Steady-State Phototropism in *Phycomyces*

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ABSTRACT The steady-state phototropic bending of *Phycomyces* sporangiophores was studied using apparatus designed to keep the growing zone vertical and the angle of illumination constant over long periods of time. The bending speed is quite constant if the intensity and angle of illumination are fixed. A phototropic inversion occurs in response to a sudden change in intensity, either an increase or a decrease. A bending component lateral to the illumination direction is strongly evident at normal incidence. It is shown that this component is due to a rotation between the stimulus and response loci about the axis of the growing zone, which is probably related to the spiral growth of the cell. The steady-state bending speed is at a maximum value for illumination directions ranging from normal incidence to about 45°. From 45 to 14° the bending speed decreases linearly with angle, reaching zero at 14°. Angles less than 14° elicit a weak negative phototropic response. Using an optical model of the growing zone, the intracellular intensity distribution was determined as a function of the angle of illumination. Several hypotheses relating the intensity distribution to the phototropic response are discussed.

INTRODUCTION

Ever since Buder (1918) showed that the normally positive phototropic response in *Phycomyces* is reversed in liquid paraffin, it has been clear that the light intensity distribution within the sporangiophore plays a crucial role in this response. More striking evidence has been provided by Shropshire (1962), who found that phototropism can be reversed merely by changing the illumination from a parallel to a divergent beam. Both types of experiments were performed under normal incidence, which simplifies the optical situation since the light rays striking the cylindrical cell remain in the plane perpendicular to the cylinder axis.

If a beam of parallel rays strikes the cell at an acute angle, the optics become more complex for two reasons: first, the section traversed by the beam is elliptical instead of circular, and second, the coplanar rays incident on the cell are refracted in such a way that they are no longer coplanar. Using living cells and an optical model, Varjú, Edgar, and Delbrück (1961) showed that as the angle of incidence (between beam and cell axis) decreases, the width of the

bright focal band on the back, or distal, side becomes narrower, reaching zero width at an angle of about 32°. For smaller angles, the band widens again, becoming at the same time much more diffuse. It was realized by these authors that this strong angular dependence of the intensity distribution implies a corresponding angular dependence of the phototropic effectiveness of a unilateral light stimulus. Thus the angle of incidence should strongly affect the phototropic response to a beam of parallel light.

The present work was undertaken to map out this relation between the phototropic response and the incident angle. Furthermore it was decided to use as the measured response the steady-state bending speed under strictly constant illumination conditions, to be sure that transient growth and tropic effects do not enter in. Also, an attempt is made to correlate these experimental facts with the intracellular intensity distribution as determined for various incident angles by means of an optical model of the sporangiophore.

MATERIALS AND METHODS

A sexually minus strain of *Phycomyces blakesleeanus* (derived from type 1555 of NRRL) was cultured using the medium and methods previously described (Dennison, 1964). All experiments employed mature stage IV sporangiophores from 5 day old cultures. The temperature was held to the range 20.5–21.5°C.

In measuring the steady-state bending speed, care was taken to maintain the angle of incident illumination constant with respect to the sensitive zone of the sporangiophore and to keep this sensitive zone vertical to avoid geotropic complications. The device developed for this purpose is diagrammed in Fig. 1. The culture vial is held in a clip which can be rotated by hand about a horizontal axis, and a microscope fitted with a vertical cross-hair reticle is aimed along this axis. The steady-state bending speed is measured by periodically rotating the culture vial about the horizontal axis so as to bring the upper 1.0 mm of the sporangiophore into a vertical orientation (as seen by the observer) at 1 minute intervals. At the beginning of each 1 minute interval, the angular position of the vial holder about the horizontal axis is measured by an angular scale attached to the support of the vial holder. The mean bending speed for the 1 minute interval is equal to the difference between two successive settings.

Care must be taken so that no part of the experimental set-up casts a shadow on the sensitive zone of the sporangiophore. To this end the sporangiophore and culture vial are first oriented vertically and illuminated by a beam of parallel blue light at an angle of 110° from vertical for 90 minutes, resulting in a right angle bend in the sporangiophore. Next the culture vial and supported sporangiophore are rotated about a vertical axis by a synchronous motor at a speed of 2 RPM for 60 minutes, while illuminated by a beam of parallel blue light fixed in a direction 60° from vertical. Under this symmetrical illumination, the sporangiophore makes a second right angle bend in the opposite sense and is thus growing vertically once again. The net result of this treatment is that the upper portion of the sporangiophore has a lateral kink

