The Lens Effect and Phototropism of *Phycomyces*

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ABSTRACT Normally, the dioptrics in air of the cylindrical sporangiophore of *Phycomyces blakesleeanus* confer on the distal side a focusing advantage of about 30 per cent for unilateral stimuli of parallel light. This advantage can be nullified or reversed to produce negative curvatures by means of diverging light stimuli. A thin cylindrical glass lens was positioned 0.15 mm from the light-adapted growing zone with its long axis parallel to the long axis of the sporangiophore. A 3 minute blue stimulus was given and the lens removed. Reproducible negative curvatures were observed with a maximum of 13 degrees occurring within 8 minutes after the beginning of the stimulus. Experiments in air were done in a water-saturated atmosphere to minimize avoidance responses due to the proximity of the lens. The data support Buder's conclusion that the focusing advantage is the principal mechanism which produces the response differential necessary for phototropism. When the lens advantage is small, the attenuation becomes important in determining the direction of the response. Data obtained from sporangiophores immersed in inert liquids indicate that the attenuation is about 14 per cent. Therefore, whenever the focusing advantage is less than 14 per cent, negative curvatures are produced by unilateral stimuli.

The dioptric properties of the cylindrical, nearly transparent growing zone of *Phycomyces* sporangiophores were suggested by Blaauw (1918) as being the principal mechanism for producing the differential elongation necessary for positive phototropism. This idea was soon confirmed by Buder (1918, 1920) who found that negative curvatures were produced by unilateral stimuli if sporangiophores were immersed in paraffin oil. The light rays within the cell in this case, diverge since the external index of refraction (n) 1.47, is higher than that of the cell, 1.38 (Castle, 1933 *a*) and the difference in elongation of the two sides of the cell is reversed.

Additional experimental tests of this lens effect were made (reviewed by Brauner, 1954 and Banbury, 1959) and general agreement reached concerning its importance. However, Ziegler (1950) found a similar reversal in the direction of phototropic response for *Avena* coleoptiles immersed in paraffin oil. The relatively massive, multicellular, sensitive tip of *Avena* led him to reject the plausibility of a lens effect for this tissue. He suggested that the reversal was caused by a change in the surface charge of the cells when irradiated, due to the dielectric properties of the paraffin oil. Thus, when he extrapolated to the responses of *Phycomyces*, he concluded that reversal of the response direction was not caused by a simple change of the light path from convergence to divergence within the cell, but rather by altered surface properties of the sporangiophore. This alteration was caused either by partially anaerobic conditions or the dielectric properties of the oil.

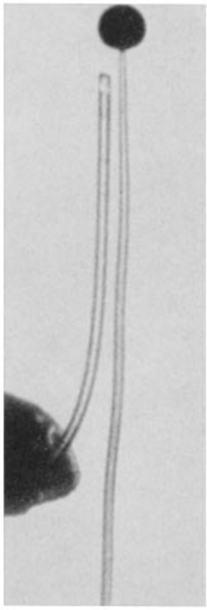

FIGURE 1. Typical stage IVb sporangiophore with cylindrical lens juxtaposed. The diameter of the lens is 0.16 mm.

In order to test whether altered surface properties of immersed sporangiophores were responsible for the change in response direction, it occurred to us that the light path within the cell could also be made to diverge by irradiating the sporangiophore in air with a diverging beam of light. If the divergence of the light stimulus could be made large enough, the resultant light path within the cell could be made to approach that in paraffin oil (see Castle's diagram, 1933 *b*).

Theoretically, the ideal situation would be to align two sporangiophores so that, for a unilateral stimulus of parallel light incident in a plane connecting their long axes, the focal region of the first sporangiophore would bisect the distance between them. In this situation the focusing advantage in the second sporangiophore would be symmetrically reversed. The first sporangiophore would give a positive curvature and the second a negative curvature. Experimentally this is not possible to do with stage IVb sporangiophores because of the large size of the sporangium. However, a thin glass cylindrical lens (Fig. 1) with approximately the dimensions of the sporangiophore might be placed close enough to the sporangiophore to reverse the focusing advantage (Fig. 2) during a unilateral stimulus and to demonstrate the function of the lens effect in determining the resultant direction of phototropic curvature.

