (Table III), and of the particular Wratten filter interposed (Wratten Light Filters, Rochester, 1922). (3) The resulting values of transmitted radiation in 10 mμ steps were plotted on a large scale against wave-length, and the total areas under the curves measured. These areas of total energy transmission were taken as the relative intensities of radiation of the source in the spectral region transmitted. (4) The intensity of a beam at a particular point was calculated by the inverse square law.

Loss of intensity through reflection by the mirrors, which were cut from the same piece, was assumed to be uniform as regards wave-length, and was not corrected for except in the case of the Wratten ultra-violet filter No. 18. Here the path of light through the glass of the mirror totalled 13 mm., a thickness of crown glass

TABLE II

Transmissions calculated from data of Gibson, Tyndall, and McNicholas (1920) for 13 mm. of "nearly neutral" crown glass.

Wave-length	Percentage transmission
(mμ)	
350	33.0
360	57.0
370	63.0
380	73.0
390	75.0

sufficient to absorb effectively all ultra-violet below 350 mμ. The transmissions calculated for this thickness of glass according to the figures of Gibson, Tyndall, and McNicholas (1920) are given in Table II. The energy of the source at each wave-length was multiplied by these factors in obtaining the total energy transmitted by Filter No. 18. Further, in order to get as much of the near ultra-violet as possible, the strongly absorbing copper chloride screen was not used in conjunction with this filter. Absorption of the near ultra-violet by the glass wall of the projection lamp was undetermined, and not corrected for, although the allowance which may be made for this in the final result will be pointed out shortly.

The apparatus comprising lamp and housing, beam housings, filters, mirrors, and optical bench on which the cultures were placed (see Fig. 2) was set up horizontally on a table in a dark room which maintained a relatively constant temperature (18.9 – 20.7°C.) during the duration of all the experiments. Pure cultures of *Phycomyces blakesleanus* Burgeff ("+" strain) were grown on wet, sterilized flour paste in small glass vials of 2.5 cm. diameter. Prior to experimen-

tation, sporangiophores were developed by keeping the cultures in a warm, moist chamber illuminated from above by light of constant intensity. At the end of some hours there was obtained a good growth of young, vertical sporangiophores 2–5 cm. tall and equally light-adapted. In order to obviate shading within a culture, and also to reduce the loss of moisture on the optical bench, each glass culture vessel was provided with a metal cover having a slit 1–1.5 mm. wide cut in it. This device allowed a limited number of sporangiophores to grow up through the slit in practically a single row, precluding shading when the slit was placed across the axis of the beams of light.

Comparable cultures of equally light-sensitive sporangiophores were placed singly between the mirrors and allowed to remain undisturbed except for the bilateral illumination, for a minimum of 4 hours. At the end of this time the number of sporangiophores bent toward each source and those remaining "indifferent" were recorded. When the general zone of phototropic balance was determined, it was systematically explored in a series of separate experiments by placing cultures at successive small intervals along it. Thus for each pair of opposed monochromatic beams there was obtained a relation between the position on the optical bench and the ratio of the sporangiophores in a culture bent in one direction to those bent in the other. These relations were plotted, and graphically interpolated to find for each the locus of equal phototropic bending. At some distance from the equalization-point the plots are frequently irregular, but are surprisingly smooth near the point of equal phototropic effect. The equalization point was determined to within 2 cm. in the case of the least phototropically effective spectral regions where orientation is least precise. Assuming the measured end-point to be near the middle of the optical bench, a maximum error of 2 cm. in the setting would produce at most an intensity change of 3 to 4 per cent in the calculated intensity of each beam.

III

Four separate experiments were performed in equating the phototropic effects of different spectral regions. These are detailed in Table III. Since it was not convenient to equate the spectral regions in turn against one constant one, the four series have to be brought to the same common denominator of intensity by simple proportionality.

The results of this calculation are given in Table IV, graphically in Fig. 3.

It is clear from Fig. 3 that the short wave-lengths of the visible spectrum are the most effective in exciting the light-sensitive system of *Phycomyces*, with a maximum probably in the extreme visible between 400 and 430 mμ. The sensitivity in the near ultra-violet is striking, and should be even greater than the calculated value since absorption by the glass wall of the projection lamp is not corrected for. Toward the longer wave-lengths there is a sharp drop in sensitivity to a value of almost nothing at 580 mμ.

TABLE III

Distance necessary for phototropic balance for each pair of opposed "monochromatic" beams of light obtained by the use of Wratten filters.