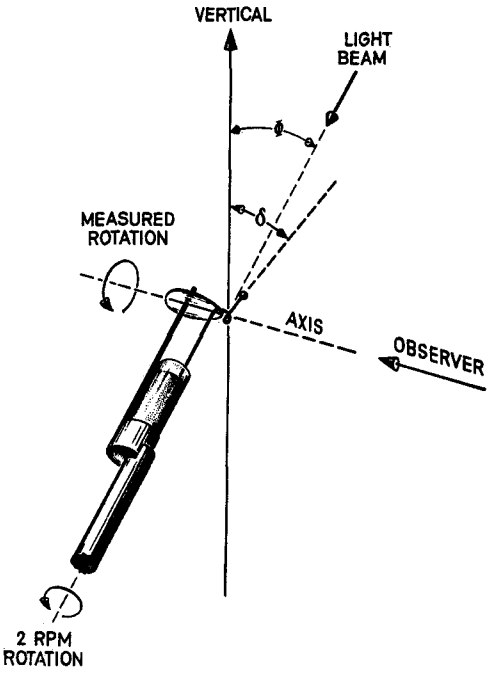

FIGURE 1. Experimental set-up for the measurement of steady-state phototropism. The upper 1.0 mm of the sporangiophore is kept in the δ-plane (the vertical plane passing through the horizontal axis) by rotating the culture vial and the sporangiophore support about the horizontal axis at 1 minute intervals. The angle δ is the deviation from vertical of the upper 1.0 mm of the sporangiophore. The light beam is in the Φ-plane (the vertical plane perpendicular to the horizontal axis and passing through the upper 1.0 mm of the sporangiophore) and makes an angle Φ with vertical. A sporangiophore is shown towards the end of an experiment, having already bent clockwise through an angle nearly 360°; consequently the culture vial has been rotated an equal amount in the opposite sense. The 2 RPM rotation occurs about an axis parallel to the culture vial axis and this is oriented vertically for the 60 minute period of 2 RPM rotation just before the beginning of each experiment. At the start of the experiment the 2 RPM rotation is switched off and the rotation about the horizontal axis is begun.

of about 2 mm, and thus is brought free from possible shadows cast by the culture vial, the support, or the old portion of the sporangiophore.

During the last 15 minutes of the 2 RPM rotation period the linear growth rate is measured, and any sporangiophores with a growth rate less than 2 mm/hr. are discarded. This period of 2 RPM rotation also ensures that light adaptation is complete before the bending speed measurements are begun.

The phototropic bending speed is measured in 3 periods of 50 minutes each. During the first and last periods (controls), $\Phi = 60°$, and during the middle period Φ is set at a test value. The first period is begun by simply switching off the 2 RPM motor, since the sporangiophore is already vertical (or nearly so), and $\Phi = 60°$. At the end of the first 50 minute period Φ is set to a test value without any interruption of the

bending speed measurements, which continue at 1 minute intervals. Similarly, Φ is returned to 60° at the end of the second 50 minute period.

It was soon noticed that the sporangiophores do not bend precisely in the vertical Φ-plane, but deviate outwards towards the observer when the illumination comes from the observer's right. This deviation is measured as follows. The observer's microscope is mounted on a micrometer-driven sliding support, so that it can move parallel to its (horizontal) optic axis. Distances may be measured along this axis by moving from one focal setting to another, and then recording the distance traversed by the microscope. The ocular micrometer scale is used to measure off a section of the upper end of the sporangiophore whose projection in the normal plane (the Φ-plane) is 1.0 mm, and then the horizontal distance between the upper and lower ends of this section is measured. This distance in millimeters is equal to tan δ, where δ is the angle of deviation from vertical. Measurements of δ are taken in this way at 5 minute intervals throughout the entire experiment.

The light source consists of a 100 watt incandescent projection lamp (General Electric designation CDS) operated on voltage stabilized alternating current. The optical train consists of a 3 cm thickness of copper sulfate solution (100 gm $CuSO_4 \cdot 5\ H_2O$/liter in 0.5 per cent H_2SO_4), a Corning 5-61 blue glass filter, and a lens which converges the radiation into an approximately parallel bundle. A calculated output spectrum of the complete source shows that at the voltage used in most of the experiments the output has a single broad peak at 470 mμ and falls to 1 per cent of this at 360 mμ and 580 mμ. The absolute intensity of the source was measured with a thermopile which was calibrated against a National Bureau of Standards radiometric standard lamp.

RESULTS

To determine the effect of light intensity on the phototropic bending speed, Φ is held at 60° throughout the experiment, and the light intensity is altered at approximately 25 minute intervals. The intensity range from 0.01 to 41 μw/cm² is covered in 6 jumps, each an intensity change of fourfold. Typical phototropic responses to these intensity jumps are shown in Fig. 2. The large phototropic inversion following the increase (lower curve) is similar to that found first by Reichardt and Varjú (1958). More unexpectedly, a similar phototropic inversion is also noted after a decrease (upper curve). In each case a lag of about 5 minutes elapses before the bending speed deviates from its initial value, but because of the inversions a much longer time lag intervenes before the new steady bending speed is established. The average bending speed at constant intensity varies only slightly with intensity, having a broad plateau from about 0.1 to 10 μw/cm². This agrees very well with the results of Reichardt and Varjú (1958) for the intensity dependence of the initial 25° of the tropic response (incident angle of illumination initially 60°). All the remaining experiments, concerned with variation in Φ, were performed at a constant intensity of 1.1 μw/cm².

