The Effect of Light on the Geotropic Responses of *Phycomyces* Sporangiophores

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ABSTRACT The geotropic responses of *Phycomyces* sporangiophores were studied under varying intensities of illumination, using a low speed centrifuge and a fixed beam of blue light. This light has a strongly inhibitory effect on the transient geotropic response, reducing it to 36 per cent of its magnitude in darkness. The inhibition does not vary systematically with light intensity over a range of 400-fold. The light sensitivity of the transient geotropic response thus differs from the light-growth response system, which shows the same growth rate in light and darkness. By contrast, the slower long term geotropic response is enhanced by light of moderate intensities, but is strongly inhibited by high intensities. At and above a mean intensity of about 1 $\mu w/cm^2$, the long term response is completely removed. If the intensity is lowered from an inhibitory level, either to darkness or to a low level, the geotropic response appears after a time lag of 20 minutes. Furthermore an increase in intensity from one level to another, both levels normally enhancing, results in a transient reversal in the long term geotropic response, also after a time lag of 20 minutes. Thus it is suggested that light is acting at some intermediate step in the long term geotropic sensory system, a step that normally requires 20 minutes for completion.

INTRODUCTION

The geotropic sensitivity of the single celled sporangiophores of the fungus *Phycomyces blakesleeanus* has long been known (Banbury, 1959), although its mechanism is poorly understood. Various effects of light on the geotropic response have also been studied, and they are of three main types—inhibition of geotropism, enhancement of geotropism, and the phenomenon of phototropic-geotropic balance. The inhibition of geotropism by light has recently been examined by Pilet (1956). Working with dark-grown cultures, he found that a light pretreatment (60 watt incandescent lamp at 25 cm for 30 min.) increased the latent period for the appearance of the geotropic response and also reduced the rate of geotropic bending. The possibility that light could also enhance the geotropic response was raised by Dennison (1958), who observed that the geotropic reaction seemed to be much weaker in darkness than in the light. The weakness and high variability of the geotropic reaction made this

conclusion rather uncertain, however. If a sporangiophore is illuminated by a horizontal beam of light, it will come to an equilibrium growth direction about 30° above horizontal. This 30° deviation is due to geotropism and is constant over nearly the entire intensity range of phototropism, a range of 10^9 (Dennison, 1958). This light-gravity equilibrium has recently been examined at intensities near the lower threshold for phototropism (10^{-8} μw/cm^2), and an action spectrum for the light-gravity balance has been measured (Varjú, Edgar, and Delbrück, 1961). This action spectrum deviates from the action spectrum found by Delbrück and Shropshire (1960), and the deviation was explained on the basis of photopigment self-screening and a variable photopigment concentration. However, it is also possible that an enhancement of geotropism by near threshold light intensities might also account for this deviation.

It seemed likely that these light and gravity interactions might be more effectively examined if the geotropic response could be increased in strength and reliability by increasing the strength of the gravitational stimulus. A preliminary study of the tropic responses of *Phycomyces* to centrifugal force (Dennison, 1961) showed that the geotropic response is materially increased in bending speed and improved in reproducibility by such means. This work also showed that sporangiophores have two distinct responses to the application of a lateral acceleration of about 4 times gravity. One of these, the transient geotropic response (active phase), is a very rapid bending against the applied force, lasting for about 6 minutes, and having a bending speed of about 5°/min. This response is a result of the deformation of the cell due to centrifugation and can equally well be elicited by the application of a purely mechanical lateral force to the sporangium. The other response is characterized by a much slower rate of bending which continues for 1 to 2 hours, until the sporangiophore has turned through an angle of about 90° and has a direction of growth antiparallel to the applied acceleration. Although slower than the transient response, this long term geotropic response is about triple the speed of the response to natural gravity.

The purpose of this work is to uncover the extent to which the geotropic and phototropic stimulus-response systems share a common sensory pathway. It is clear that the final step in both these pathways is the establishment of differential elongation rates on opposite sides of the cell, causing bending. It is hoped that a study of the interaction between the sensory systems will suggest the points where these separate pathways converge to a common mode of response.