Series	Left beam			Right beam		
	Wratten filter no.	Percentage transmission of neutral filter (if used)	Critical distance from culture to lamp for phototropic balance	Wratten filter no.	Percentage transmission of neutral filter (if used)	Critical distance from culture to lamp for phototropic balance
			(*cm.*)			(*cm.*)
1	76	—	38.5	75	—	32.7
2	74	—	41.0	75	0.38	30.2
3	74	50.8	40.0	73	—	31.2
4	76	23.6	39.8	18*	—	51.2

* Copper chloride screen not used in right beam of this experiment.

Blaauw (1909) determined the relative efficiencies of different wave-lengths of light in causing just perceptible bending of the sporangiophores of *Phycomyces nitens*. His results are also plotted in Fig. 3, and show a maximum at 495 mμ with decreasing sensitivity in the blue. The displacement of the maximum some 80 mμ toward the blue end of the spectrum in the present experiments may reflect a specific difference between the photosensitive systems of the two species of *Phycomyces*, or distinct states of sensitivity due to the different circumstances of experimentation. Blaauw worked with relatively brief exposures (between 16 and 192 sec.) to a narrow region of a dispersed spectrum of low intensity, whereas in the present experi-

TABLE IV

Relative effectiveness of different spectral regions in stimulating *Phycomyces*.

Wratten filter no.	Wave-lengths between which are contained 1/2 of energy transmitted by filter as used in the experiment	Relative energy necessary to produce same phototropic effect	Relative effectiveness in stimulation = reciprocal of relative energy necessary to produce the same phototropic effect; No. 76 = 100
	($m\mu$)		
76	429–448	0.0124	100.0
18	356–376	0.0248	50.0
75	477–495	0.0474	26.2
74	521–535	7.82	1.6
73	564–580	10.3	0.12

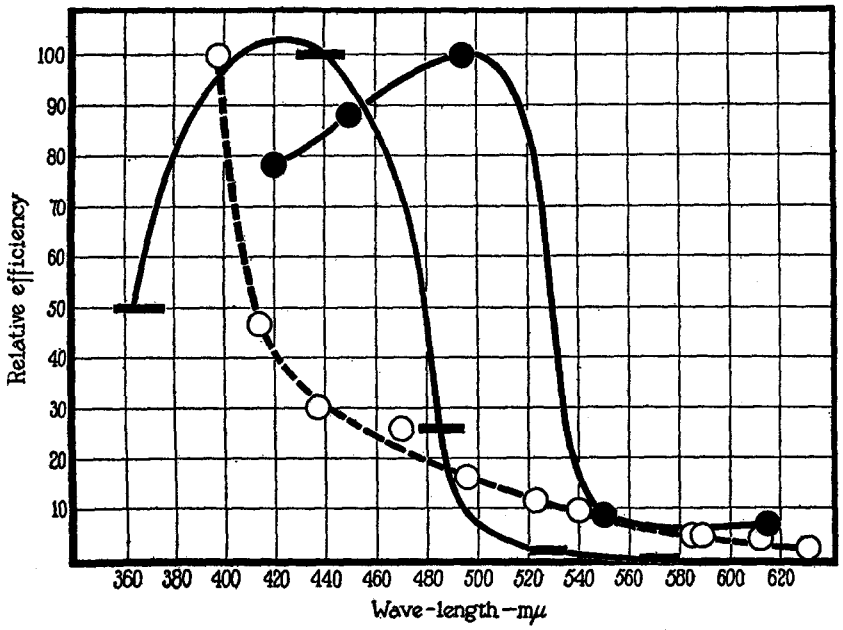

FIG. 3. Relative efficiency in stimulation of different wave-lengths. Solid bars, *Phycomyces blakesleanus*, data from Table IV. The length of each bar represents the range within which one-half of the energy transmitted by each combination of filters is contained. Solid circles, *Phycomyces nitens*, data from Blaauw (1909). Open circles, *Pilobolus*, calculated from data of Parr (1918). The most effective point in stimulation of each series of measurements is arbitrarily put at 100.

ments wider spectral regions of much greater intensity were allowed to act for 4 hours. More pigment initially present in Blaauw's sporangiophores would cause relatively less effective absorption in the blue, assuming a strong screening action in that region by the pigment. Preliminary experiments indeed show this to be the region of greatest absorption of the pigments extracted from the sporangiophores by acetone or other fat solvents.