Next, consider the results of a typical experiment shown in Fig. 3. It is clear

that after the change of Φ from 60 to 25° there is an abrupt decrease in bending speed, after the usual 5 to 7 minute time lag. After the return to 60° the bending speed increases, approximately to its former magnitude. The deviation, δ, is also affected by the change in Φ, although a longer time is required to reach a steady value than is the case with the bending speed. Note the complete absence of a phototropic reversal following the change in Φ. A low amplitude oscillation in the bending speed with a period of about 8 minutes can also be seen in this record. These fast oscillations, first noted by Dennison (1958), are associated with many diverse phototropic phenomena, but their origin remains unknown.

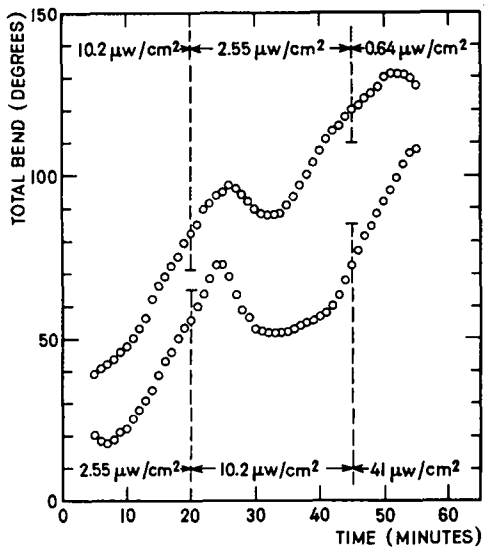

FIGURE 2. Phototropic inversions following an increase in intensity (lower curve) and a decrease in intensity (upper curve).

For all experiments the bending speed characterizing each 50 minute interval is defined as the mean bending speed during the final 40 minutes of that interval. The initial 10 minutes of each interval are discarded because the bending speed is not constant during this period. Thus each experiment provides a single bending speed value for the test Φ and two control bending speed values for $\Phi = 60°$. Only rarely are the two control speeds identical, the change being sometimes an increase but more often a decrease. Experiments in which this change is greater than 30 per cent were discarded from the analysis that follows. Four such discards were made out of a total of 42 experiments. The mean control bending speeds (axial correction applied, see below) for the 38 remaining experiments were 3.30° per minute for the first control period and 3.04° per minute for the second control period.

The bending speed during the first control period varied among different specimens, ranging from 1.8 to 4.5° per minute. Measurements of the growth

rate of these specimens just before the first control period also show a great variability, ranging from 2.3 to 5.2 mm/hr. But when a plot of growth rate *versus* bending speed is made for the 38 specimens, the points form a symmetrical area with no evidence of a correlation between bending speed and growth rate.

The bending speed ratio, defined as the ratio of the speed during the test period to the mean speed during the two control periods, is given in Fig. 4

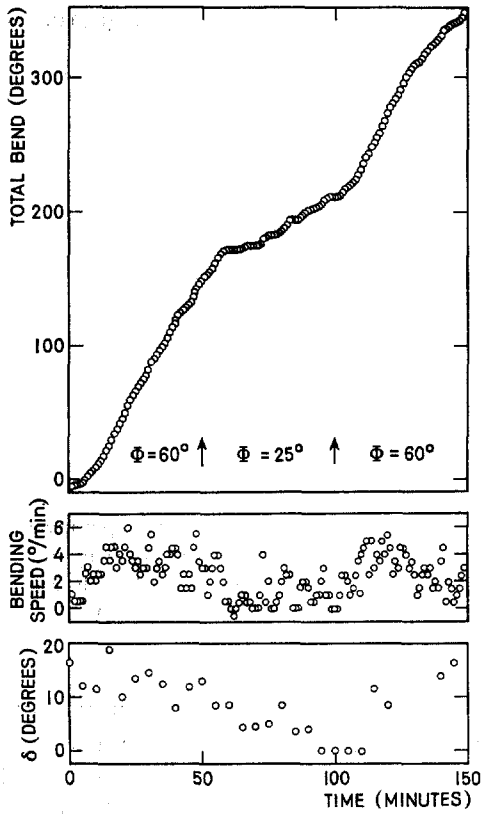

FIGURE 3. Typical phototropic experiment, showing the accumulated total bend (upper plot), the bending speed (middle plot), and the deviation, δ (lower plot), as a function of time. During the first 50 minutes $\Phi = 60°$, during the middle 50 minutes $\Phi = 25°$ (test angle), and during the final 50 minutes $\Phi = 60°$. Note the lack of phototropic reversal following the change in Φ.

