INTRACELLULAR ROTATION AND THE PHOTOTROPIC RESPONSE OF *PHYCOMYCES*

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ABSTRACT Experimental evidence indicates that during phototropism, *Phycomyces* sporangiophores use their own net rotation to convert an apparently spatial stimulus to a temporal one. Conversion to a continuous temporal stimulus insures that phototropism never adapts as long as the spatial asymmetry in illumination is maintained. If this temporal stimulus is circumvented by rotating the cell backwards so that there is no net rotation of some of the receptors relative to the light, the response can be reduced by two-thirds. The system thus adapts to the incident light, resulting in a reduced response. For the illumination of a transparent cell, this compensating rotation speed is 10°/min counterclockwise and probably corresponds to the photoreceptor rotation in the most effective part of the growing zone. We infer that this region is in the upper portion of the growing zone and that the receptor system rotates integrally with that region of the cell.

INTRODUCTION

One of the great mysteries of the phototropic response of *Phycomyces* has been that, unlike the light growth response, it fails to adapt. Under balanced bilateral illumination the photo-growth system responds to changes in light intensity, but after this intensity change, the growth rate always returns within a few minutes to the same level. A steady unilateral illumination, however, causes a continuous phototropic response, which persists indefinitely (Dennison, 1965). The cell is cylindrical, and therefore unilateral light is focused in a bright band on the back side (Fig. 1 *a*). Growth is faster in this bright band than on the opposite side, causing the cell to bend towards the light. The mystery is apparent when we ask why the brightly lit part of the cell continues to grow faster than other parts. We would expect that the photo-growth response system in each part of the cell would adapt to its own local light level and thus in the steady state grow at the same rate as in any other part. The possibility that locally autonomous adaptation does not occur but is rather spread across the cell was examined by Dennison and Bozof (1973). One side of the sporangiophore was exposed to a short period of ultraviolet illumination, and then a second ultraviolet stimulus was used to probe the responsiveness of various parts of the cell at a later time. The results of these experiments support the view of localized adaptation.

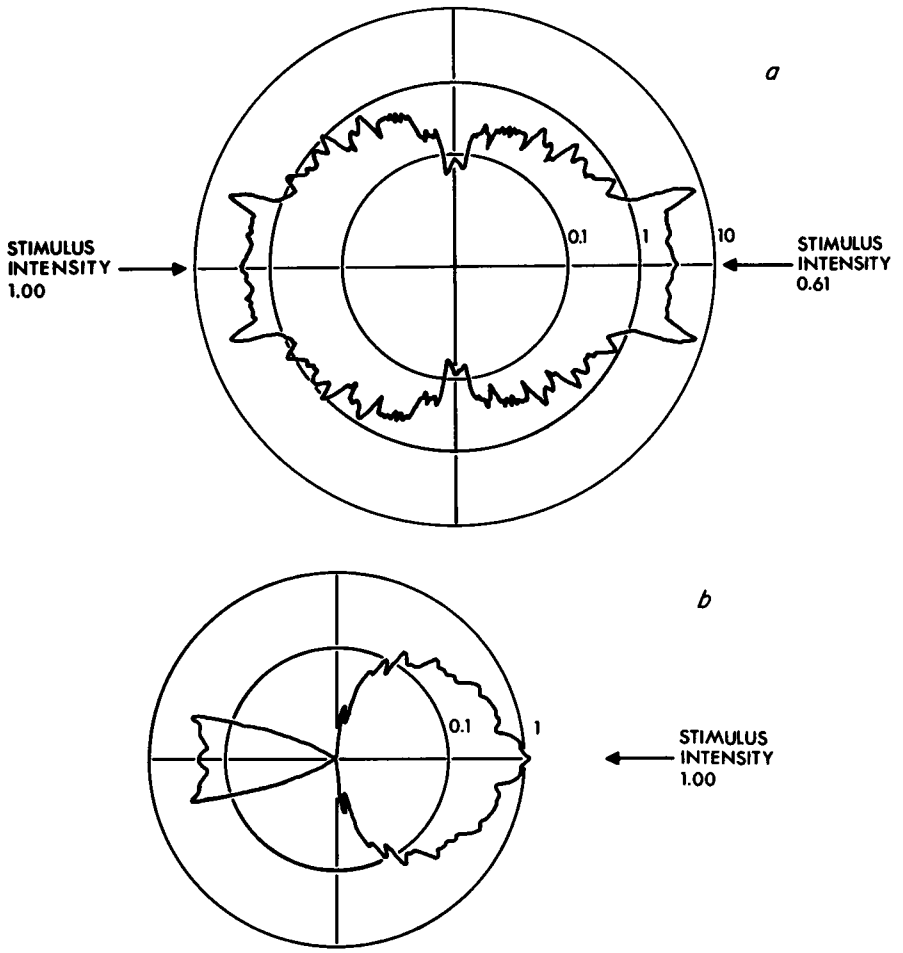

FIGURE 1 Theoretical polar logarithmic plots of the light intensity adjacent to the inner surface of the wall in a 0.01-μm-thick pigment layer when the cell is illuminated by unpolarized light incident perpendicular to its axis. The circles are separated by a factor of 10 in intensity. The circle marked "1" is normalized to one for an incident intensity of one. All reflections have been taken into account, but no correction for small particle scattering has been made. Assumed radial dimensions are 50 μm to outer surface, 49.4 μm to inner surface of wall, and 23 μm to tonoplast. (*a*) The transparent cell. Refractive indices are assumed to be 1.50 for the wall, 1.38 for the cytoplasm, and 1.36 for the vacuole (wavelength: 450 nm). Absorption coefficients are assumed to be 0.23 cm^{-1} in the wall, 27.6 cm^{-1} in the cytoplasm, and 0.23 cm^{-1} in the vacuole. The illumination is assumed to be a beam of intensity 1.00 from the left and one of intensity 0.61 from the right. (*b*) The opaque cell. Refractive indices are assumed to be 1.69 for the wall, 1.46 for the cytoplasm, and 1.38 for the vacuole (wavelength: 285 nm). Absorption coefficients are assumed to be 361.5 cm^{-1} in the cell wall, cytoplasm, and vacuole. The illumination is assumed to be a single beam from the right at intensity 1.00.

Although the unilateral stimulus is constant in time as far as the whole cell is concerned, intracellular movement of photoreceptor units could convert spatial variation in intensity into temporal variation. The sporangiophore grows by both stretch and twist. Unless the photoreceptors are in an inner structure, slipping with respect to the wall (for which there is no evidence), they would rotate at the same rate. In the maximally responsive region of the growing zone, this twist at the cell wall amounts to about 10°/min (Cohen and Delbrück, 1958; Ortega et al., 1974). As the photoreceptors are carried around the cell, they would be subjected to variations in light intensity. Specifically, the receptors would receive a strong step-up stimulus when they cross from the dark zone through the edge of the bright focal band. Although the receptors would also receive a step-down stimulus when they cross the second edge of the band into the other dark zone, the magnitude of the negative growth response to a step down in intensity is known to be smaller than that of the positive growth response to a step up (Foster, 1972; Foster and Lipson, 1973). This rotational theory of phototropism can be tested experimentally in a very simple way. If the sporangiophore is mechanically rotated on a turntable during a constant unilateral stimulus, in a direction opposite to the internal system and at exactly the same speed, such mechanical rotation would just cancel out the effect of the normal internal rotation. Under these conditions, the net rotation of photoreceptors would be zero, and if the theory is correct the phototropic response should disappear.

Although this situation is straightforward in general, it is more complex in detail. What one observes is the net bending rate of the end of the cell, the dynamic sum of responses to stimuli at each longitudinal level of the growing zone both at that point and as displaced downward and around the cell. For experimental reasons we have used conditions that yield moderately slow bending rates, thus nearly equalizing the cell stimulation on each side. Under these conditions several additional effects may become important. Clearly one is that the receptors (like the cell wall) rotate at different rates at different levels along the growing zone. This means the minimum stimulus will occur at different turntable rates, depending on the longitudinal position of the stimulus. A second effect is that at different net rotation rates the local response will be different in both magnitude and latency, since these parameters are influenced by the rate of change of the stimulus intensity (Foster and Lipson, 1973). A third effect is that when the responses are almost in perfect balance on opposite sides there will be a minimum bending rate. A fourth effect is that the way a cell has responded in one region may affect the way it responds in another.

These effects also introduce shifts in the direction of phototropic bending. As the turntable rotation rate passes through the cancellation speed for the photoreceptors, the locus of stimulus shifts from one edge of the bright band to the other, causing an abrupt shift in bending direction. A shift in direction may also arise when a change in turntable rotation rate causes the opposed responses to shift in azimuth relative to each other. Suppose the intensity distribution on one side is relatively smooth while on the opposite side it is focused. Since the time to maximum response of the smooth stimulus would be longer than that of the focused band (Foster and Lipson, 1973),

there will be only one turntable rate that precisely opposes these responses. At rates near the point of balance the bending direction will shift dramatically.