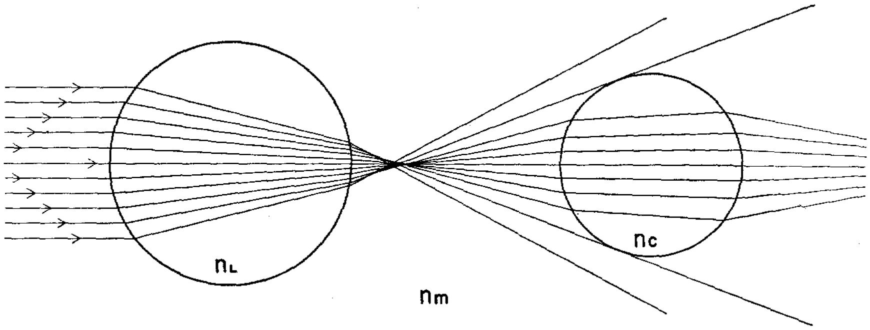

FIGURE 2. Ray diagram of light path for the cylindrical glass lens and sporangiophore for experiments described in Figs. 4 and 5. n_L, n_M, and n_C designate the index of refraction of the lens (1.50), medium (1.00), and cell (1.38) respectively. The lens diameter is 0.16 mm and cell diameter is 0.12 mm. The lens is positioned 0.14 mm from the cell.

MATERIAL AND GENERAL METHODS

Stage IVb sporangiophores of *Phycomyces blakesleeanus* (sexually negative strain 1555 of the National Regional Research Laboratory) were grown on 5 per cent potato-dextrose-agar in glass shell vials. All experiments were performed at 22°C and observations made with a phototropically inactive red safelight.

For unilateral stimulation, parallel blue light was obtained from a monochromatic blue source, consisting of a tungsten projection lamp, collimating system, 10 cm path length of 5 per cent aqueous $CuSO_4$ to remove infrared, and a 440 mμ interference filter. The collimated beam had a divergence of less than 3.5 degrees and an intensity of 0.18 μw/cm^2. For obtaining straight sporangiophores, plane mirrors were inserted into the beam so that the light was incident at 60 degrees to the long axis of the sporangiophore and the sporangiophores were rotated at 2 RPM (Fig. 3). For the lens experiments the sporangiophores and lens were enclosed in a lucite chamber in which the relative humidity was maintained at 98 per cent or greater. For the liquid immersion experiments, the same system was used with the lucite cover and micromanipulator removed.

METHODS AND RESULTS

A. *Tropic Responses with and without Lens* Sporangiophores were adapted to parallel blue light incident at 60 degrees to the long axis of the sporangio-

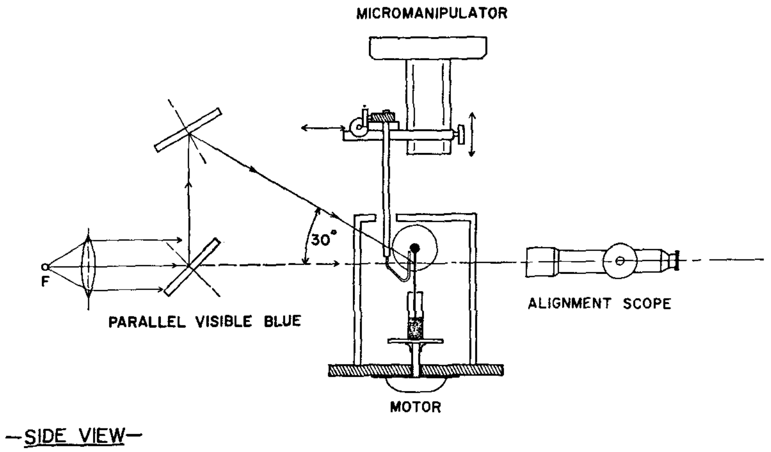

FIGURE 3. Schematic diagram of apparatus for producing negative curvatures to diverging blue light stimuli. A measuring scope was also used normal to the plane of the diagram with a red observing field designated by the circle around the sporangium. An eyepiece protractor measured the angular position of the sporangiophore to the nearest degree. During alignment of the lens a second red safe-light was used to illuminate the field of the alignment scope.