(dashed curve) as a function of the test Φ. This curve has several simple and yet remarkable features. From 90 to about 45° the curve is substantially flat, so that within this range the bending speed is *independent* of the angle of incidence. From 45 to 14° the bending speed decreases linearly with decreasing Φ. For angles less than 14°, the bending is *negative*; *i.e.*, away from the light source, with a speed one-tenth that at 60°.

The deviation, δ, is slower to respond to a change in Φ than the bending speed, and therefore the deviation corresponding to a 50 minute interval is defined as its mean value during the final 30 minutes of that interval. The mean deviation for all experiments was 18.0° for the first control period and

15.5° for the second control period. The deviation is given as a function of Φ in Fig. 5. Clearly, δ does not become appreciable until Φ exceeds about 45° and becomes extreme for normal incidence ($\Phi = 90°$). Note, however, that δ is always positive; *i.e.*, the deviation is always towards the observer when the

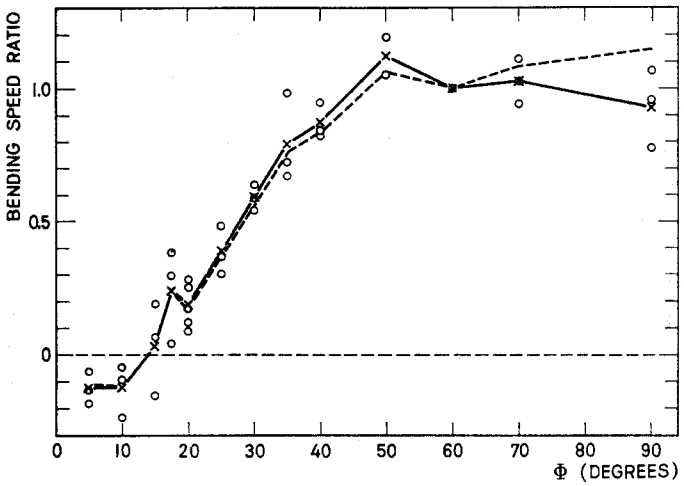

FIGURE 4. Phototropic bending speed as a function of Φ. On the ordinate is plotted the ratio of the mean bending speed during the middle 50 minute period to the mean bending speed during the control 50 minute periods. Circles, individual observations with the axial correction applied (see text). Crosses with solid line, mean values with the axial correction applied. The dashed line indicates the mean values without the axial correction.

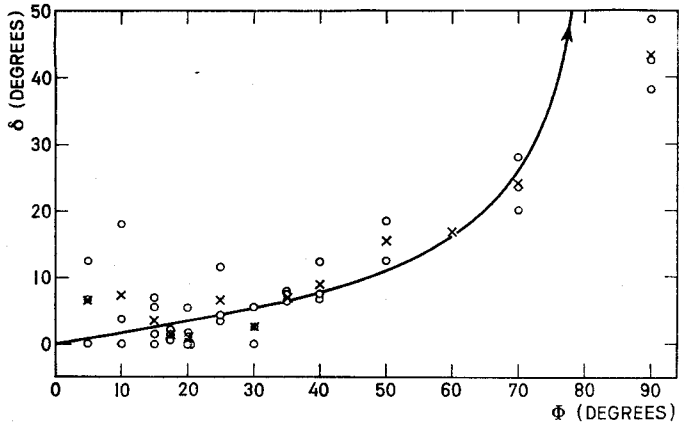

FIGURE 5. Steady-state deviation, δ, as a function of Φ. Plotted is the mean value of δ during the final 30 minutes of each 50 minute interval. Crosses, mean values. Circles, individual observations. The smooth curve is calculated from equation (1) solved for δ with $\beta = 80.9°$:

$$\cos^2 \delta = 1.026 - \frac{0.0256}{\cos^2 \Phi}$$

light comes from the right. This agrees with earlier observations of this phenomenon, in connection with observation of the 3-dimensional trajectory of phototropism (Dennison, 1958) and photogeotropic equilibrium (Varjú, Edgar, and Delbrück, 1961), in that the deviation (or declination) from the direction of illumination is always clockwise when viewed from above.

When δ becomes substantial, a systematic error is introduced into measurements of the phototropic bending speed. The angle measured by the observer at 1 minute intervals is the angle between vertical and the projection in the vertical Φ-plane of the upper 1.0 mm of the sporangiophore. If α is the true angle through which the sporangiophore has bent in 1 minute in its own tilted plane of bending, and α' is the angle as measured by the observer in the vertical plane, then $\tan \alpha = \cos \delta \tan \alpha'$ which for small α becomes $\alpha = (\cos \delta)\, \alpha'$. If the observed bending speeds are corrected by the factor $\cos \delta$, one arrives at the bending speed referred to the cell's *axis*, or the *axial bending speed.* The *axial* bending speed ratio is given as a function of Φ in Fig. 4 (solid curve). The principal difference between the two curves in Fig. 4 is in the region above 45°.