The experiments consist of two sets. In the opaque cell series, ultraviolet narrow-slit stimuli were used in the hope that the high absorbance of the cell at this wavelength would simplify the optical situation and that the restricted extent of the stimulus would reduce the complexity of the response. In the transparent cell series bilateral unbalanced blue stimuli were used, covering the entire cell. Data collection and analysis are complicated by the need for a description, in three dimensions, of the cell's position at successive times and by the possibility that the cell's real bending might be confused with the apparent bending caused by the turntable rotation. Such considerations led us to the experimental arrangement and the mathematical analysis described below.

EXPERIMENTAL METHODS

The cultures are a sexually minus strain of *Phycomyces blakesleeanus* (No. 1555 of the Northern Regional Research Laboratory, Peoria, Ill.), obtained from the *Phycomyces* stock center at the Division of Biology, California Institute of Technology, Pasadena, Calif. Vegetative spores were heat-shocked for 10 min at 45°C and seeded on vials containing 4% potato dextrose agar (Difco Laboratories, Detroit, Mich.) at a density of about five spores per vial. Before the medium was autoclaved, thiamine hydrochloride was added at a concentration of 0.5 mg/liter of medium. Immediately after inoculation, the vials were placed in an illuminated culture box humidified at about 80%. The temperature and illumination were different in the two experimental series. In the opaque cell (ultraviolet radiation, 285 nm) series, the temperature was between 23°C and 25°C and the illumination was from three 15-W incandescent lamps located 50 cm above the vials. In the transparent cell (blue radiation, 450 nm) series, the temperature was between 20°C and 21°C, and the illumination was from two 7-W incandescent lamps 40 cm above the vials. After the opaque cell series was completed, it was discovered that the sporangiophores were growing more slowly than normal, which may have affected the rate of phototropic bending. To correct this problem, the illumination and temperature of the culture box were reduced before the transparent cell series was begun. The first crop of sporangiophores (appearing 3 or 4 days after inoculation) were cut or pulled out, and only sporangiophores from cultures 5 days old or more were used for experiments. Uniformly mature sporangiophores were insured by selecting only those between 25 and 35 mm long.

The turntable is mounted on a three-way platform connected by a splined slipshaft and universal joints to a 15:1 reduction gear, driven by a Slo-Syn SS50-1008 stepping motor (Superior Electric Co., Bristol, Conn.). The motor was driven by an ST-1800V translator module, whose input pulses came from an external source, such as a Tektronix Type 162 waveform generator coupled to a Type 161 pulse generator (Tektronix, Inc., Beaverton, Ore.). The turntable rotated 0.12°/pulse.

The optical source for the opaque cell series was a Bausch & Lomb high-intensity grating monochromator (Bausch & Lomb Inc., Rochester, N.Y.) with a deuterium lamp, set at a wavelength of 285 nm. The output radiation was focused on an adjustable slit imaged on the sporangiophore by a quartz lens. The slit was adjusted so that the illumination falls on a 0.25-mm-wide band of the sporangiophore's growing zone centered at 0.5 mm from the base of the sporangium. A slit was chosen partly to restrict the region stimulated and partly to control the rate of phototropic bending, since ultraviolet is a particularly effective phototropic stimulus. Although satisfying these objectives, the slit introduced its own problems due to the stimulus that may arise from the sharp edges of the illuminated area. The turn-

table was enclosed in a polystyrene foam box, with openings for the entry of the 285-nm beam, red observing light, and two horizontal microscopes. The box was humidified to about 80% and the temperature (regulated by the room air conditioner) ranged from 22°C to 24°C. A blue light source, consisting of a 6-V incandescent lamp whose output was filtered by 3.5 cm of water and a Corning 5-61 blue filter (Corning Glass Works, Corning, N.Y.), was used for straightening and light-adapting the sporangiophore before each run. This light beam impinged on the sporangiophore at 30° above horizontal and had an intensity of about 1 $\mu W/cm^2$.

For the transparent cell series, the slit system was replaced by an unbalanced bilateral arrangement, which permitted control of the phototropic stimulus by adjustment of the amount of imbalance of two opposed beams (Fig. 2). The optical source was a Bausch & Lomb 500-mm grating monochromator with tungsten incandescent lamp, set at a wavelength of 450 nm. The emergent beam passed through a beam-splitter cube, and the two resulting beams were brought into 180° opposition by a pair of mirrors. The intensities of the beams were equalized by the insertion of glass plates. A sporangiophore centered in the apparatus received an equal phototropic stimulus on each side and thus a net stimulus of zero. The stimulus was unbalanced by placing a neutral density (N.D. 0.20) filter in the left beam, which lowered the intensity of the beam to 61% of its former value. The intensity was calibrated with a thermopile and the calibration extended to low intensity with a photomultiplier photometer. All experiments in the transparent cell series were performed at an intensity of 0.30 $\mu W/cm^2$ per beam. The sporangiophore was enclosed in a small aluminum housing fitted with windows for entry

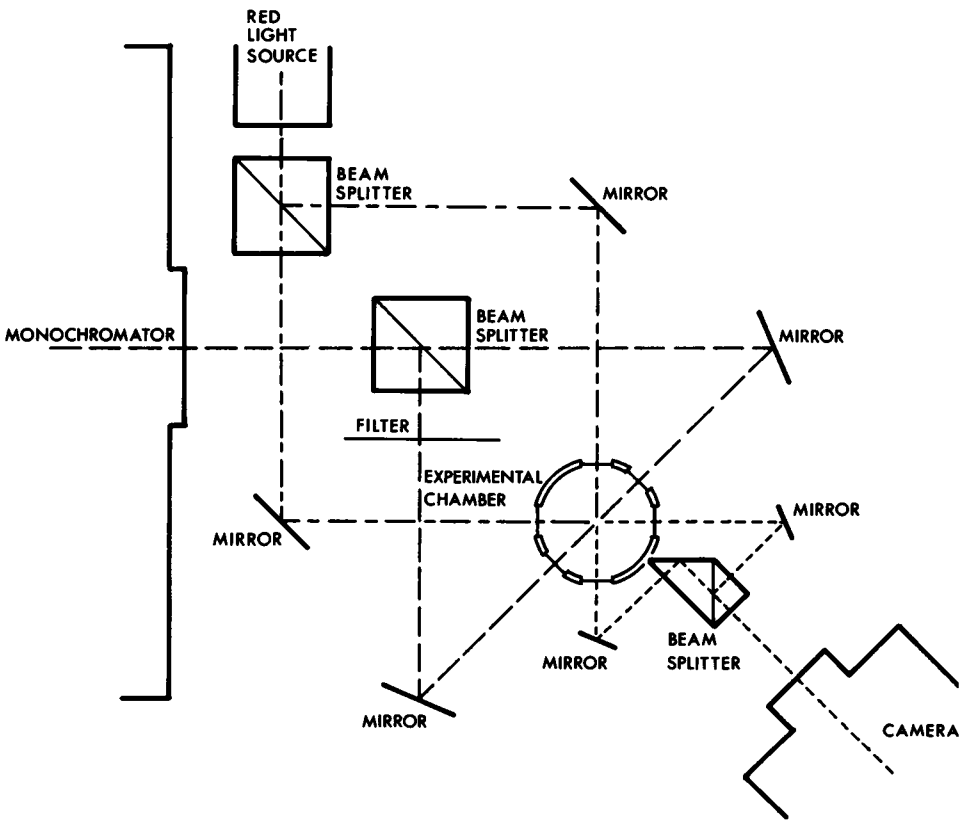

FIGURE 2 Top view of the optical arrangement for stimulation and photography in the transparent cell series. See text for explanation.

of the stimulating and recording light beams. The inside of the housing was humidified by moistening the walls with water, and the temperature was controlled by the room air conditioner. A small thermister probe continuously monitored the air temperature inside the housing. In all the transparent cell series experiments the temperature range was between 20°C and 21°C.

Data collection in the opaque cell series was by a pair of Gaertner long-focus microscopes with goniometer eyepieces (Gaertner Scientific Corp., Chicago, Ill.). These were aligned horizontally and 90° apart. The 285-nm stimulus entered at 30° from the axis of the left microscope. Angle measurements of the upper 0.75 mm of the sporangiophore were made with each microscope simultaneously, at 1-min intervals throughout the experimental run. The sporangium rotation rate was determined before each run by measuring the amount of turntable rotation needed per minute to maintain the sporangium at a constant angular position. The sporangium was marked by a 70-μm Sephadex sphere (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) applied with a micromanipulator.

