Interplay between the Reactions to Light and to Gravity in *Phycomyces*

D. VARJÚ, LOIS EDGAR, and M. DELBRÜCK

From the Division of Biology, California Institute of Technology, Pasadena. Dr. Varjú's present address is the Max Planck Institut für Biologie, Abteilung Reichardt, Tübingen, Germany

ABSTRACT Sporangiophores of *Phycomyces* do not grow directly towards a horizontal beam of light, but equilibrate at an angle of about 30° above the horizontal. After describing several related observations, this paper suggests that the dioptric properties of an obliquely illuminated cylindrical lens, illustrated by a dummy cell, as well as a negative geotropic response, play major roles in determining the direction of growth. The shift of the equilibrium direction of growth towards the vertical, or a purely geotropic response, over a tenfold range of very low intensities (around 10^6 quanta/cm^2 sec., or 10^{-13} watt/cm^2) has been studied, and an action spectrum made, measuring the quantum fluxes producing a standard intermediate equilibrium direction of growth at different wavelengths. This may differ from the action spectra at higher intensities in lacking conspicuous maxima from 370 to 490 mμ. However, in the ultraviolet it parallels the other spectra, although without showing the much higher quantum efficiency of ultraviolet relative to visible light previously noted. Possible interpretations are discussed.

The sporangiophores of the mold *Phycomyces* are cylindrical single cells which, when mature and growing at a nearly steady rate display responses to light and to gravity. Since these sporangiophores offer unique properties for the study of photoreception, there exists a rich literature on their responses to light. Much less is known about the geotropism of these cells. This paper attempts to gain a deeper insight into the interaction of these two sensory mechanisms.

When the sporangiophores of *Phycomyces* are illuminated with a horizontal beam of light, they do not grow directly towards the light, but in a direction 30° up from the horizontal (Fig. 1*a*). The "up" part of this statement is undoubtedly due to gravity. Even if the experiment is started with the sporangiophore pointing straight down, the final direction of growth is as described. The 30° part merits closer analysis. Dennison (1958) showed that this angle is constant over an enormous range of intensities (a factor of 10^9). He also showed that if the angle of incidence of the light is varied, the re-

sulting direction of growth behaves approximately as the resultant of two vectors of constant length, a vertical gravity vector, and a phototropic vector, in the direction of the light, about twice as long as the gravity vector. That the length of the phototropic vector should be independent of the intensity is reasonable enough in view of the fact that the sporangiophores are known to adapt to light over a wide range of light intensities.

There are, however, other aspects which do not fit the interpretation of the limiting angle of 30° as a resultant between a geotropic and a phototropic

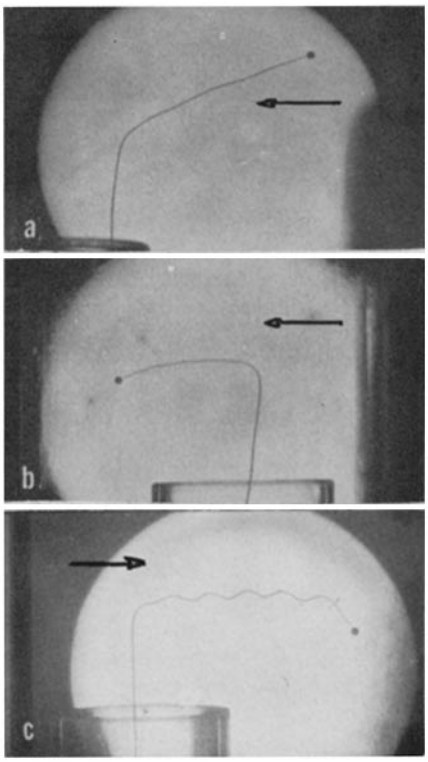

FIGURE 1. Photogeotropic equilibria at medium intensities. (*a*) Illumination with visible light. Sporangiophores in air. (*b*) Illumination with visible light. Sporangiophores in mineral oil. (*c*) Illumination with light of wavelength 280 mμ, sporangiophores in air. The arrows indicate the direction of light incidence. The "frozen waves" which are present in all three cases, but most pronounced in case (*c*), are the result of "hunting" during growth (Dennison, 1958). The direction of the terminal part of the specimen shows the momentary direction of the growing zone. Because of hunting, this direction is not an indication of the average direction of growth. The "hunting cycle" varies from 40 to 60 minutes.