DISCUSSION

A. *Phototropic Inversion*

The phenomenon of phototropic inversion as a response to a sudden increase in intensity (Fig. 2, lower curve) is explained by Castle (1962) in terms of the laterally asymmetric distribution of a substance called M, which is consumed in the growth process. He supposes that during the course of phototropic bending the consumption of M is greater on the more rapidly elongating convex side (distal to the light source) than on the opposite side and hence the inequality, $M_p > M_d$, becomes established, where M_p and M_d are the M levels on the proximal and distal sides, respectively. Castle also assumes that the local growth rate is dependent on the local M level and on the light intensity, the latter acting after a certain time delay. An increase in intensity produces a more rapid growth rate on both sides of the cell and hence a more rapid consumption of M_p and M_d. The inversion is then explained by this temporary increase in M consumption and the consequent exhaustion of M_d, causing a temporary slowing of growth on the distal side relative to the proximal side.

The present work shows that such inversions are not restricted to intensity increases, but also occur following intensity decreases (Fig. 2, upper curve). This result would be consistent with Castle's theory if it could be shown that the temporary decrease in growth rate caused by the intensity drop leads to a reduced consumption of M and hence a temporary increase in both M_d and

M_p. Since $M_p > M_d$ due to the previous bending, it might be that this increase could raise M_p temporarily to a high enough level to increase the growth rate on the proximal side above that on the distal side and thus cause the inversion.

B. *Phototropic Bending Speed*

The dependence of bending speed on incident angle of illumination (Fig. 4) explains in part the phenomenon of phototropic-geotropic equilibrium. Since the phototropic bending rate approaches zero for $\Phi = 14°$, one would expect that in such an equilibrium, where the phototropic response to a horizontal beam is balanced by an upwards (negative) tropic response to gravity, the angle between the sporangiophore and the beam could not fall below 14°. Dennison (1958) reported that when precautions were taken to reduce stray reflected light, a light-gravity balance of 14.1° from the horizontal beam direction was found. Varjú, Edgar, and Delbrück (1961) reported angles definitely less than 30°, and Varjú, Burkhardt, and Delbrück (unpublished manuscript) found an angle of 13.7°. All these values are for intensities within the plateau of maximum phototropic response. On going to lower intensities, Varjú, Edgar, and Delbrück (1961) found a slight increase in this angle to about 30° at an intensity of 8×10^{-7} μw/cm^2 (monochromatic radiation at 480 mμ). Below this intensity the angle increases sharply as the absolute intensity threshold for phototropism is approached. Since the bending speed curve shows no pronounced features in the region near $\Phi = 30°$, it does not explain the fact that the phototropic-geotropic balance point remains close to 30° down to such low intensities.

C. *Deviation*

As shown in Fig. 5, there is a well defined quantitative relation between the deviation, δ, and Φ. In what follows I will try to show that this relation can be explained by the spiral growth of the cell.

The photographs in Fig. 6 (explained more fully below) show that, for all values of Φ, the intensity distribution within the cell has a plane of symmetry containing the illuminating beam and the sporangiophore axis. Therefore, any differential light stimulus arising from this pattern must be set up, initially at least, in a direction parallel to this plane of symmetry. Now if there is no deviation ($\delta = 0$) the growing zone is perfectly vertical and the plane of symmetry through it coincides with the Φ-plane and makes a right angle with the δ-plane (see Fig. 1). But when δ is greater than zero the plane of symmetry through the growing zone is no longer perpendicular to the δ-plane. In particular, this symmetry plane now makes an angle, β, with the δ-plane less than 90° (see Fig. 8). Application of solid geometry shows that β depends on Φ and δ as

follows (see Appendix for derivation):

$$\cos \beta = \frac{\sin \delta}{\sqrt{\sec^2 \Phi - \cos^2 \delta}} \quad (1)$$

It is now possible to calculate the experimental values of β, based on the observed values of δ as a function of Φ given in Fig. 5. The resulting values of β