Data collection in the transparent cell series was by an automatic system. As shown in Fig. 2, a camera recorded the image of the sporangiophore as seen from two directions 90° apart. The left and right images were combined by a pair of mirrors, a right-angle prism, and a beam-splitter cube. Illumination was provided by a Zeiss UKATRON UN60 microflash unit (Carl Zeiss Inc., New York), which contains an incandescent lamp as well as a xenon strobe lamp. The output of this unit was rendered phototropically inert by a red filter and passed through a beam-splitter cube. The two resulting beams were brought into the proper alignment with the left and right camera fields by a pair of mirrors. During an experimental run the sporangiophore was kept centered by the incandescent lamp of the microflash unit. At 1-min intervals an automatic timer turned off the incandescent lamp and triggered the camera shutter, which fired the xenon lamp synchronously to make the exposure. The camera consisted of a Leitz 80-mm focal length Summar macro objective (E. Leitz, Inc., Rockleigh, N.J.), coupled with a Leitz focusing telescope that allowed the camera field to be observed continuously. Completing the optical train were an Ilex electronic shutter (Ilex Optical Company, Inc., Rochester, N.Y.) and the film magazine (ULE, Inc., Stoneham, Mass.), which carries a 100-ft roll of 35-mm film. The film used was Kodak Plus-X (Eastman Kodak Co., Rochester, N.Y.), processed according to the manufacturer's instructions. Each frame consisted of a double exposure, the left image superimposed on the right (Fig. 3). To distinguish them, the camera prism assembly was tilted slightly, raising the left image relative to the right. Before each experimental run, a double-pointed crown of platinum wire (25-μm diameter) was placed on the sporangium, previously coated on its top with mineral oil. The rotation speed of the sporangium was computed from the changing separation of the two points, as seen in the two images.

The processed films were analyzed in a Vanguard Motion Analyzer (Vanguard Instrument Corp., Melville, N.Y.), equipped with a tape punch output for angle and one rectangular coordinate. The angle of the upper 0.75 mm of the sporangiophore and the vertical position of the sporangium were measured for each image, resulting in two lines of data for each frame of the film. The punched paper tape was fed into a computer terminal, and the data were stored and analyzed in the central computer.

The experimental protocol for the opaque cell series was as follows. The sporangiophore was removed from the culture box. In red light the Sephadex marker was attached to the sporangium, and its rotation rate was measured. This takes about 12 min. Then the sporangiophore was rotated at 6 rpm under blue illumination for a 30–40 min equilibration. During the final 5 min of this period, the growth rate was measured. Next, the blue light was turned off and the turntable speed adjusted to the desired value. After the centering of the sporangiophore was carefully adjusted, the 285 nm stimulus began. At 30-s intervals throughout the entire run, the vertical position was readjusted to keep the microscope crosshairs at 0.75 mm from the base of the sporangium; in this way the 0.25-mm band of 285 nm illu-

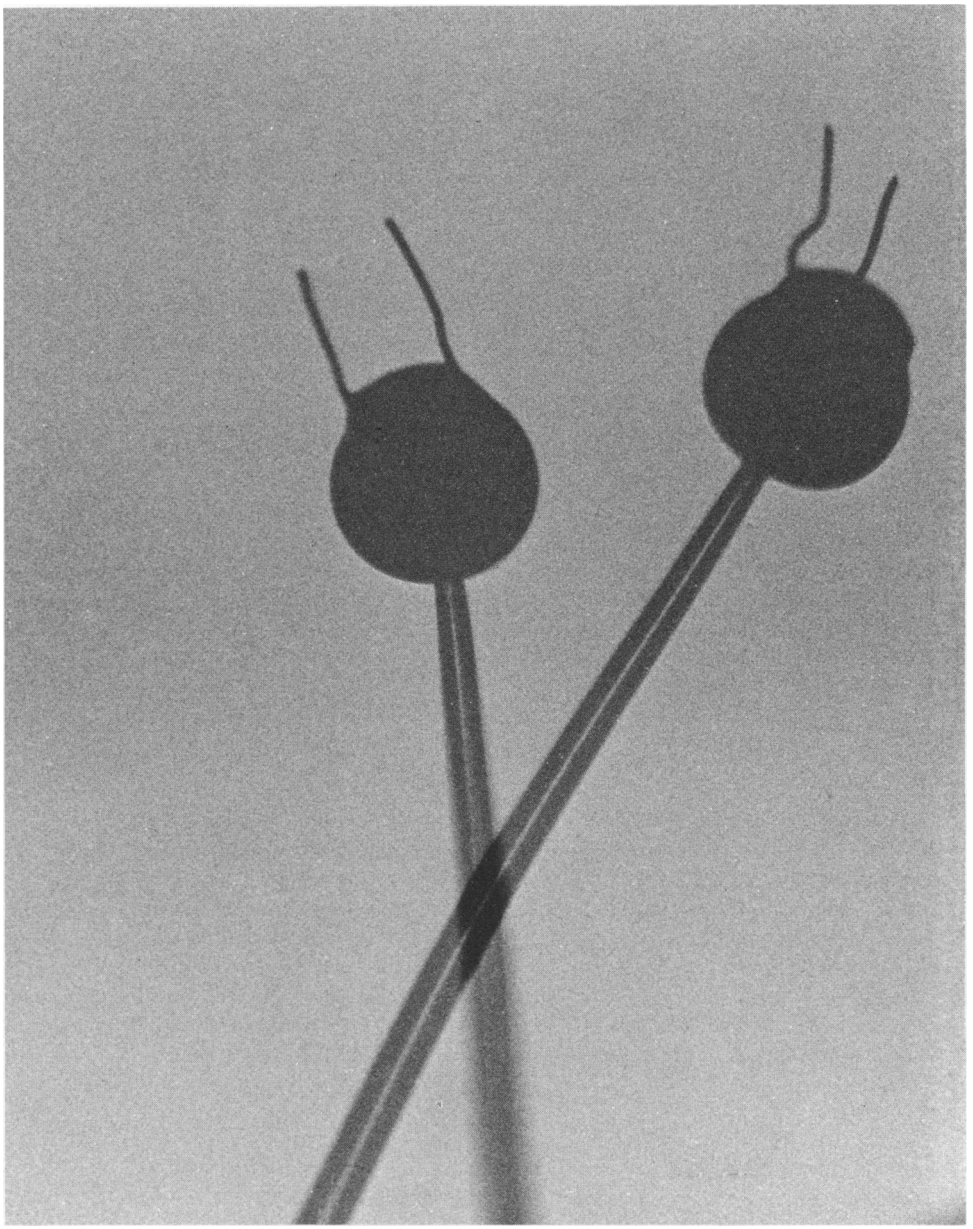

FIGURE 3 Sample film frame obtained from the apparatus of Fig. 2. On the top of the sporangium may be seen the double-pointed wire crown used to measure the sporangium rotation rate.

mination was kept at a nearly constant distance from the sporangium. The run was terminated after 47 min, or if the sporangiophore deviated by more than 40° from vertical in either microscope.

The experimental protocol for the transparent cell series was as follows. The sporangiophore was removed from the culture box, and in red light the wire crown was added. Then, still in red light, the sporangiophore was placed on the turntable and centered. The total time in red light was from 3–10 min. With the turntable stopped, the bilateral 450-nm illumination was then turned on with equal intensity on each side. This equilibration period lasted 60 min, and during this time the temperature, growth rate, and straightness of growth were monitored. Note that this equilibration period differed from that of the opaque cell series in several ways: it was static, bilateral, horizontal, and 60 min long. During the final 10 min of this period, 10 frames were exposed for the measurement of the sporangium rotation rate. At 30 s before the run began, a filter (N.D. 0.20, 61% transmission) was placed in the left beam, and the turntable rotation began at the predetermined rate. The timer was then activated, which began exposing at one exposure per minute. The images were monitored visually throughout the run to make sure the focus and centering were correct. The run was terminated after 60 min, or if the sporangiophore deviated more than 50° from vertical.