vector: the sporangiophores grow *strictly* in the horizontal direction under two conditions where the above argument would lead one to expect a similar compromise angle. These conditions are: (*b*) when immersed in mineral oil and illuminated with visible light (Fig. 1 *b*), and, (*c*) when in air and illuminated with light of wavelengths below 300 mμ (Fig. 1 *c*).

Under both these conditions the specimens are *negatively* phototropic. Qualitatively we understand the reasons for this reversal in the sign of the phototropism. One assumes following Buder (1918) that it is the strong *convergence* of the light beam on the distal side which normally gives that side an advantage over the proximal side. This advantage is lost in mineral oil,

because in this condition the lens is not a converging one, and it is lost in the ultraviolet, because the distal side is shielded by an internal screen (gallic acid, Dennison, 1959).

These are good reasons for explaining why phototropism is negative under these two conditions, but they do not explain why the geotropic component does not seem to compete the same way as it appears to do under normal conditions. The geotropic component *does* show up also under conditions (*b*) and (*c*), if the intensity is lowered to exceedingly low levels, to levels, in fact, at which also for blue light in air the equilibrium direction of growth deviates more than 30° from the horizontal. This, therefore, is the range where we are dealing with a true competition of the *intensity* of the light *versus* gravity, whereas at higher intensities other aspects of the illumination must be involved in the explanation of the equilibrium direction of growth.

One might think that shading of the sporangiophore by the sporangium causes the bending to stop at the limiting angle of 30°. It is true that some shading takes place in the case of the normal positive tropic reactions where we find the deviation of 30°, and not in the two cases of negative tropic reactions where we do not find it. It is easily shown, however, that at an angle of 30° the shadow covers only the top 0.25 mm of the sporangiophore. This area contributes only a negligible amount to the photosensitivity of the specimens (Delbrück and Varjú, 1961). Even at an angle of 10° from the horizontal less than half the growing zone would be shaded.

A likely explanation can be developed from a closer analysis of the dioptric properties of the sporangiophore when exposed to oblique illumination. On the proximal side the distribution of intensity is a cosine function of the azimuth angle φ around the periphery of the sporangiophore, modified by losses from reflection. On the distal side the distribution as a function of azimuth is very different. When illumination is at right angles to the axis of the specimen ($\alpha = 90°$) a sharply bounded band is illuminated covering about one-fifth of the distal side, with an intensity distribution which has maxima at the edges. The sharpness of the edges, and the maxima at the edges, are brought about by the fact that the most marginally incident rays are refracted so as to cross the less marginally incident ones inside the cell (*cf.* Fig. 13C of Reichardt and Varjú, 1958). In terms of wave fronts this feature can be described by saying that the wave front "folds in" during its passage through the specimen. When the specimen is tilted relative to the incident light, the illumination on the distal side changes as follows: the band at first narrows while remaining sharply edged and with maxima at the edges. At a certain angle, about 37°, before the band has narrowed to zero width, it develops "wings," *i.e.*, there is now at the edges not a drop to zero intensity but to a finite lower intensity, followed by a gradual decline to zero. These

wings are due to marginal rays which are refracted so strongly that they hit the distal side beyond the heterolateral edge. As the specimen is tilted further the band finally narrows to zero width (at about 32°) and has very pronounced wings. At this point the paraxial rays are in focus on the distal side. The intensity distribution now has a cusp. On further tilting the cusp is smoothed out rapidly so that the distribution becomes somewhat similar to that on the front side with a maximum in the middle and a smooth decline towards the margins.

Fig. 2 illustrates these facts with photographs of a dummy specimen.