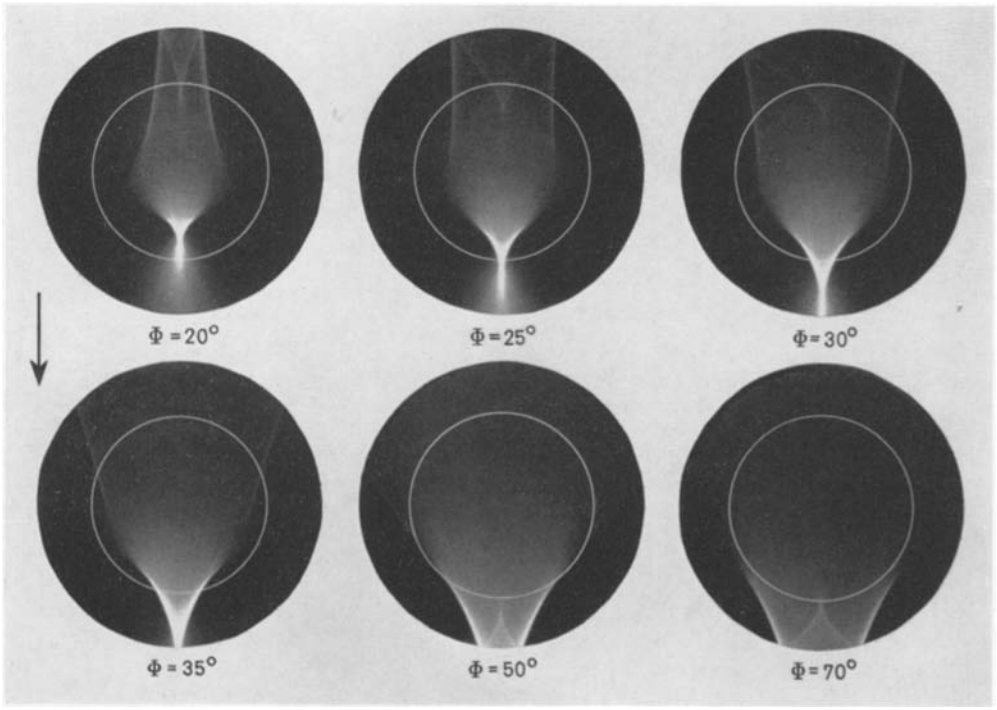

FIGURE 6. Photographs of an optical model of the sporangiophore showing the intracellular intensity distribution as a function of Φ. The cross-section corresponds to a point 1.7 mm below the sporangium, and the circle indicates the approximate location of the outer boundary of the vacuole. The direction of illumination is indicated by the arrow. For further details, see text.

cluster remarkably closely about a mean of 80.9°, for a range of Φ from 15 to 70°, and furthermore β shows no increasing or decreasing trend over this range of Φ.

What is the significance of this unexpected constancy of β at 80.9°? First of all it means that the growing zone orients itself in such a way that the light source direction as seen from the growing zone is rotated by 9.1° about the cell axis from the direction of bending. In other words, the cell creates and maintains an aiming error that has a constant value of 9.1°, independent of Φ

over a wide range. To take a step further, suppose that this aiming error (counterclockwise as seen from above) is of just the correct size to compensate for an aiming error in the opposite direction (clockwise) introduced by the spiral growth of the cell. The cell grows in the manner of a left-handed screw and thus a cross-section of the growing zone seen from above would indeed be rotating in a clockwise direction. My hypothesis is simply this: the spiral growth tends to introduce an aiming error in the clockwise sense, and the resulting bending increases the deviation, δ, until a balance point is reached at which the error due to spiral growth just cancels the error due to the deviation. The spiral growth error is presumably dependent only on the internal properties of the cell and hence independent of Φ, therefore the compensating error must likewise be constant, as indeed it is found to be.

The mechanism of the spiral error is not at all evident. A representative value for the rate of rotation of the cell wall at the upper end of the growing zone is about 15° per minute (Castle, 1937). Using the "twist" curves of Cohen and Delbrück (1958) one may estimate that the twist rate in the middle portion of the growing zone (1 to 1.5 mm below the sporangium) is from $\frac{1}{3}$ to $\frac{1}{2}$ of the total and hence ranges from 5 to 7° per minute. The minimum value of 5° per minute leads to the conclusion that the cell wall rotates by 9.1° in 1.8 minutes in this portion of the growing zone. A naive explanation for the spiral error is that a time delay intervenes between the primary reception of the tropic stimulus and the initiation of the bending reaction and that during this time the receptor structure has rotated clockwise by a certain amount, causing the error. Now it is unlikely that the cell wall is in fact the structure bearing the photoreceptors because, among other reasons, its measured rate of rotation is too rapid, requiring a time delay of 1.8 minutes or less. This is unreasonably short, compared with a growth response delay of about 2.5 minutes and a phototropic response delay of about 5 minutes. The experiments of Delbrück and Varjú (1961) led them to the hypothesis that the photoreceptors are not in fact fixed to the cell wall but instead are associated with a "system" which moves upwards with the same speed as the sporangium. They also postulated the existence of another structure, which contains the responding elements and which is fixed relative to the mature, non-growing part of the sporangiophore. These two structures are thus in continual longitudinal motion relative to each other and to the cell wall. It seems reasonable to suppose that these two hypothetical structures might also rotate relative to one another, although there is no evidence for this. Such a relative rotation could then be the cause of the spiral error, if we assume that the rotation velocity lies in the range from 3.6 to 1.8° per minute, corresponding to a delay time in the range from 2.5 to 5 minutes, respectively.