DATA ANALYSIS

The sporangiophore is imaged from two directions 90° apart, completely specifying the cell's orientation in space. We consider the sporangiophore axis to consist of a number of straight line segments. The orientation of each segment defines a vector[1], $\mathbf{e}_i$. We define the uppermost vector, $\mathbf{e}_0$, as corresponding to the orientation of the region of the cell above the bend, and $\mathbf{e}_i$ for all vectors below. We will be concerned later only with $\mathbf{e}_0$, but the general analysis is instructive and not much more complicated. We have directed the stimulus beam, $\mathbf{b}$, down the negative X-axis. For any cell segment, the stimulus plane, designated by its normal vector, $\mathbf{s}_i$, is defined by the beam direction, $\mathbf{b}$, and the sporangiophore segment orientation, $\mathbf{e}_i$:

$$\mathbf{s}_i = \mathbf{b} \times \mathbf{e}_i / |\mathbf{b} \times \mathbf{e}_i| .$$

If the turntable rotation is zero, the bending plane of the ith segment is defined by the vector $\mathbf{e}_i(0)$ and the vector $\mathbf{e}_i(\Delta t)$. The vector normal to this plane is: $\mathbf{p}_i = \mathbf{e}_i(0) \times \mathbf{e}_i(\Delta t)/|\mathbf{e}_i(0) \times \mathbf{e}_i(\Delta t)|$. The error angle, ϕ_i, is the angle between the stimulus plane and the bending plane. As shown in Fig. 4, we are using a right-handed coordinate system with ϕ_i the angle from the stimulus to the bending plane ($\mathbf{e}_i$ is the "rotation axis" of ϕ_i). The orientation of this bending plane may also be given in terms of the angle ϕ_i and the orientation of the stimulus plane. The formula for the rotation of a vector through an angle γ, about a defined axis of rotation, $\mathbf{q}$, whose direction cosines are c_1, c_2, and c_3, is:

$$B(\gamma, \mathbf{q}) = \cos\gamma \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} + (1 - \cos\gamma) \begin{bmatrix} c_1^2 & c_1c_2 & c_1c_3 \\ c_2c_1 & c_2^2 & c_2c_3 \\ c_3c_1 & c_3c_2 & c_3^2 \end{bmatrix}$$

$$+ \sin\gamma \begin{bmatrix} 0 & -c_3 & c_2 \\ c_3 & 0 & -c_1 \\ -c_2 & c_1 & 0 \end{bmatrix}.$$

[1]This and all subsequent vectors are understood to be of unit magnitude.

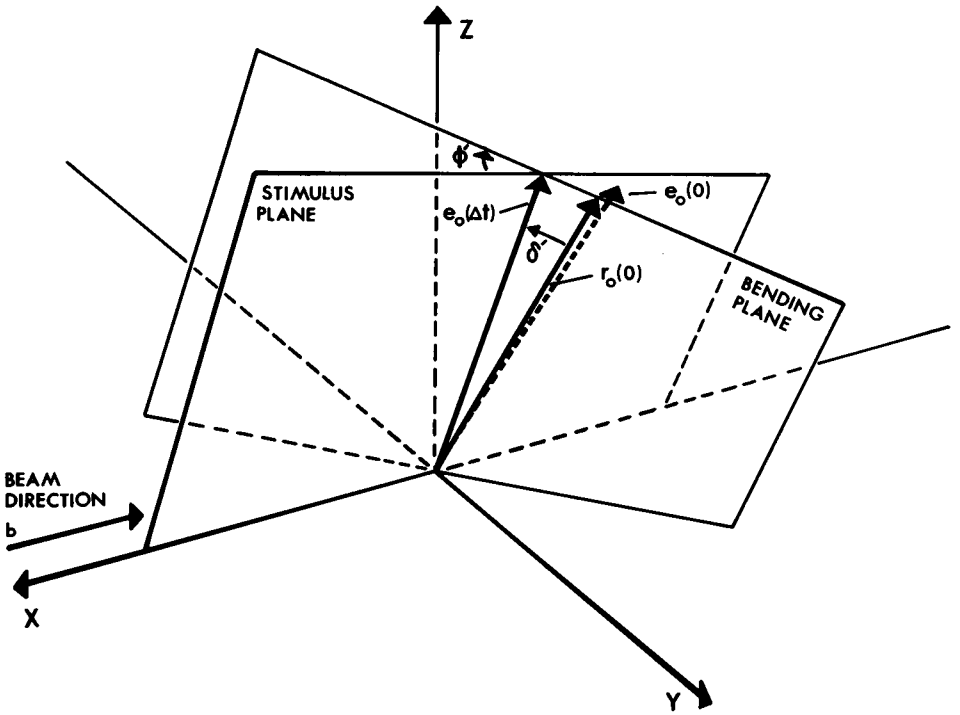

FIGURE 4 The relationships between the stimulus beam direction, the stimulus plane, the sporangiophore bending plane, the error angle, ϕ', and the bending speed, δ'. The sporangiophore orientation at the beginning of the interval is given by $\mathbf{e}_0(0)$ and $\mathbf{r}_0(0)$; the former is the actual initial orientation and the latter is the initial orientation after a correction is made for the turntable rotation during the interval. The orientation at the end of the interval is given by $\mathbf{e}_0(\Delta t)$. During the interval the sporangiophore is assumed to bend in the bending plane, determined by $\mathbf{r}_0(0)$ and $\mathbf{e}_0(\Delta t)$, and the amount of bend is the angle δ'. The error angle, ϕ', is positive for the transparent cell when taken counterclockwise from the stimulus plane to the bending plane; δ' as shown in the diagram is thus negative. For the opaque cell, the aiming error is 180° plus ϕ' as shown.

We may then substitute our error angle, ϕ_i, for γ, and the direction cosines of $\mathbf{e}_i$ for the c's, redefining the vector normal to the bending plane as $\mathbf{p}_i = B(\phi_i, \mathbf{e}_i)\mathbf{s}_i$. This normal is the appropriate "rotation axis" for the bending of that segment of the sporangiophore. One may then use the same transformation as above to describe the bending of the cell during a short time, Δt. Since the bend will be small, the operator B may be approximated by

$$\begin{bmatrix} 1 & -c_3'\delta_i & c_2'\delta_i \\ c_3'\delta_i & 1 & -c_1'\delta_i \\ -c_2'\delta_i & c_1'\delta_i & 1 \end{bmatrix},$$

where δ_i is the angle through which the ith segment bends during Δt, and c_1', c_2', c_3' are the direction cosines of $\mathbf{p}_i$. Therefore if the lowest bending segment was oriented at $t = 0$ in the direction $\mathbf{e}_n(0)$, it will be oriented in the direction $\mathbf{e}_n(\Delta t) = B(\delta_n, \mathbf{p}_n)\mathbf{e}_n(0)$ after an interval Δt. The bending of this lowest bending segment will of course cause the segment supported above to appear to bend even if it does not itself bend. If spiral growth causes the segment

to twist, the segment above will also be twisted. A twist during Δt corresponds to a rotation, ψ, about the sporangiophore axis. Since the bending plane is defined by rotation about the same axis, both effects may be combined to give a new $\phi' = \phi + \psi$. We may therefore write for the uppermost segment: $\mathbf{e}_0(\Delta t) = B(\delta_n, \mathbf{p}_n)B(\delta_{n-1}, \mathbf{p}_{n-1}) \ldots B(\delta_0, \mathbf{p}_0)\mathbf{e}_0(0)$. If additionally an external turntable rotation is applied, then the result observed in the experimental coordinates is: $\mathbf{e}_0(\Delta t) = B(\rho, \mathbf{k})B(\delta_n, \mathbf{p}_n) \ldots B(\delta_0, \mathbf{p}_0)\mathbf{e}_0(0)$, where ρ is the angle through which the turntable rotates in the interval Δt about the Z-axis, $\mathbf{k}$. Since Δt is 1 min in our experiments, ρ is also the rate of turntable rotation. If all the $\mathbf{e}_i$ are measured, it is possible to obtain all the B's and therefore a description of the phototropic event in terms of δ_i's and ϕ_i''s as a function of position along the growing zone. We have chosen to analyze only the top segment.

In our experiments we are interested in the instantaneous direction in which the cell bends relative to the stimulus plane. Therefore we first calculated the new orientation assumed by a rigid cell after being rotated through an angle ρ by the turntable: $\mathbf{r}_0(0) = B(\rho, \mathbf{k})\mathbf{e}_0(0)$. The real bend is from $\mathbf{r}_0(0)$ to $\mathbf{e}_0(\Delta t)$, and these vectors now define the bending plane. The angle between the normal to the stimulus plane, $\mathbf{s}(\Delta t) = \mathbf{b} \times \mathbf{e}_0(\Delta t)/|\mathbf{b} \times \mathbf{e}_0(\Delta t)|$, and the normal to the bending plane, $\mathbf{p} = \mathbf{r}_0(0) \times \mathbf{e}_0(\Delta t)/|\mathbf{r}_0(0) \times \mathbf{e}_0(\Delta t)|$, is the error angle, ϕ'. We actually use a corrected version of the angle ϕ', given by $\phi' = \cos^{-1}(\mathbf{s}(\Delta t) \cdot \mathbf{p}) + \rho\Delta t/2$, where $\rho\Delta t/2$ is a correction required because the direction of bending changes during Δt due to the turntable rotation. The angle ϕ' is assumed constant during Δt. The angle through which the sporangiophore bends during Δt is $\delta' = \cos^{-1}[\mathbf{r}_0(0) \cdot \mathbf{e}_0(\Delta t)]$. Since Δt is always 1 min, δ' is also the bending rate. To visualize the bending direction and speed during the entire experiment, a graphic method is used in which δ' is represented as a length in a two-dimensional rectangular coordinate system. In this system, which we call the $X'Y'$ plane[2], the direction of bending (error angle) is represented by a line segment making an angle of ϕ' with the X' axis and δ' is represented by the length of that segment. Although δ' and ϕ' determine the length and orientation of the first line segment, these parameters do not specify its location in the $X'Y'$ plane. We therefore arbitrarily locate the segment's origin at a point corresponding to the first $\mathbf{e}_0(0)$ of the experiment, projected onto the XY plane. The length and orientation of the next segment are determined similarly, and we stipulate in addition that the second segment begins at the end of the first segment. Continuing in this manner, the successive segments are laid end to end, forming the $X'Y'$ plane bending trajectory (see Fig. 5).