We now postulate that the intensity of the tropic stimulus changes appreciably with the light *distribution* on the distal side, and we note that this distribution changes rapidly near 30°, the angle of equilibrium under normal conditions. Under this postulate it is still true, then, that this angle is a compromise angle between geo- and phototropic tendencies, but it is a compromise which at present is unsuited for the study of the interplay between the two reactions, because the tropic reaction by itself doubtless changes rapidly, and in a fashion as yet unknown in its details, in the vicinity of this compromise angle.

We therefore turn our attention to a situation in which the compromise between the two tropic tendencies is more clearly defined, namely, at very low intensities. As mentioned earlier, there exists an intensity range in which the angle of equilibrium gradually moves from 30° above the horizontal to the vertical as the intensity decreases. We have studied this transition for the whole wavelength range of the action spectrum.

METHODS

At the lowest intensities the time course of the tropic reaction is somewhat different from that in the normal range. It may take more than an hour before the equilibrium direction of growth is reached. At higher intensities the tropic reaction generally starts much earlier (the precise time depending on the intensity and on the previous adaptation history), and it may show "hunting," *i.e.* an oscillation around the equilibrium direction of growth, with a period of 40 to 60 minutes, and sometimes very large amplitudes (Dennison, 1959 *b*). If hunting is present the specimen shows in the end a "frozen wave" in the stalk, and the tangent to such a frozen wave is an unambiguous measure of the equilibrium direction of growth. Hunting is most pronounced in the ultraviolet and Fig. 1 *c* shows a good example of it. However, frozen waves are never present at the lowest intensities. At all intensities an exposure of 5 to 6 hours is sufficient for obtaining a reasonably accurate measure of the equilibrium direction of growth.

For any one specimen this direction remains fairly constant for many hours, except for the hunting at higher intensities just described. In contrast to this intraspecimen constancy there is, at all wavelengths and for all strains, a considerable interspecimen variability. Presumably this has to do with slight variabilities in such

properties as diameter, pigmentation, and stiffness, each of which could affect strongly the very delicate equilibrium between the two tropic tendencies. In terms of experimentation, this means that averages have to be taken over large numbers of specimens and a wide intensity range has to be covered. To accomplish this conveniently an