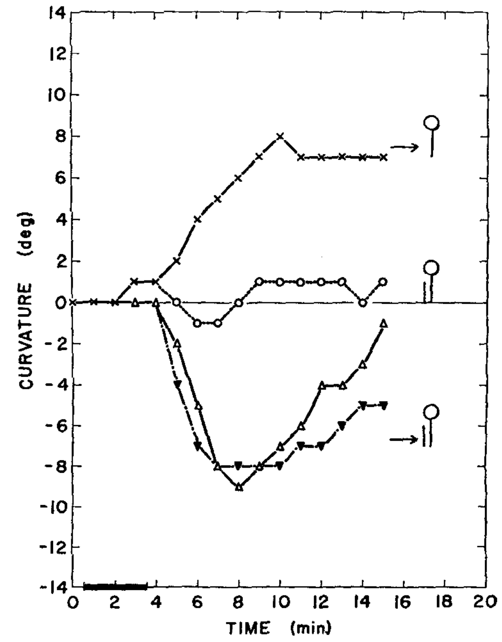

FIGURE 4. Responses to unilateral stimuli. The experimental conditions for each curve are represented schematically with the arrow denoting parallel blue light. The vertical position of the long axis of the sporangiophore is taken as zero degrees curvature with positive curvatures toward the source and negative curvatures away from the source. The heavy bar on the time axis designates the stimulus interval.

phore while rotating for 45 minutes. At 30 seconds before time zero, the blue source was turned off, the rotation stopped, and the glass lens, mounted on a micromanipulator, positioned at 0.16 to 0.14 mm from the sporangiophore and parallel to it. Simultaneously, the first plane mirror (Fig. 3) was removed so that the light stimulus would be incident at 90 degrees to the long axis after passing through the cylindrical lens. At time zero the blue source was turned on and a stimulus (diverging stimulus; Fig. 2) given for 3 minutes. The blue source was then turned off and the cylindrical lens removed.

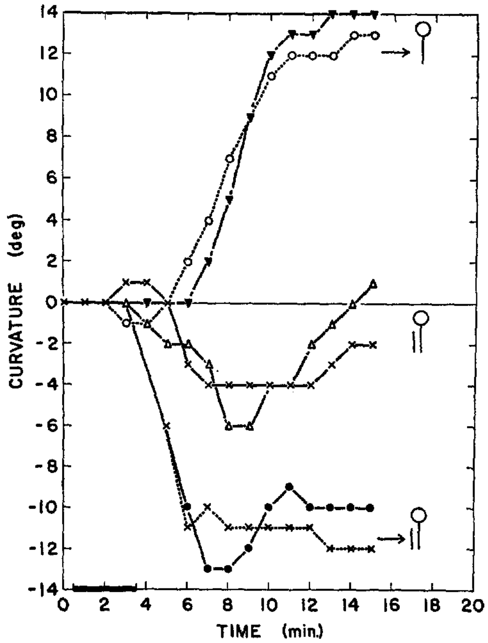

FIGURE 5. Responses to unilateral stimuli for a sporangiophore which gave avoidance responses due to the presence of the lens alone.

For the control experiments, the lens either (*a*) was juxtaposed for 3 minutes with no light stimulus, or (*b*) a 3 minute stimulus was given without the lens (parallel stimulus). The results for two typical series of experiments are presented in Figs. 4 and 5. Each figure represents the results obtained with a single sporangiophore. The angular position of the sporangiophore was determined at 1 minute intervals for 15 minutes with a protractor eyepiece and after each response, the sporangiophore was allowed to readapt while rotating for 30 minutes.

For parallel light stimuli positive curvatures (toward the source) of 7 to 14 degrees were produced. For diverging stimuli negative curvatures of 8 to 14 degrees were produced. It was found in initial experiments that the presence of the lens alone in the dark, for periods of time greater than 3 minutes would produce negative curvatures (avoidance responses) of the same order of magnitude as those produced by continuous diverging stimuli. The magnitude of these responses was found to be smaller as the relative humidity was increased within the lucite chamber and could almost be eliminated for a relative humidity of greater than 95 per cent.

Thus to minimize the spurious effects of these avoidance responses (Walter, 1921), the lens was juxtaposed for only 3 minutes and the relative humidity within the lucite chamber maintained as high as possible (greater than 98 per cent). A sliding lucite collar was placed around the lens holder at the point where it was inserted into the lucite chamber to prevent dry air from entering. Occasionally negative curvatures still occurred with these precautions. However, for such a sporangiophore showing avoidance responses, the curvature due to the diverging stimulus was even more negative and could be clearly separated from the avoidance responses (Fig. 5).