The stimulus from the sporangiophore's view is rotated at a rate somewhat different from the turntable rotation. We define the angle through which the stimulus rotates during Δt as the stimulus rotation rate (SR), which is calculated as:

$$\mathrm{SR} = \cos^{-1}(\mathbf{s}(0) \cdot (B)(\rho, \mathbf{k})\mathbf{s}(0)) + \cos^{-1}(B(\rho, \mathbf{k})\mathbf{s}(0) \cdot \mathbf{p})) - \cos^{-1}(\mathbf{s}(\Delta t) \cdot \mathbf{p}).$$

In characterizing the overall phototropic response of an entire experimental run, the average response was taken from 20 min after the start of the run to the end of the run, usually 40 min later. We did not analyze the data covering the first 20 min because we are principally interested in steady-state bending, which does not become established until after about 20 min.

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[2]Although the $X'Y'$ plane is useful for conceptual purposes, it does not correspond to any physical system. In the case of a nearly vertical sporangiophore, the line segment in the $X'Y'$ plane corresponds roughly to a projection in the XY plane of a line connecting the termini of the vectors $\mathbf{r}_0(0)$ and $\mathbf{e}_0(\Delta t)$ (Fig. 4). However, if the cell is far from vertical, this XY plane projection would shorten the line segment considerably; to avoid this we plot a segment whose length is always numerically equal to δ'. All subsequent calculations concerning the bending trajectory in the $X'Y'$ plane similarly assume δ' to have the quality of a length.

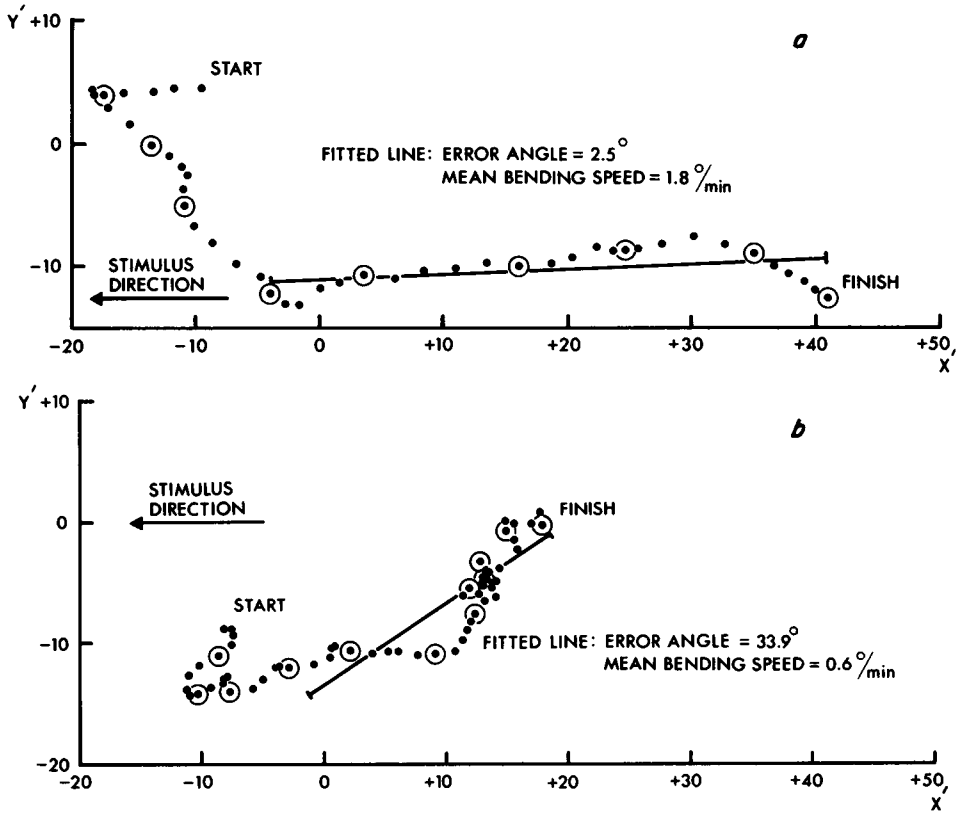


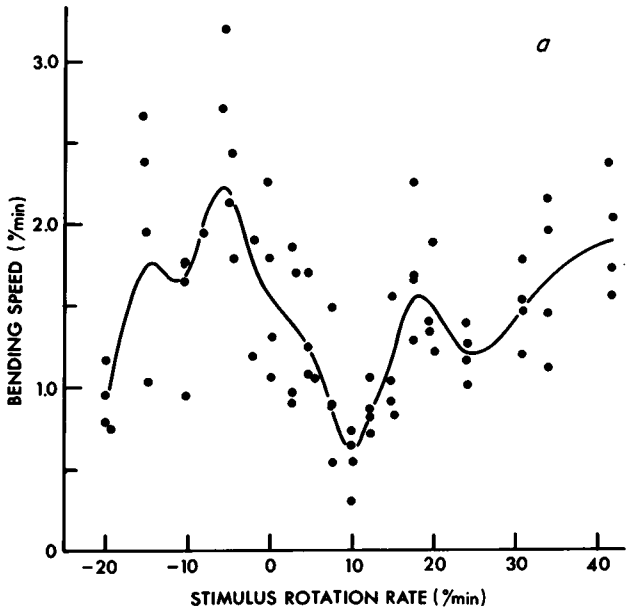
FIGURE 5 Experimental bending trajectories plotted in the $X'Y'$ plane. Coordinates are in units numerically equivalent to those of δ', the angle through which the sporangiophore bends during each 1-min interval (see text). Every fifth point is circled. In (*a*) the turntable rotation rate is zero and in (*b*) it is 10°/min counterclockwise (SR = +10°/min).

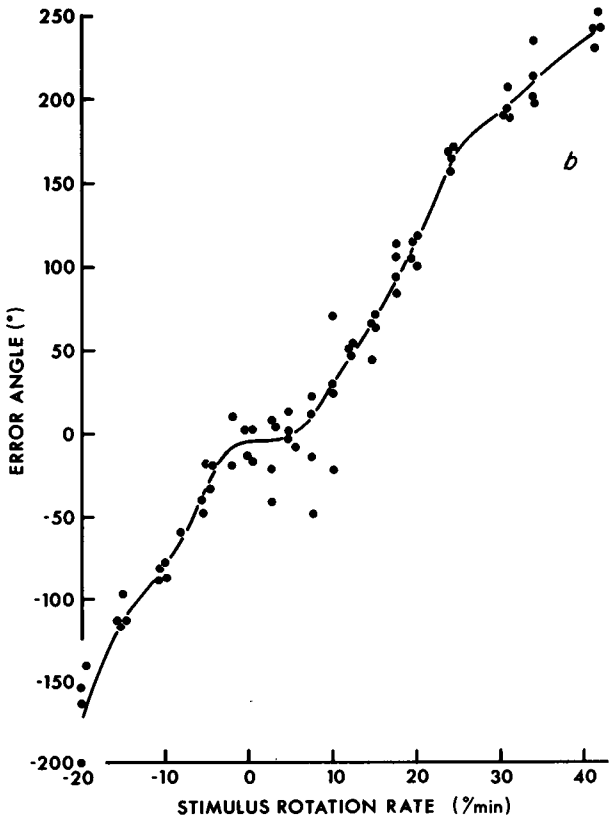
The average response from 20 min to the end of the run is obtained by fitting a straight line by the method of least squares to the points in the $X'Y'$ plane. The average bending direction is defined as the angle of this fitted line relative to the X' axis. The average speed is defined as the distance between the point of the 20th min and the last point in the direction of the fitted line, divided by the time interval.