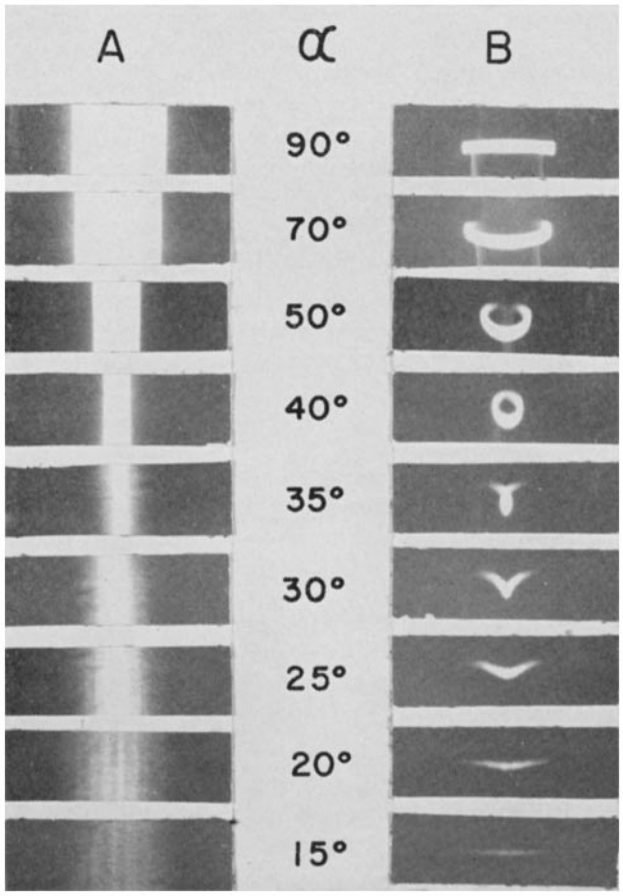

FIGURE 2. Intensity distribution on the distal side of a transparent rod, illuminated obliquely with a parallel beam of light. A thin-walled glass tube is filled with a sucrose solution matching the ptotoplasm in refractive index. Parallel light strikes the proximal side at an angle α with the long axis of the tube. The distal side is covered along its entire length with transparent diffusing tape exhibiting the illuminated portions. For $\alpha > 40°$ one obtains a sharply edged illuminated strip. For smaller angles the intensity distribution changes radically as described in the text. The photographs in series A show the intensity distribution in this strip for different angles α. The striations for $\alpha < 20°$ are an artifact due to multiple reflections in the glass wall of the tube. Series B shows the intensity distribution on the distal side of light entering through a narrow equatorial slit on the proximal side. It exhibits more clearly the nature of the singularity at $\alpha = 30°$, where the paraxial rays are in focus on the distal side. Note that the illuminated strip at its broadest ($\alpha = 90°$) covers only about one-fourth of the distal side.

arrangement was made by which eight batches of specimens could be tested simultaneously at eight different intensities, differing in steps of two or four. The arrangement is described in detail in Fig. 3 and its legend.

As light source the monochromator of a Beckman DU spectrophotometer was used. The emergent flux was controlled by changing the slit widths of the monochromator. In the visible region a diffusing plate and a condenser lens were used to obtain a large and homogeneous field.

For the work described in this paper, strain DEL, a sexually negative wild-type strain, NRRL 1555, was used and cultured on potato dextrose agar in small shell vials.

General procedure for measurements: a wavelength is selected and the intensity at the last mirror is set to a value near the threshold intensity: four to six shell vials, each containing one to three sporangiophores, are placed in each of the reflected

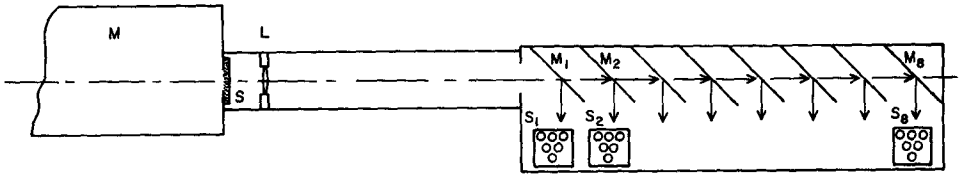

FIGURE 3. Illumination arrangement for simultaneous tests at eight different intensities. Top view. Light from the monochromator M passes through a scatterer S, and a collimating lens L, and then enters a light-tight box. Here it is partially reflected, partially transmitted by each of eight mirrors (M_1; M_2 . . . M_8). The specimen holders (S_1; S_2 . . . S_8) each hold six vials with one to three specimens each. For work in the visible, projection slide glass plates were used (3¼ × 4 inches) coated for 50 per cent transmission with MgF_2. For work in the ultraviolet, Corning 9-54 filters were used (3⅛ × 3⅛ inches, 2 mm thick), coated for 25 per cent transmission.

light beams. The specimens are left for 5 to 6 hours. At the end of this period the equilibrium angles are measured by shadowing the specimens onto a protractor, the direction of projection being at right angles to the direction of the stimulating light. Previous observers have noted that at these low intensities the direction of the tropic reaction is not exactly in the plane of incidence of the light, but deviates from it by an "angle of declination" (Buder, 1946), which is always closkwise when looking at the specimen from above. We, too, have found declinations up to 30° at the lowest intensities. These declinations do not introduce ambiguities into the measurement of the tropic reaction in the plane of incidence.

RESULTS

Fig. 4 shows typical curves relating the equilibrium direction of growth to the logarithm of the intensity, expressed in terms of quantum flux. At any one wavelength the shift from one limiting angle to the other occurs over a range of three to four $\log_2$ units. Sample curves are given for the wavelengths 480, 380, and 280 mμ. Between 480 and 380 mμ phototropism is positive and

the limiting angles are 30° and 90°. Throughout this range of wavelengths the curves are uniformly steep. At 280 mμ phototropism is negative and the limiting angles are 0° and 90°. In this range below the inversion point the curves are again unformly steep, and steeper than on the other side of the inversion point. They cover the greater range between the two limiting angles in the same intensity interval.