MATERIALS AND METHODS

The cultures (*Phycomyces blakesleeanus*, sexually minus strain, derived from strain 1555 of the National Regional Research Laboratory) are grown from heat-shocked vegeta-

tive spores inoculated on potato dextrose agar medium in small glass vials as described previously (Dennison, 1961). The potato dextrose agar medium is improved by the addition of small amounts of cottonseed oil in emulsified form. A medium containing 5 per cent potato dextrose agar (Difco), 0.1 per cent Wesson oil (Wesson Oil and Snowdrift Sales Co.), and 0.001 per cent tween 40 (Atlas Powder Co.) as an emulsifier produces a crop of large uniform mature sporangiophores 4 days after inoculation. The plain 5 per cent potato dextrose agar medium often produces many fine sporangiophores (useless for experimentation) on the 4th day, and not until the 5th or 6th days do the large sporangiophores appear. Similar findings have been reported by De Boer (1929). The cultures are kept in a humidified (about 80 per cent relative humidity) ventilated chamber illuminated continuously from above by a 15 watt incandescent lamp at 60 cm and held at a temperature of 23°C. All experiments are performed at 20.5 to 21.5°C. in a room darkened except for the red-filtered phototropically ineffective lights required for photography and manipulation, and the blue source described below.

The centrifuge is basically that described previously (Dennison, 1961). Sporangiophores are rotated at a radius of 20 cm about a vertical axis at a constant speed. Two speeds are used, a high speed which produces a horizontal centrifugal force of 4.1 times gravity (4.1 $\times$ *g*), and a very low speed, which produces about 0.02 $\times$ *g*. At the high speed, sporangiophores are subjected simultaneously to 4.1 $\times$ *g* of horizontal force and 1.0 $\times$ *g* of vertical force (natural gravity), which combine to produce a resultant force of 4.22 $\times$ *g* in a direction 13.7° below horizontal and outward. The sporangiophores are mounted in holders as described previously, and are protected from air currents by a cylindrical cover of clear plexiglass, which encloses the entire upper surface of the centrifuge disk.

A photographic recording device is used to make nearly simultaneous observations on ten sporangiophores during centrifugation. A Cenco-Edgerton stroboscope, fitted with a red filter to exclude phototropically effective light, illuminates the sporangiophores with a single flash per revolution of the centrifuge. The stroboscope is triggered by sliding electrical contacts fastened to the underside of the disk and arranged so that by suitable switching the stroboscope flash is triggered when any of the ten sporangiophores passes through a single fixed point. A camera lens (Roussel 135 mm focal length, f/4.5), its axis tangent to the arc of rotation of the sporangiophores, forms an image of the flash-illuminated sporangiophore in the focal plane of a bolex H-16 reflex 16 mm motion picture camera. The over-all magnification is approximately unity. Exposures are made by opening the camera shutter and holding it open while the stroboscope trigger circuit is closed just long enough for a single flash, which makes the exposure. Then the camera shutter is closed and the film advanced for the next exposure. The controls for the shutter and stroboscope circuit are interlocked mechanically so that this entire process requires only 3 seconds. Each of the ten sporangiophores is brought successively into focus by connecting different contact pins into the stroboscope trigger circuit. In this way photographs can be taken of all ten sporangiophores successively in a total time of about 30 seconds. During the first 20 minutes of each centrifuge run, exposures are generally taken at 1 minute intervals, and subsequently at 5 minute intervals. The exposed film (Kodak Plus-X) is processed accord-

ing to the manufacturer's recommendations and the negatives analyzed by direct projection and measurement. A 16 mm still projector is used to provide an enlarged image on a ground glass screen. The angular orientation of the image of the upper 1.0 mm of the sporangiophore is measured directly on the screen by a Keuffel and Esser Paragon, Jr., drafting machine with protractor head. This instrument measures angular orientations (with a precision of 0.5°) independently of side-to-side or up-and-down displacements of the image.

Illumination of sporangiophores during rotation is provided by a fixed beam of parallel filtered light, 2 cm by 5 cm in cross-section, extending across the diameter of the centrifuge disk (Fig. 1). Each sporangiophore passes through the 2 cm width of this beam twice in each revolution, once on the near side and once on the far side. The sporangiophores receive equal phototropic stimuli from opposite sides (in alternation) and are thus in a state of phototropic indifference. The light source consists of a 100 watt, 120 volt tungsten filament projection lamp (General Electric designation CDS) powered by a Variac variable transformer, which draws its power from a Sola voltage-

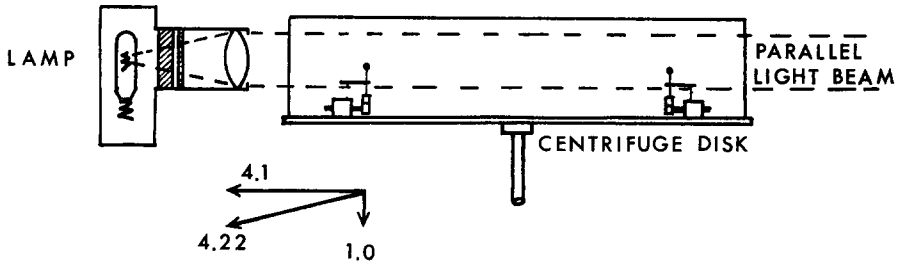

FIGURE 1. Centrifuge and blue light source. At high speed, the sporangiophores are under a steady centrifugal force of 4.1 × g, which combines with gravity to give a resultant of 4.22 × g. The centrifuge axis is vertical.