RESULTS

Rate of Phototropic Bending

When the $X'Y'$ plane trajectories are examined, it is apparent that the major prediction of the hypothesis is supported by the data of the transparent cell series; counterclockwise turntable rotation at approximately 10°/min strongly suppresses the phototropic response. Fig. 5 presents the trajectories of two experiments, one with no turntable rotation and the other with a turntable rotation of 10°/min counterclockwise. The first of these (*a*) shows three phases. During the first 5 min, the sporangiophore shows a latent period, during which there is no response to the stimulus (in this case


a
BENDING SPEED (°/min)
3.0
2.0
1.0
0
-20
-10
0
10
20
30
40
STIMULUS ROTATION RATE (°/min)


b
ERROR ANGLE (°)
250
200
150
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-50
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-150
-200
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30
40
STIMULUS ROTATION RATE (°/min)

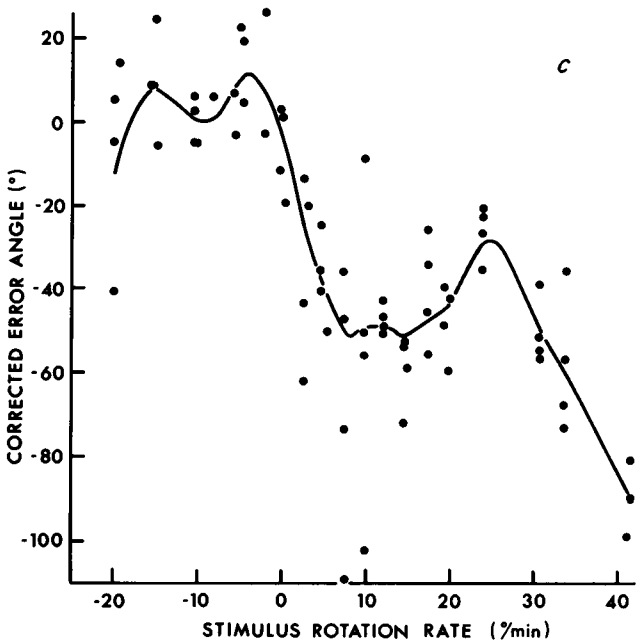
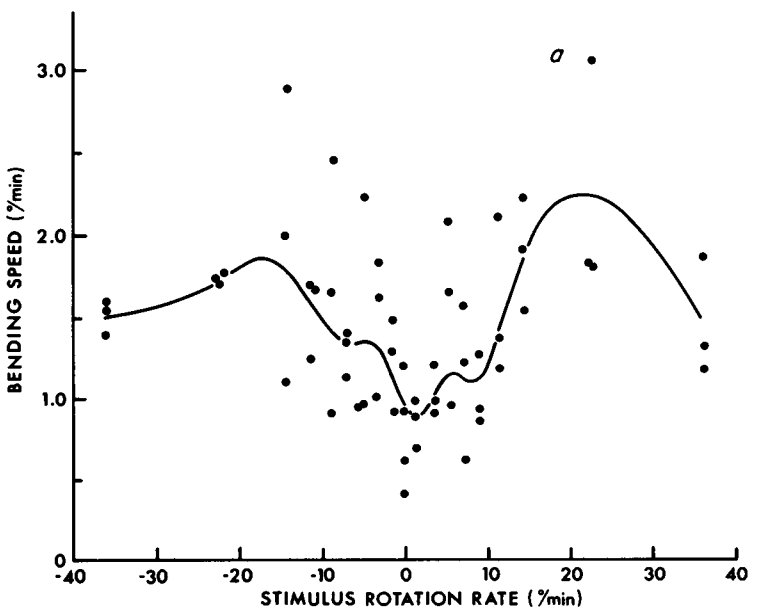


FIGURE 6 Results for the transparent cell. Points are individual data values and the curve is a computer-calculated smoothed spline fit (Reinsch 1967, 1971), consistent with the variance observed in the data. (*a*) Bending speed vs. stimulus rotation rate. (*b*) Error angle, ϕ', vs. stimulus rotation rate. (*c*) Corrected error angle vs. stimulus rotation rate. The corrected error angle is obtained by subtracting from the measured error angle a delay of 8 min multiplied by the stimulus rotation rate. The stimulus rotation rate refers to the rotation rate of the intensity pattern as viewed from the cell. For a vertical cell it is identical to the turntable rotation rate; for a nonvertical cell a geometrical correction is applied (see text).

there was a movement to the left, probably a random motion unrelated to the stimulus). From 5 to 20 min the response is in a direction about 45° to the right of the stimulus. Finally, from 20 to 45 min (when the experiment was terminated because the sporangiophore was too far from vertical) there is steady bending in a direction towards and roughly parallel to the stimulus. The line is fitted by computer (see Data Analysis above) to the last 26 points. The length of the line is 44.85°, and the mean bending speed for this 25-min interval is 1.79°/min. The line makes an angle of +2.53° with the stimulus direction, and we will call this the error angle. We will use the right-handed coordinate convention that in the transparent series the error angle is zero if exactly towards the stimulus, negative if deviating clockwise (to the right), and positive if deviating counterclockwise (to the left). In the opaque cell series, the error angle is zero if exactly away from the stimulus direction, negative if deviating clockwise, and positive if deviating counterclockwise. In the second of these experiments (*b*), the turntable rotation is +10°/min. Although distinct phases of the response are not apparent here, it is clear that the bending speed is greatly reduced.

Not only are the dots in the $X'Y'$ plane closer together in this experiment, but for 16 min the sporangiophore wanders in a series of tiny looping movements with a net bending speed essentially zero. The line fitted to the last 41 points has a length of 23.9°, which corresponds to a mean bending speed of 0.65°/min for this 40-min interval. The line makes an error angle of +33.9° with the stimulus direction.

The data of the transparent cell series of 57 experiments, in which the whole growing zone is illuminated bilaterally, are collected in Fig. 6*a*. The bending speed is the mean speed corresponding to the line fitted to the points from 20 min to the end of the experiment (usually at 60 min). The stimulus rotation rate (SR) is the actual rotation of the intensity pattern within the sporangiophore, averaged over the same interval as the bending speed. The most striking feature of Fig. 6*a* is the sharp dip in bending speed extending from an SR value of +5°/min to +15°/min, with the lowest value at +10°/min. At an SR of +10°/min the bending speed is reduced to about 30% of its value with no rotation. For increasingly negative (clockwise) turntable speeds, the bending speed first exceeds and then falls below the rate for no rotation. At −5°/min the bending speed is about 140% of that for no rotation, and at −20°/min, it falls to about 60% of the speed for no rotation. Between −5 and −20°min, there may be a secondary dip and peak, but such possible fine structure is all but obscured by the experimental scatter. For rotation speeds more positive than +10°/min, the bending speed rises to a peak at +17.5°/min, where its level is comparable to that observed with no rotation. As the rotation rate becomes more positive, the bending speed drops to a shallow minimum (at +25°/min) and then rises uniformly, so that at +41.5°/min it is slightly greater than the speed for no rotation. Thus the curve appears to be symmetrical about a center at +10°/min, but only out to rotation