TABLE I

INDEX OF REFRACTION FOR INERT FLUORINATED LIQUIDS

Fluorochemical	Index of refraction
FC-75	1.277
FC-43	1.290
Kel F-3	1.408

B. *Tropic Responses with Immersion Fluids* Once it was clearly established that negative curvatures could be produced by diverging stimuli and the deduction made that no spurious effects were occurring with immersed sporangiophores, a series of immersion experiments were made to determine the balancing external medium for which no curvature would occur for unilateral stimuli. Presently, there are available totally inert liquids, perfluorotributyl amines and fluorinated hydrocarbons, which are miscible in all proportions (Table I). Thus, it is possible to obtain any index of refraction from 1.28 to 1.41.

Sporangiophores were inverted and immersed in fluorochemicals in a glass cuvette, 25 × 25 × 100 mm. They were adapted to the unilateral parallel blue light by rotating the cuvette with immersed sporangiophores for 45 minutes. The rotation was stopped with one face of the cuvette normal to the incident beam and the bending speed measured at 15 minute intervals and averaged over the initial 2 hour period. The angular position was also measured after a total exposure of 4 hours.

The results of average bending speeds are tabulated in Table II and the average position after 4 hours plotted in Fig. 6. For both methods, the balancing index of refraction interpolated for no curvature is about 1.295. For smaller external indices of refraction positive curvatures are produced and for larger indices of refraction, negative curvatures are produced. The large errors in determining the individual averages for Fig. 6 are probably due to variations in the index of refraction of the individual cells used, to complicating geotropic responses, and/or the variable bending speeds. The growth rate was determined for the immersed sporangiophores and for all experiments was between 2.5 and 4.0 mm per hour.

TABLE II

Index of refraction of immersion liquid	1.28	1.29	1.30	1.31	1.32	1.33
Bending speed, *degree/min.*	0.2 ± 0.1	0.1 ± 0.1	− 0.1 ± 0.1	− 0.2 ± 0.2	− 0.4 ± 0.1	− 0.3 ± 0.2

Average bending speeds of tropic responses for initial 2 hour interval of response for immersed sporangiophores. The angular position was determined at 15 minute intervals and the bending speeds for each 15 minute interval averaged, since the curvatures were measured to only the nearest degree and since the speeds were extremely variable from one interval to the next. At least 10 sporangiophores were followed for each value and the standard errors of the mean are indicated.

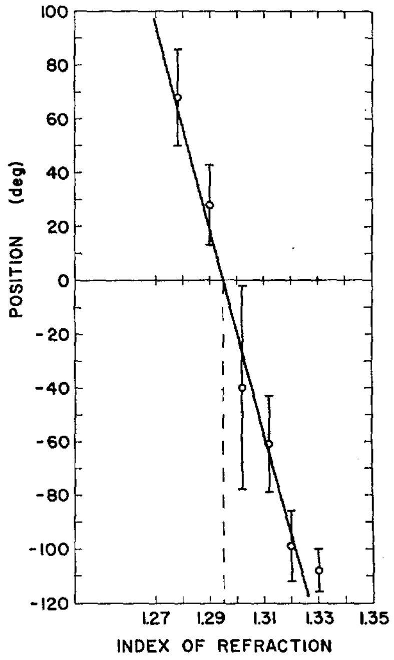

FIGURE 6. Average position, after 4 hours, of sporangiophores irradiated unilaterally while immersed in inert liquids of various indices of refraction. At least 10 sporangiophores were used for each average and the standard errors of the mean are represented. Positive curvatures denote bends toward the source from the initial inverted vertical position of the long axis of the sporangiophore.

DISCUSSION

The fact that negative curvatures can be produced by changing the focusing advantage with a diverging stimulus in air proves the function of the "lens effect" in determining the resultant direction of the phototropic response. The scale drawing in Fig. 2 is a ray diagram of the actual light path during the experiments described in Figs. 4 and 5, and it is clear that the light path through the sporangiophore is diverging.

Negative curvatures in air also indicate that Buder's interpretation of the results obtained in paraffin oil was correct. It must be concluded that neither the dielectric properties of paraffin oil nor other surface changes are involved in the negative phototropic curvatures of *Phycomyces*. Indeed, it seems more reasonable to suggest that a lens effect should be looked for in the upper, semitransparent, 200 micron long, sensitive tip of *Avena* coleoptiles (compare Nuernbergk, 1927).

The negative curvatures are complicated occasionally by avoidance responses even at these high relative humidities and short stimuli, but in every case the response was much larger than could be explained by the avoidance response alone.