stabilized 115 volt AC power supply. The lamp output intensity is varied by changing the output voltage of the variable transformer. The radiation from the lamp passes through 2 cm of water in a glass cell and then through a Corning 5-61 blue glass filter. Finally the radiation is converged into a rectangular beam of parallel light by a lens (focal length 211 mm, diameter 58 mm) with a rectangular aperture. At the normal operating lamp voltage the output of this filtered source is spectrally characterized by a broad principal peak centered near 460 mμ, having a width at half-maximum extending from about 400 mμ to 500 mμ and dropping to negligible values at 350 mμ on the short wavelength side and 600 mμ on the long wavelength side. A weak secondary band extends from about 680 mμ out to about 1.4 μ in the infrared where the water cell begins to absorb strongly. This red and infrared band is due to a very weak transmission band in the 5-61 filter (about 0.5 per cent transmission) which is greatly accentuated by the characteristically high lamp output in the red and infrared regions relative to the blue region (Forsythe and Adams, 1945). It is unlikely, however, that the infrared radiation is biologically active at wavelengths greater than 800 mμ. Since the total energy contained in the region from 680 to 800 mμ is a small fraction of the

energy in the main blue band, this contamination is judged to be insignificant.[1] Absolute intensity measurements of the source beam are made with a Reeder RUM-4C thermopile, (C. M. Reeder and Co., 171 Victor Ave. Detroit Michigan), whose output is amplified by a Beckman model 14 DC breaker amplifier. The thermopile-amplifier system is calibrated (intensity units, microwatts per square centimeter) against a National Bureau of Standards radiometric standard lamp. Since the presence of infrared would preclude accurate measurement of the source intensity in the principal blue band, a 3 cm thickness of 100 gm./liter $CuSO_4 \cdot 5H_2O$ in 0.5 per cent H_2SO_4 is placed between the source and thermopile. The $CuSO_4$ cell transmits significantly (greater than 0.1 per cent) only at wavelengths shorter than 650 mμ and transmits in excess of 70 per cent in the region from 380 mμ to 530 mμ. The measured intensities are corrected for the transmission loss through the $CuSO_4$ cell, which is fairly constant at 15 per cent in the region (380 to 530 mμ) containing 90 per cent of the energy of the source. The transmission data for the $CuSO_4$ cell and the 5-61 filter were obtained with a Cary model 15 recording spectrophotometer.

RESULTS

In a typical experiment ten culture vials are selected, each vial containing a mature stage IV sporangiophore. A sporangiophore is considered mature if its total height is over 25 mm for 5 day old cultures, over 27 mm for 6 day old cultures, and over 30 mm for 7 day old cultures. Using only non-phototropic red light, the vials are mounted in holders on the disk, as described previously (Dennison, 1961), with the sporangiophores approximately vertical. Then the centrifuge cover is applied (a pad of wet paper is included to maintain humidity) and the centrifuge is rotated very slowly ($0.02 \times g$) for a pretreatment period of 2 hours. During this period the blue source is set at the same intensity to be used during the high speed centrifugation. The pretreatment thus serves as a period of temperature and humidity equilibration, light adaptation, and a check on phototropic indifference. Any sporangiophores deviating by more than 30° from vertical at the end of the pretreatment period are excluded. After the pretreatment the centrifuge is speeded up to $4.1 \times g$ and held at that speed for 2 or 3 hours. Exposures are taken immediately before the speed-up, at 1-minute intervals for 20 minutes after the speed-up, and at 5-minute intervals thereafter. The high exposure frequency is needed at the beginning of the run to record the fast-moving transient geotropic response. Occasionally after an hour or two at high speed, some of the sporangiophores begin to deviate from the radial plane (defined by vertical and radial lines through the sporangiophore). If this deviation exceeds 45°, the angle measurement is judged to be too imprecise and the sporangiophore is excluded.