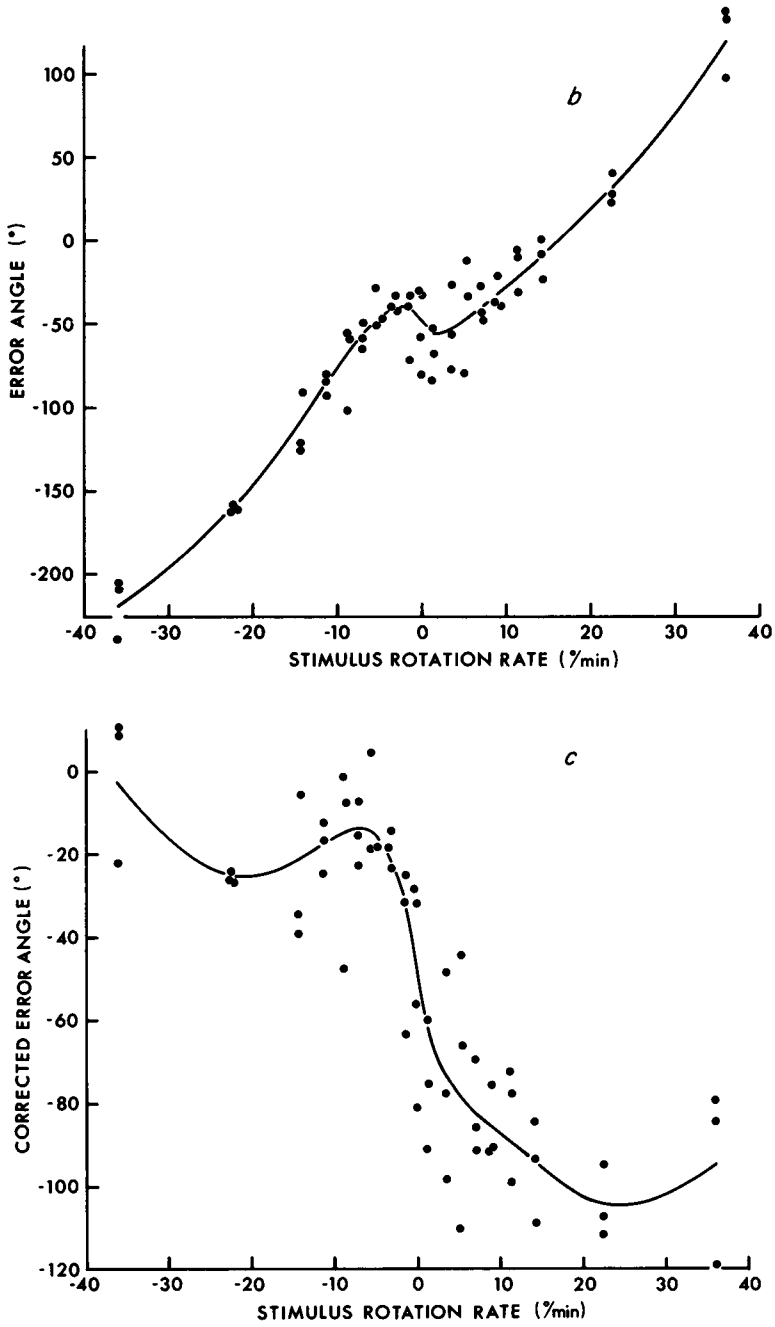


FIGURE 7 Results for the opaque cell. Points are individual data values and the curve is a computer-calculated smoothed spline fit. (*a*) Bending speed vs. stimulus rotation rate. (*b*) Error angle, ϕ', vs. stimulus rotation rate. (*c*) Corrected error angle vs. stimulus rotation rate. The corrected error angle is obtained by subtracting from the measured error angle a delay of 6 min multiplied by the stimulus rotation rate.

rates 10°/min greater or 10°/min less. Beyond these limits, the symmetry clearly breaks down.

The data for the opaque cell series, in which a narrow band of the growing zone is illuminated unilaterally, are collected in Fig. 7 *a*. This figure shows an increase in bending speed for rotation rates greater than +10°/min and less than 0°/min. In contrast to Fig. 6 *a*, there are two dips in bending speed in the region of positive

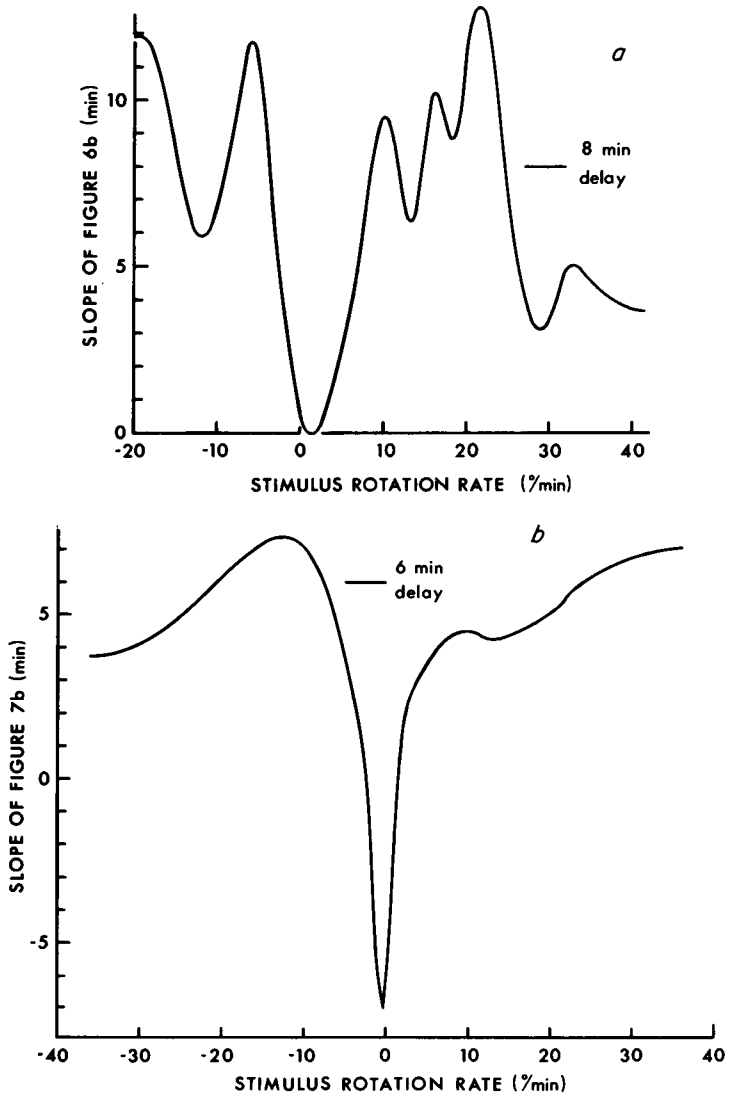


FIGURE 8 (*a*) Slope of Fig. 6 *b*, or $\partial\phi'/\partial(SR)$ for the transparent cell. This curve is obtained by taking the derivative of the spline-fit calculated curve of Fig. 6 *b*. (*b*) Slope of Fig. 7 *b*, or $\partial\phi'/\partial(SR)$ for the opaque cell. This curve is obtained by taking the derivative of the spline-fit calculated curve of Fig. 7 *b*.

rotation rate. The dips are at 0–2°/min and 8–10°/min. The conditions of the transparent cell and the opaque cell series differ markedly in illumination pattern, region of the growing zone illuminated, and the physiological state of the sporangiophore, as mentioned earlier.

Error Angle (ϕ')

As mentioned in the Introduction, shifts in bending direction are expected in the vicinity of stimulus rotation speeds for which the locus of the stimulus switches from one side to the other. In Figure 6*b* the data for the transparent cell series are presented. To arrive at each value of the error angle plotted here, a line is fitted by computer to the points in the $X'Y'$ plane from 20 min to the end of each experiment, usually a total of 41 points (see Data Analysis above).

To see the data more clearly, they have been replotted (Fig. 6*c*) with an angle subtracted from the error angle corresponding to a fixed delay multiplied by the stimulus rotation rate. We call this the corrected error angle. Such a delay and consequent rotation is anticipated since for the light growth response there is a delay to the point of maximum response, typically 6 or 7 min at 20°C (Foster and Lipson, 1973). In Fig. 6*c* an arbitrary value of 8 min was chosen to make the curve approximately horizontal on both sides of the major shift. The angle plotted (corrected error angle) is the counterclockwise angle from the positive X'-axis to the fitted direction *minus* this fixed delay (8.0 min) multiplied by the rotation rate. Note the shift of about 60° centered at about 1°/min rotation rate.

In Fig. 8*a* the slope of Fig. 6*b*, i.e., the quantity $\partial\phi'/\partial(\mathrm{SR})$ (in minutes), is plotted as a function of SR. The large shift occurs in the region between 0 and 6°/min, centered at 1°/min. On both sides of this minimum there are larger than average values and at high positive rotation rates there are much lower than average values (see Discussion).

For the opaque cell series the dependence of the error angle on stimulus rotation rate is given in Fig. 7*b* and in Fig. 7*c* is plotted (corrected error angle) with a delay of 6.0 min. A shift of 60° is seen in the region centered at about 0°/min. The slope of Fig. 7*b* is shown in Fig. 8*b*. As might be expected for a narrow band stimulus corresponding to a narrow range of growing zone rotation rates, the minimum has a width of only 5°/min, as compared to 10°/min. Also, there is no correspondingly reduced $\partial\phi'/\partial(\mathrm{SR})$ at large positive rotation rates (see Discussion).

DISCUSSION

The stimulus rotation rate giving minimum bending speed in the transparent cell series is about 10°/min. Measurements of the sporangium rotation rate for these same 57 sporangiophores give an average value of 9.9°/min (±1.3). This coincidence suggests that the sporangium and the upper, sensitive, part of the cell rotate together as one unit and that the adaptive-responsive system is simply rotated integrally with the whole rotating cell at that level in the growing zone. Since the only rotational element is the cell wall and since cytoplasmic plug flow (due to viscosity) is antici-

pated within the cell, all the adjacent cytoplasm probably rotates nearly at the same rate as the wall. The receptors and light adaptation mechanism could then lie anywhere from the tonoplast to the plasma membrane. Light adaptation has been shown to remain fixed with respect to the position of the sporangium (Delbrück and Varjú, 1961), which probably implies that most of the cytoplasm slips up the cell as a body, relative to the cell wall, as the cell grows in length. The cytoplasmic mass in the growing zone leaves behind it only a thin trace of cytoplasm below the growing zone, and the cell tapers slightly as it grows. The fact that a portion of the bend moves down from the sporangium (as is necessary for it to be made permanent) probably implies that some product responsible for cell bending moves down the cell at a rate near that of the cell's growth. The fact that the cell rotation rate decreases rapidly as a function of position from the top of the growing zone (Ortega et al., 1974) means that the product responsible for bending is not rotated very far around the cell. As Castle (1962) has noted in the transparent case, the top portion of the cell becomes balanced after the initial bending and the continuous bend is from the lower (slower rotating) portion. As a result there will be neither an unacceptably large aiming error nor a spiralled cell.