The experiments with immersion liquids have the advantage that it is experimentally possible to determine the optical conditions for which no curvature is produced. For no curvature to occur, the effectiveness of the stimulus in the two halves must be equal. As shown previously (Delbrück and Shropshire, 1960) for a stimulus of unit effectiveness on the proximal side to be balanced on the distal side, the attenuation (b), due to any cause, must equal the focusing advantage (a).

Effect proximal = effect distal for no curvature.

$$1 = 1 + a - b$$

If the focusing advantage is less than that of the attenuation or negative, negative curvatures result. If greater, positive curvatures are produced.

It should be emphasized, moreover, that this derivation does not say anything critical about the relative importance for the phototropic response mechanism of total path length, intensity distribution, greater mechanical advantage of receptors near the midline, or photoreceptor distribution within the sporangiophore. a and b are simply the *fractional* changes in stimulus effectiveness due to refraction and absorption, respectively. In order to compare these effects quantitatively, it is assumed that a is directly proportional to 1 minus the ratio of the surface area intercepted on the distal side after refraction to the area intercepted on the proximal side. (For the values calculated below, an incident area bounded by rays having an angle of incidence to the cell surface of 45 degrees was considered.) The attenuation b is assumed directly proportional to the average decrease in per cent transmission across the growing zone; *i.e.*, to the absorptance (1 minus the transmission).

The external medium required for no curvature to a unilateral stimulus of parallel light has an index of refraction near 1.295. The calculated value of the focusing advantage for this medium indicates that there is a residual focusing advantage of about 14 per cent necessary to balance the attenuation. Previous direct spectrophotometric estimates for the attenuation of the growing zone gave an optical density of about 0.10 in the visible (Delbrück and Shropshire, 1960).

Stifler (1961) observed that sporangiophores immersed in water were negatively phototropic to unilateral stimuli. For such an external medium, the residual focusing advantage is much smaller than the attenuation. Thus the negative curvature found is in agreement with the results obtained for fluorochemical immersion with an index of refraction of 1.33.

The importance of attenuation for determining the resultant response direction could be demonstrated near the balance condition in two ways. Several sporangiophores were allowed to bend in liquid, $n = 1.30$, while being irradiated unilaterally at 480 mμ. The average position after 3 hours was found to be -21 degrees. When the source was changed to 518 mμ, the average position 3 hours later had shifted to $+34$ degrees. This shift can be explained by a decrease in the attenuation since the focusing advantage remains the same. Such a decrease would be expected for this wavelength change since the absorption of carotenoids known to be present, with an order of magnitude of 0.03 OD units superimposed on the background attenuation, decreases by about tenfold in this wavelength region. It is doubtful from action spectra comparisons (Delbrück and Shropshire, 1960), that a significant proportion of this decrease in attenuation could be explained by a decrease in self-screening of the photoreceptor.

Second, sporangiophores which had been cultured on agar with 0.1 mg/ml of 2,3,5-triphenyltetrazolium chloride (Abbot and Grove, 1959), resulting in a bright pink appearance of the growing zone, were immersed in the same liquid. The tropic response was negative and the average position after 3 hours shifted from -21 degrees for normal sporangiophores to -95 degrees. In this case, the attenuation is certainly increased by foreign screening and is much greater than the residual focusing advantage. In exactly the same manner, the increased attenuation due to gallic acid absorption in the ultraviolet produces the phototropic insensitivity to unilateral stimuli at 310 mμ and negative curvatures at shorter wavelengths by balancing out or exceeding the focusing advantage (Delbrück and Shropshire, 1960).

The bending speeds are less significant than the use of the average position determinations for determining the balancing external medium. Two reasons are: (*a*) the variable onset time for curvature, primarily an experimental difficulty for such small bending speeds, and (*b*) the variable viscosity of the fluorochemical solutions (0.65 to 5 centistokes). However, the bending speed does appear to depend on the net difference in effectiveness on the two sides of the sporangiophore. It is a curious fact, without apparent explanation other than a complication due to the avoidance response, that for the negative curvatures in air, the time of onset was consistently earlier (Figs. 4 and 5) than for positive curvatures. There was no significant change in bending speed when all experiments with the lens were compared for positive *versus* negative curvatures.

The final conclusion from these experiments is that the resultant direction of the phototropic response can be explained readily and solely in terms of the lens effect and attenuation, without in any way resorting to two photoreceptor systems (Thimann and Curry, 1960) or to changes in the surface properties caused by immersion in liquid media (Ziegler, 1950).

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