IV

Since the work of Blaauw (1909) little of quantitative significance has been learned about the photosensitivity of fungi as related to wave-length. An exception is the study by Parr (1918) of the phototropic responses of *Pilobolus*. If a proper measure of sensitivity is chosen, namely, the reciprocal of the amount of energy necessary at each wave-length to produce the same phototropic effect, the data of Parr can be plotted in the form of an absorption spectrum (see Fig. 3). In order to compute the amount of energy needed, the validity of the Bunsen-Roscoe law must be assumed for exposure times of 50–80 minutes duration. While there are objections to this assumption, the procedure is the only way of properly expressing the results of the experiments, which the author failed to do. Thus interpreted, the data of Parr show that the young sporangiophores respond to light over the whole visible range of wave-lengths, with regularly increasing sensitivity down to 398 mμ, where the experiments ceased. No maximum of sensitivity was found within this range.

Numerous studies relating wave-length and sensitivity have been made with other sessile plants such as the coleoptiles of *Avena* (Blaauw, 1909; Koningsberger, 1922; Sonne, 1929; Bachmann and Bergann, 1930). With certain differences, all agree in showing the greatest sensitivity to be within the region 425–475 mμ, although there is no reason to suppose that the plants used in the different experiments were equally light- or dark-adapted, or were similarly etiolated. Studies of the photosensitivity of free-swimming, green flagellates show their respective maxima to be even more displaced toward the red, *e.g.*, at 483 mμ for *Euglena* and *Gonium* (Mast, 1917); at 494 mμ for *Volvox* (Laurens and Hooker, 1920).

It is clear that the photosensitive systems of fungi about which

we have significant information absorb more effectively in the short wave-lengths of light than do those of chlorophyll-containing organisms or of animals. It is therefore of particular interest to know what rôle, if any, in the photic responses of such fungi is played by their pigments which absorb the short wave-lengths.

SUMMARY

Under the circumstances of experimentation described, the sporangiophores of *Phycomyces* are found to be most sensitive to stimulation by light in the violet between 400 and 430 mμ. Toward the red, sensitivity falls to nearly zero near 580 mμ, while in the near ultra-violet around 370 mμ, sensitivity is still high. The previous experiments of Blaauw had placed the point of greatest sensitivity some 80 mμ nearer the red end of the spectrum. Because of the known presence in the sporangiophores of *Phycomyces* of "accessory" pigments, care must be taken in identifying such results with the absorption spectrum of *the* photosensitive substance.

REFERENCES

Allen, R. F., and Jolivette, H. D. M., *Tr. Wisconsin Acad. Sc.*, 1914, **17**, Part I.
Bachmann, F., *Planta*, 1929, **8**, 486.
Bachmann, F., and Bergann, F., *Planta*, 1930, **10**, 744.
Bergann, F., *Planta*, 1930, **10**, 666.
Blaauw, A. H., *Rec. trav. bot. Néerl.*, 1909, **5**, 209.
Blaauw, A. H., *Z. Bot.*, 1914, **6**, 641.
Castle, E. S., *J. Gen. Physiol.*, 1929–30, **13**, 421.
Forsythe, W. E., 1919 Report of Standards Committee on Pyrometry, *J. Opt. Soc. America*, 1920, **4**, 305.
Gibson, K. S., Tyndall, E. P. T., and McNicholas, H. J., *Technolog. Papers Bureau Standards, No. 148*, 1920.
Hecht, S., *J. Gen. Physiol.*, 1920–21, **3**, 375.
Koningsberger, V. J., *Rec. trav. bot. Néerl.*, 1922, **19**, 1.
Laurens, H., and Hooker, H. D., Jr., *J. Exp. Zool.*, 1920, **30**, 345.
Massart, J., *Bull. Acad. roy. Belgique*, 1888, **16**, 590.
Mast, S. O., *J. Exp. Zool.*, 1917, **22**, 471.
Oehlkers, F., *Z. Bot.*, 1926, **19**, 1.
Parr, R., *Ann. Bot.*, 1918, **32**, 177.
Pringsheim, E. G., and Czurda, V., *Jahrb. wissensch. Bot.*, 1927, **66**, 863.
Sonne, C., *Strahlentherapie*,, 1929, **31**, 778.
Van der Wey, H. G., *Proc. Acad. Sc. Amsterdam*, 1929, **32**, 65.
Wratten Light Filters, Rochester, Eastman Kodak Company, 5th edition, revised, 1922.