Assuming that β is constant at 80.9°, theoretical values of δ as a function of Φ were calculated, using equation (1) (solved for δ), and are given as a smooth

curve in Fig. 5. Agreement with experimental values is generally good, with exceptions at $\Phi = 5$ and 10° and at $\Phi = 90°$. Equation (1) predicts that δ should reach 90° when $\Phi = 80.1°$. However, extreme values of δ would doubtless bring geotropism into play, and it is likely that the δ values between 40 and 50° observed at $\Phi = 90°$ represent the maximum values possible in the presence of gravity. The disagreement at $\Phi = 5$ and 10° is more puzzling and may perhaps be related in some way to the negative phototropism observed at these angles.

D. *Intracellular Intensity Distribution*

A simple optical model was constructed to determine the intracellular intensity distribution as a function of angle of illumination. A glass cylinder 500 mm long, 43 mm in diameter, and having a wall thickness of 1.2 mm is closed at the bottom by a thin plate of diffusing glass and filled with a sucrose solution of refractive index 1.38. At the top of the cylinder is a simulated opaque sphere, 180 mm in diameter. Thus the entire assembly is an optical model of the upper 1.7 mm of a sporangiophore built to a scale of 1:300, with the exception of the wall, which is about 10 times too thick. The slight taper of the growing zone is judged optically unimportant. When the tube is illuminated by parallel light of uniform intensity, the image formed on the diffusing plate is equivalent to the intensity distribution across the cell 1.7 mm below the sporangium. The diffusing plate is photographed from directly beneath, using illumination incident on the tube at varying angles.

Patterns corresponding to six values of Φ are given in Fig. 6. A striking feature of these patterns is the bright focal line, which is inside the cell for Φ less than 30–35° and inside the vacuole for Φ less than 20°. The vacuole boundary at 1.7 mm below the sporangium, indicated by the circles in Fig. 6, is based on the cell measurements of Shropshire (personal communication).

An apparently attractive hypothesis is one ascribing a major growth effect to the strong intensity gradients that occur near this bright line. Such a hypothesis might explain phototropism for angles above 25°, but is unable to account for the vigorous tropic reaction (negative) to a divergent beam, reported by Shropshire (1962). As Shropshire's ray diagram clearly shows, no bright line and hence no strong gradient exist under these conditions. Therefore a phototropic mechanism based on a strong intensity gradient is thought unlikely.

I will now discuss a group of models which make the following two assumptions:

1. The photoreception process occurs in the periphery of the protoplasm inside the cell wall, and the effect at each point is some function of the intensity at that point.
2. The steady rate of phototropic bending is some function of the ratio of

the integrated light effect in the distal cell half (away from the light source) to that in the proximal cell half (towards the light source).

Measurements of the peripheral intensity distribution were carried out by microdensitometry of the photographic negatives (Fig. 6). The scanning spot diameter was 3 per cent of the cell radius and the radius of the circular scanning path was 90 per cent of the cell radius. Integration of the resulting intensity values shows that the energy drop across the glass tube varies from

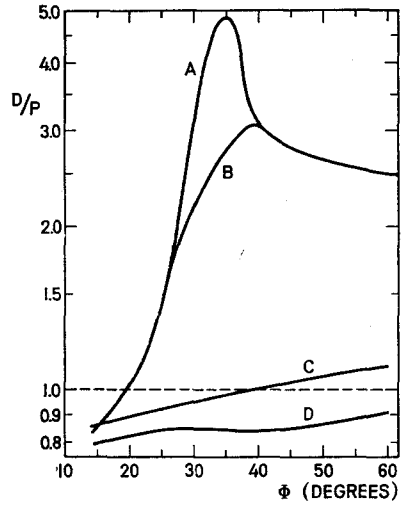

FIGURE 7. Integrated light effect ratios as a function of Φ, for four different models. In the ratio D/P, P is the integrated effect on the periphery of the proximal (illuminated) half of the cylinder and D is the integrated effect on the distal half. In curve D, the effect at each point is assumed proportional to the intensity I. In curve C, the effect at each point is proportional to $I \times m$ where m is the distance to the midplane. In curve A, the effect at each point is proportional to $I^2 \times m$. In curve B, the effect at each point is proportional to $I^2 \times m$, with the additional assumption that the effect reaches saturation when $I = 9\bar{I}$, $\bar{I}$ being the mean peripheral value of I.

19 per cent for $\Phi = 15°$ to 8 per cent for $\Phi = 60°$, with a mean of 15 per cent (curve D, Fig. 7). Thus it is clear that any model based simply on the ratio of the total absorbed energy in the proximal and distal halves would predict negative phototropism for all angles, contrary to experimental fact.