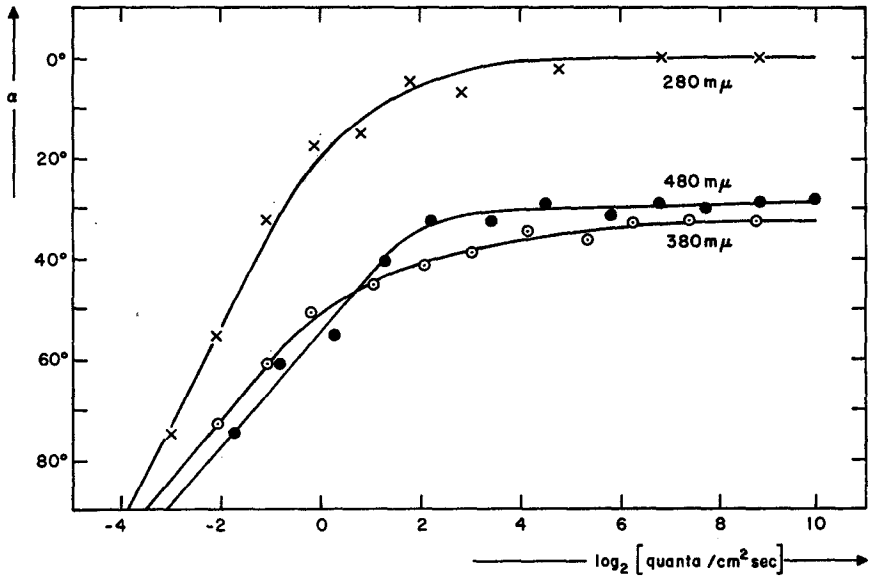

FIGURE 4. Geotropic-phototropic equilibrium direction of growth at different wavelengths, *versus* $\log_2$ intensity. The intensity is expressed in units of 10^6 quanta/cm² sec., with logarithms taken to the base 2. For blue light this unit intensity corresponds to $I = -24$ on the scale used in previous publications (Delbrück and Reichardt, 1956). The angle plotted on the ordinate is the angle between the horizontal direction of light and the direction of growth.

The data for Fig. 4 were taken up to the highest intensity available with our monochromatic set-up, and in each case this intensity was high enough to clearly exhibit a plateau region for the equilibrium direction of growth. However, these intensities are still very low in physiological terms. When 1,000 times higher intensities are used, limiting angles are obtained which are somewhat variable and generally smaller; *i.e.*, the direction of growth is more nearly horizontal. At least part of this effect is due to scattered and reflected stray light. When this was reduced as much as possible the limiting angle increased, though not quite to 30°. It is possible, therefore, that part of the effect is connected with the different qualities of the illumination. In the high intensity range the light was not monochromatic.