The proposed sequence of events begins with the carousel of receptors being brought into the lit band after a period of relative dark adaptation. Both the increases and the decreases in growth velocity must be considered along with the azimuthal location. The observed bend is thus a summation of the light growth response delivered locally and spread around the cell. We believe this adaptive-responsive system extends all along the growing zone and rotates with the cell wall at a rate that varies from about 10°/min in the upper portion to zero at the bottom of the growing zone.

What are the implications of these ideas with respect to the detailed results of each experiment? One idea we stressed in the Introduction was that in this delicate tropic balance (bilateral unequal intensities, transparent cell) the azimuthal position of the opposed responses is important. We also noted that the delay to the point of maximum response is shorter for a focused band than for a smooth one. Let us now apply these principles to the specific instance of the transparent cell, for stimulus rotation rates greater than 25°/min. Fig. 8 *a* indicates that from 25 to 40°/min the value of $\partial\phi'/\partial(\mathrm{SR})$ drops from about 8 min to about 4 min. This shift can be considered a reduction in the effective delay between stimulus and response, and since the turntable rotation is counterclockwise, this shift amounts to a clockwise change in bending direction. Also at this rotation rate (25°/min) there is a well-defined secondary minimum in bending speed, and from 25 to 40°/min there is a steady increase in bending speed. We believe that these facts can be explained by assuming that at 25°/min the position of the response to the focused band of light is opposite that of the smooth band, because at this speed the longer delay to maximum response of the smooth band just compensates for the larger distance to be covered (from Fig. 1 *a*: focused band is about 35°, smooth band nearly 180°). Although in opposite directions, these responses are unequal in magnitude, and we suppose that the focused band is dominant. If now the counterclockwise turntable rotation is speeded up, the

difference in delays of the two responses will cause a relative angular shift between them, and they will no longer be opposite. Specifically, if the delays differ by 2–3 min, then for each extra 1°/min of rotation speed, these responses will shift relative to each other by 2–3°. This will result in a significant and progressive clockwise shift in the net bending vector for increased counterclockwise turntable rotation rates, as reflected in the shift in effective delay ($\partial\phi'/\partial$(SR)) from 8 to 4 min. And finally, the unbalancing of the two response vectors would increase the magnitude of the net vector, increasing the bending rate as observed.

Let us now consider the general features of the response in both the transparent and the opaque cells. In the region of about 10°/min, a minimum bending speed is observed in both cases (Figs. 6*a* and 7*a*), and we argue that this is due primarily to light stimulus adaptation. The constant component of the $\partial\phi'/\partial$(SR) curves (Figs. 8*a* and *b*) is most certainly due to the time it takes for the response to be expressed. Because of the much greater light contrast in the opaque case, the delay to maximum response is several minutes shorter than that in the low contrast transparent case. In the opaque cell, the average error angle for zero stimulus rotation is about 50° clockwise (−50° in Fig. 7*b*), which implies that the responding region must be rotating at about 8–9°/min clockwise (assuming a delay of 6 min). This is consistent with the fact that the stimulus is confined in the opaque cell to the upper part of the cell, rotating nearly at the speed of the sporangium (about 10°/min). In the transparent cell the error angle is only about −6° to −7° for zero stimulus rotation (Fig. 6*b*), which correspondingly implies a rotation rate of 0.7–0.9°/min (assuming a delay of 8 min). This is consistent with a broad region of stimulation and a responding region near the basal, slowly rotating part of the cell.

In the opaque case, the stimulus is confined to a band 0.5 mm from the sporangium, a region rotating from 7 to 10°/min. Recall the light distribution for the opaque case, as illustrated in Fig. 1*b*. In spite of the "opaque" designation, we believe the far focused band may have some effect at slow relative rotation speeds. Although this band rises to a peak value of only about 30% of the incident intensity, it is exceedingly sharp, with high contrast at its edge. When the net rotation is slow the response to the focused band may be comparable to that of the smooth band on the opposite side, leading to a nearly balanced condition and low bending speed. On the other hand, at higher net rotation rates the smooth band is moving rapidly enough across the receptors to give a sizable response and the focused band now acts like a brief pulse stimulus and produces a weaker response. This dominance of the smooth band at higher rotation rates may explain the increased bending speed for rotation rates of 20°/min and −20°/min, compared to that for rotation rates of −10 to 10°/min. The failure to find a more sharply defined minimum in phototropic bending is puzzling. This may be due to the use of a narrow band stimulus, whose sharp upper and lower edges may act as continual sources of tropic stimulation. Also there may be too great a distribution of cell rotation rates within the 0.25-mm slice of growing zone or too great a variation in cell rotation rate from experiment to experiment or from time to time during a single experiment.

The direction of relative rotation determines on which edge of a focused band the stimulus occurs. If the turntable speed is such that the net rotation rate of the receptor system is slow and clockwise, then the locus of stimulus will be on the counterclockwise edge of the band. On the other hand, if the net rotation is instead slow and counterclockwise, the stimulus will shift to the other side of the band. Thus a large shift in bending direction is anticipated when the stimulus rotation rate is swept through the value that just cancels the cell's rotation. Such a shift is indeed found, but it is centered around zero rotation rate and not 10°/min, as would be expected from the cell's rotation rate at the point of stimulus. We have no adequate explanation for this discrepancy.

In the transparent cell case the stimulus is distributed throughout the growing zone, which rotates typically from 0 to 10°/min. The bending speed drops to a sharp minimum at 10°/min stimulus rotation rate. The fact that phototropism fails to vanish completely may be due to the distribution of rotation speeds within the growing zone or to variation in the cell's rotation rate during the course of an experimental run. As already discussed there is a slight minimum at 25°/min due to the opposition of responses. The error angle (Fig. 6 *b*) is less negative then in the opaque case because the more slowly rotating, lower region of the cell is dominant. Between −2 and 7°/min there is a gradual shift in bending direction as a result of the stimulus shifting from one side of the lit band to the other. Although the situation is obviously complex, given the wide range of relative rotation speeds along the growing zone, the shift centers at 1°/min (Fig. 8 *a*). As in the opaque cell, this speed is unaccountably different from the speed of minimum phototropism (10°/min).

At moderate negative stimulus rotation rates (−4 to −16°/min), both opaque and transparent cases show increased bending speed as a consequence of the greater effectiveness of the rotation mechanism at higher than normal rotation rates. Quite possibly, the fact that *Phycomyces* bends so precisely towards a light stimulus is due to the evolutionary selection for the "correct" rotation rate. Although the cell might bend faster if its natural rotation rate were twice as high, its aim would be seriously affected.

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REFERENCES

CASTLE, E. S. 1962. Phototropic curvature in *Phycomyces*. *J. Gen. Physiol.* **45**:743.

COHEN, R., and M. DELBRÜCK. 1958. Distribution of stretch and twist along the growing zone of the sporangiophore of *Phycomyces* and the distribution of response to a periodic illumination program. *J. Cell. Comp. Physiol.* **52**:361.

DELBRÜCK, M., and D. VARJÚ. 1961. Photoreactions in *Phycomyces*. Responses to the stimulation of narrow test areas with ultraviolet light. *J. Gen. Physiol.* **44**:1177.

DENNISON, D. S. 1965. Steady-state phototropism in *Phycomyces*. *J. Gen. Physiol.* **48**:393.

DENNISON, D. S., and R. P. BOZOF. 1973. Phototropism and local adaptation in *Phycomyces* sporangiophores. *J. Gen. Physiol.* **62**:157.

FOSTER, K. W. 1972. The photoresponses of *Phycomyces:* analysis using manual techniques and an automated machine which precisely tracks and measures growth during programmed stimuli. Ph.D. Thesis. California Institute of Technology, Pasadena. Xerox University Microfilms, Ann Arbor, Mich.

FOSTER, K. W., and E. D. LIPSON. 1973. The light growth response of *Phycomyces*. *J. Gen. Physiol.* **62**: 590.

ORTEGA, J. K. E., J. F. HARRIS, and R. I. GAMOW. 1974. The analysis of spiral growth in *Phycomyces* using a novel optical method. *Plant Physiol.* (Bethesda). **53**:485.

REINSCH, C. H. 1967. Smoothing by spline functions. I. *Numer. Math.* **10**:177.

REINSCH, C. H. 1971. Smoothing by spline functions II. *Numer. Math.* **16**:451.