This shortcoming has long been known, and Buder (1946) proposed to remedy it (for normal incidence) by assuming that the contribution of each element of the cell wall to the over-all bend is proportional to the distance of that element from the midplane (the plane separating the proximal from the distal half). This mechanical advantage assumption can be introduced here by multiplying the intensity at each peripheral point by the distance from that point to the midplane and integrating the result in each cell half. When this is

done (curve *C*, Fig. 7) we find that the effect on the distal side is indeed increased but that it exceeds the proximal effect only for Φ greater than 40°. For Φ less than 40°, the mechanical advantage factor is not able to overcome the light losses, and the integrated effect is greater on the proximal side. Thus the peripheral mechanical advantage model, assuming a local effect proportional to intensity, predicts negative phototropism for angles of incidence below 40°, contrary to the experimental results.

The above suggests that there exists some kind of amplifying mechanism which permits a high intensity area to have a greater effect than a low intensity area encompassing the same total energy. Although no direct evidence exists for a mechanism with this feature in *Phycomyces*, it is nevertheless possible. A system with such an amplifying effect would be a two step photochemical sequence, requiring for example a chemical reaction between two diffusible photoproducts. The reaction rate would be a function of the product of the photoproduct concentrations and hence would increase rapidly with intensity. Thus the effect at any point might well be proportional not to the intensity, I, but to I^2.

To test this idea, calculations were made in which the effect at each peripheral point is equal to the square of the intensity at that point, and this effect, multiplied by the distance to the midplane, is integrated over the two cell halves. The results (curve *A*, Fig. 7) show two important features of agreement with the experimental results. First, the I^2 model shows a zero point at $\Phi = 19°$, with positive phototropism above this angle and negative phototropism below it. This is reasonably close to the experimentally observed zero crossing at $\Phi = 14°$. The second feature of the model is that for $\Phi = 40°$ and greater it predicts a high and relatively constant distal-to-proximal ratio, in agreement with the plateau of bending speed observed for $\Phi = 45$–$90°$. The quantitative relation between the distal-to-proximal ratio and the resulting bending speed is left open.

At $\Phi = 30$ and 35° the ratio goes above the plateau level in curve *A*, reaching a peak of 4.8 at $\Phi = 35°$, which is clearly in disagreement with the experimental results. The I^2 model can be modified in several ways to remove this peak; one way is to assume that there exists a saturation intensity, and intensities above this produce no additional effect. If the intensity saturation level is chosen at 9 times the mean peripheral intensity, curve *B* of Fig. 7 results. Although the factor of 9 is arbitrarily chosen, there is ample evidence that the light-growth system as a whole saturates for a sufficiently strong stimulus. Another way to modify the I^2 model is to replace the I^2 assumption by I^k. By giving k a value less than 2, it might be possible to eliminate the offending peak at $\Phi = 35°$, without changing the other features of the curve too greatly. It is felt, however, that such refinements should wait until a direct experimental test can be made of the most important assumption underlying the I^2

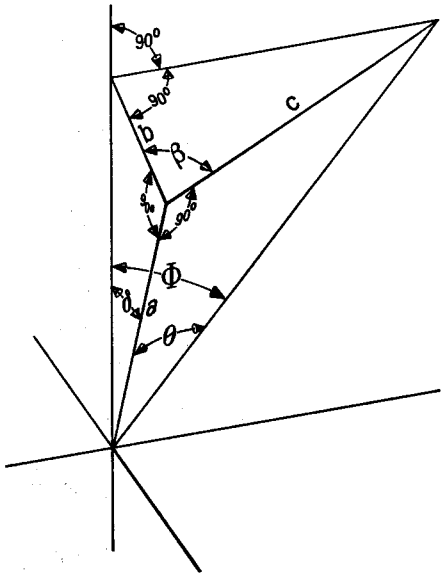

FIGURE 8. The relation between β, δ, and Φ. The angles δ and Φ are as in Fig. 1. The origin of this diagram represents an arbitrary point along the longitudinal axis of the upper 1.0 mm of the sporangiophore. This axis is represented by the line of length a and is perpendicular to the plane containing the angle β. The plane containing the angle θ contains both the sporangiophore axis and the illuminating beam axis and is thus the plane of symmetry of the intracellular intensity distribution (see text).

model, namely that an amplifying mechanism of the sort discussed above is actually operative.

Appendix

Derivation of equation (1)

In Fig. 8 the quantity a is arbitrary, so we may set $a = 1$. Then $c = \tan\theta$ and $b = \tan\delta$, and thus,

$$\cos\beta = \frac{\tan\delta}{\tan\theta}. \tag{2}$$

Since Φ, δ, and θ form a right spherical triangle,

$$\cos\theta = \cos\delta\cos\Phi \tag{3}$$

Combining (2) and (3) to eliminate θ, we obtain,

$$\cos\beta = \frac{\sin\delta}{\sqrt{\sec.^2\Phi - \cos^2\delta}} \tag{1}$$

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