Let us now consider the problem of constructing an action spectrum for

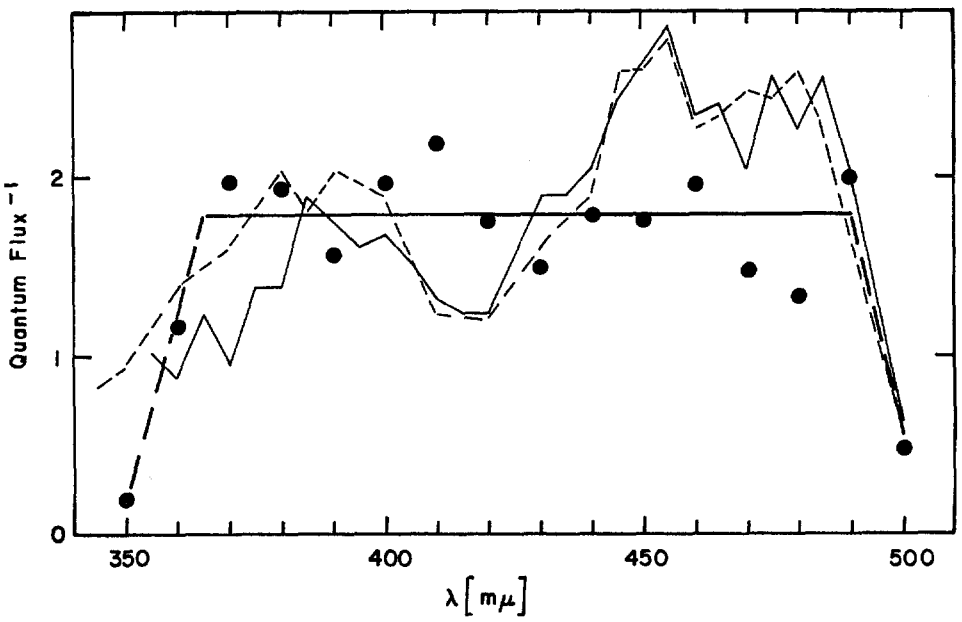

FIGURE 5 *a*

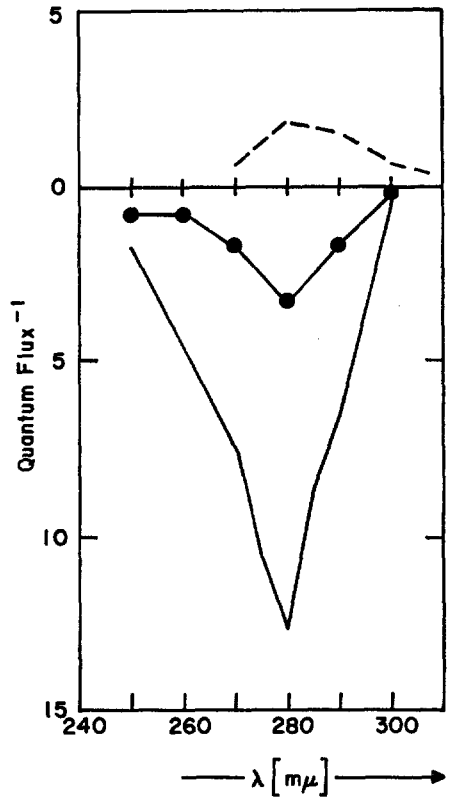

FIGURE 5 *b*

FIGURE 5. Action spectrum of geotropic-phototropic equilibrium. The reciprocal quantum flux needed to produce a standard equilibrium direction of growth is plotted *versus* wavelength (filled circles). The standard direction of growth is half-way between the two limiting angles, *i.e.* 60° above the horizontal in the visible and near ultraviolet (Fig. 5 *a*), where phototropism is positive, and 45° for wavelengths below the inversion point (Fig. 5 *b*), where phototropism is negative. For comparison, there are also plotted the action spectra of the growth response (dashed curve) and of the purely phototropic equilibrium (solid curve) (Delbrück and Shropshire, 1960). The ordinate scale for the geotropic-phototropic equilibrium differs from that for the other two by a factor of 50,000. For the geophototropic curve, the unit on the ordinate scale is 10^{-6} cm² sec./quantum.

this equilibrium. Within either of the wavelength regions (above and below the inversion point) we need only compare relative shifts of the curves. In tying the two regions together, an ambiguity arises. Clearly the rule of assessing an action spectrum by determining quantum fluxes producing equal effects cannot be applied. In one region we have positive, in the other negative phototropism, with different limiting directions of growth in the two regions. Since this difference is not clearly understood, we must proceed somewhat arbitrarily. In Fig. 5*a* and *b* we have chosen the midpoint between the two limiting directions of growth as the reference point for comparison, *i.e.* 60° above horizontal for the wavelengths of positive phototropism and 45° for the wavelengths of negative phototropism, and present the two portions of the action spectrum in separate sections, *a* and *b* of Fig. 5. For comparison, the action spectra for the growth response and for the purely phototropic response are also shown (Delbrück and Shropshire, 1960).

The three spectra agree with respect to the total range in which photoresponses occur, extending from 500 to 250 mμ and beyond. The phototropic and the geotropic-phototropic spectra agree in the location of the inversion point. In the range 370 to 490 mμ, however, in contrast to the comparison spectra, the geotropic-phototropic spectrum appears to be flat. The experimental variability is about ± 25 per cent of the ordinate value for each point. Therefore one cannot exclude the possibility of a hidden fine structure. Below the inversion point, where the phototropic effectiveness per quantum of light is about six times larger than in the visible when matched with a standard phototropic stimulus, it is at most only about twice as effective as the visible when matched with the geotropic stimulus.

DISCUSSION

The experiments reported in this paper are concerned with the equilibrium between geotropic and phototropic effects. One determines the intensity of horizontal illumination for which the equilibrium direction of growth is intermediate between the limiting direction in complete darkness (the vertical), and the limiting direction over an enormous range of higher intensities (horizontal for the ultraviolet, 30° above for the visible wavelengths).

The intensities producing a direction between the two limiting ones are very low indeed, about 10^6 quanta/cm^2 sec. The cross-section of the growing zone intercepting this flux is about 2×10^{-3} cm^2 and absorption by the visual pigment during the passage through the growing zone is certainly not more than 10 per cent (Delbrück and Shropshire, 1960). Thus at most about 100 quanta are absorbed per growing zone per second.

Some authors have spoken of and reported "threshold" intensities for the phototropic reaction (Buder, 1946). Presumably the intensity above which the equilibrium direction begins to deviate from the vertical is meant by this

term. This intensity is necessarily a poorly defined quantity, in view of the gradual transition between the two limiting directions. If one extrapolates the linear part on our graphs to 90°, one obtains a threshold about four times smaller than the one for which the equilibrium direction is midway between the extremes. This result depends considerably on the type of extrapolation. With these qualifications in mind, the values we find are quite compatible with those reported by Buder. We have also confirmed Buder's statement that in stage I, before the sporangium is formed, the sporangiophores are about 10 times more sensitive than in stage IVb, the steady-state growth phase after formation of the sporangium. However, we have not made any detailed studies on stage I specimens.

In one sense our measurements refer to an absolute threshold: they indicate the general level of intensities at which the mechanism of dark adaptation begins to fail. This mechanism, whose nature is still completely unknown, works with almost uniform efficiency for an intensity range of about a factor of 10^9, beginning at intensities only slightly higher than those discussed here. It would be reasonable to think that at least one component of this mechanism would be an increase in the concentration of visual pigment during dark adaptation. One must bear in mind, though, that an increase in concentration would fail to increase the sensitivity to phototropic stimuli if carried to the point where it produces an optical density large enough to reduce by more than a few per cent the quantum flux reaching the distal side. Such an absorption would reduce the increased stimulation of the distal side mediated by the focussing effect, and necessary for positive phototropism. Indeed, for larger increases in the optical density produced by the visual pigment we would have to anticipate a reversal of the direction of the tropic effect, for the same reason that we actually find it in the ultraviolet.

Let us consider, then, what effect on the action spectrum we should anticipate if, under conditions of maximal dark adaptation, the concentration of visual pigment does increase to the limit compatible with positive phototropism. Clearly this limit will be reached first for those wavelengths for which the absorption of the visual pigment is maximal. At these wavelengths, then, the action spectrum of the tropic effect would be depressed. Our reasoning leads us to anticipate, therefore, a depression of the maxima of the action spectrum, or, in other words, a flattening. This is what our experiments do indeed suggest. Whether this theory is correct could only be shown if a method were found to assay the visual pigment directly.

If this idea is correct we can use it to estimate the amount of pigment per unit volume of the growing zone. Assuming a molecular extinction of 40,000, similar to that of rhodopsin, and near the theoretical upper limit for absorption bands about 5,000 cm^{-1} wide, one obtains a concentration of about 10^{-7} moles/ml. To isolate this pigment it would be very important to know whether

the pigment is limited to the growing zone or distributed more or less uniformly throughout the sporangiophore. Attempts to answer this question have not yet given any unequivocal answers, though the finding (Delbrück and Varjú, 1961) that the adapting system moves relative to the wall suggests that the pigment may also move, possibly through protoplasmic streaming, which would distribute it widely.

Below the inversion point, we find a clear parallelism between the wavelength dependence of the action spectrum for the geotropic-phototropic equilibrium and of those spectra obtained earlier for the growth response and for the purely phototropic equilibrium at much higher intensity levels. In each of these cases we are matching the ultraviolet stimulus against some standard stimulus. In considering the wavelength dependence of the effectiveness of the ultraviolet stimulus we have to remember that the protoplasm contains a screening pigment (gallic acid) which absorbs in this part of the ultraviolet much more strongly than the visual pigment, and that the visual pigment is probably embedded in the protoplasm. Thus all of the incident light is absorbed within a short distance after entering the sporangiophore. The fraction absorbed by the visual pigment is then proportional to the ratio of the extinction coefficients of visual and screening pigments, and the wavelength dependence of this ratio should be the same in the three experimental situations corresponding to the three action spectra. The parallelism of the three spectra is therefore not unexpected.

Earlier it was found for the purely phototropic spectrum, at intensity levels about 50,000 times higher than those considered here, that the efficiency of the ultraviolet at its peak at 280 mμ is about five times higher, per incident quantum, than at the maximum in the visible, in contrast to a much less pronounced peak in the growth action spectrum. This excess height of the tropic action spectrum in the ultraviolet was explained by the fact that in the visible the tropic effect depends on a differential between the proximal and the distal side, whereas in the ultraviolet the tropic effect is due to the stimulation of the proximal side alone. One should expect the same reasoning to be applicable to the geotropic-phototropic equilibrium. Here, too, we are matching the phototropic effect against a standard effect, the geotropic one, and in comparing the visible and the ultraviolet below the inversion point, we should find the excess height in the ultraviolet because again we are dealing in the visible with a differential between the proximal and the distal side and in the ultraviolet with an effect on the proximal side only.

Our experiments do not show this enhanced efficiency of the ultraviolet and at present we have no clue as to the cause of this discrepancy. It is possible that under conditions of complete dark adaptation, there are other changes, besides the concentration of visual pigment conjectured above. For example, the concentration of the gallic acid, or of some other internal

screen, might change, and some of it might be located peripheral to the visual pigment. Conversely, the whole distribution of protoplasm might change, and the average depth below the surface of the photoreceptors might increase, thus leading to an increased attenuation of the efficiency of the wavelength below the inversion point. Other possible explanations might be found in azimuthal inequalities in the level of adaptation, which might be more pronounced at these low levels than in the normal range, or in complex interaction between phototropic adaptation and geotropic sensitivity.

This investigation was supported by a grant from the National Science Foundation.
Received for publication, February 17, 1961.

REFERENCES

1. BUDER, J., 1918, Die Inversion des Phototropismus bei *Phycomyces*, *Bot. Ber.*, **36**, 104.
2. BUDER, J., 1946, Uebersicht über Ergebnisse einiger noch ungedruckter Arbeiten aus den botanischen Anstalten der Universität Breslau, mimeographed private communication.
3. DELBRÜCK, M., and REICHARDT, W., 1956, System analysis for the light growth reactions of *Phycomyces*, *in* Cellular Mechanisms in Differentiation and Growth, (D. Rudnick, editor), Princeton University Press, 3.
4. DELBRÜCK, M., and SHROPSHIRE, W., JR., 1960, Action and transmission spectra of *Phycomyces*, *Plant Physiol.*, **35**, 194.
5. DELBRÜCK, M., and VARJÚ, D., 1961, Photoreactions in *Phycomyces*. Responses to the stimulation of narrow test areas with ultraviolet light, *J. Gen. Physiol.*, **44**, 1177.
6. DENNISON, D. S., 1958, Studies on phototropic equilibrium and phototropic-equilibrium in *Phycomyces*, Ph.D. thesis, California Institute of Technology, Pasadena.
7. DENNISON, D. S., 1959 *a*, Gallic acid in *Phycomyces* sporangiophores, *Nature*, **184**, 2036.
8. DENNISON, D. S., 1959 *b*, Phototropic equilibrium in *Phycomyces*, *Science*, **129**, 775.
9. REICHARDT, W., and VARJÚ, D., 1958, Eine Inversionsphase der phototropischen Reaktion, *Z. physik. Chem.*, **15**, 297.