Consider the effect of steady illumination on the transient geotropic re-

[1] The red and infrared energy is estimated to be about 3 per cent of the blue energy for the highest lamp voltage used and about 10 per cent of the blue energy for the lowest lamp voltage used.

sponse (Fig. 2). This response begins with the so called passive phase, an outward bending of the sporangiophore during the centrifuge speed-up. The passive phase is not a true biological response but only a result of the mechanical flexibility of the sporangiophore. From 80 to 90 per cent of the

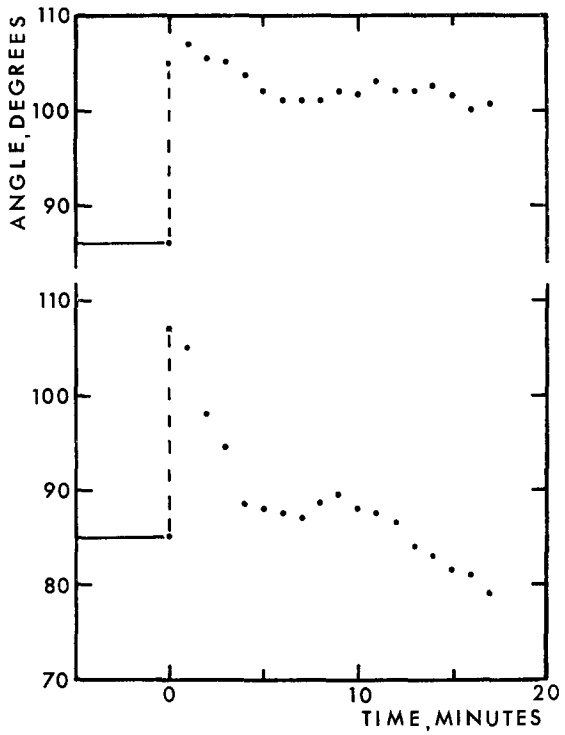

FIGURE 2. Transient geotropic responses. The upper curve shows a typical response under illumination (beam intensity 1.14 $\mu w/cm^2$). The lower curve shows a typical response in darkness. The ordinate is the angle between the upper 1.0 mm of sporangiophore and the centrifuge radius passing through the sporangiophore. This angle is zero when the sporangiophore is growing horizontally towards the centrifuge axis. The vertical dashed line represents the passive phase of the response, when the sporangiophore is bent outwards during the centrifuge speed-up at time zero. The centrifuge acceleration is complete within 30 seconds. Typically, the active phase of the transient geotropic response consists of rapid inward bending for a period of 4 to 8 minutes after the speed-up. Following this, there may also be reversed bending (outwards) at a slower rate for another interval of 4 to 8 minutes. This reversed bending is highly variable and may be absent.

total passive bend measured at the top 1.0 mm arises from flexure in the growing zone (upper 4 mm), the remainder being due to flexure in the lower, non-growing portion. Table I shows that the amount of passive bending is not significantly different in darkness and in the light. Since the amount of passive bending is dependent on the elastic and plastic properties of the cell

(mainly in the growing zone), this result implies that these mechanical properties are not affected by steady illumination.

The active phase of the transient response is a brief and rapid bending of the growing zone against the direction of the applied centrifugal force. The size of this active phase is greatly reduced by illumination. In darkness this response has a mean value of 19.7° ± 2.6° and in steady illumination the response has a mean value of 7.0° ± 1.0°. This inhibition does not vary significantly with intensity over the range from 0.13 to 50.8 $\mu w/cm^2$. Some sporangiophores show practically no responses under illumination, while others show nearly normal responses. This pattern of variability shows no systematic trend with illumination intensity. The duration and general character of the active phase of the transient geotropic response are un-

TABLE I

EFFECT OF LIGHT ON THE SIZE OF THE TRANSIENT GEOTROPIC RESPONSE

The passive phase of the response is the angle through which the upper 1.0 mm of sporangiophore bends during the centrifuge speed-up. The active phase of the response is the angle through which the upper 1.0 mm of sporangiophore bends during the first 6 minutes of centrifugation, or until a clear minimum in angle is reached. The total number of sporangiophores is given in parentheses. Cultures are all 5 days or more in age.

Illumination	Passive phase	Active phase
	(*degrees* ±2 SEM)	(*degrees* ±2 SEM)
Dark	20.3±1.9(33)	19.7±2.6 (33)
Light	19.7±0.9 (98)	7.0±1.0 (108)

affected by illumination intensity in the range used. These results show that the light sensitivity of the active phase of the transient geotropic response is in marked contrast to the light sensitivity of linear growth. The system responsible for the active phase of the transient geotropic response is maintained in different steady states in darkness and in the light, as opposed to linear growth, which proceeds at the same rate in darkness as in the light.

The long term geotropic response is affected by steady illumination in two distinct ways, depending on the intensity. At low intensities the response is more rapid than in darkness and at high intensities the response is reduced from that in darkness, becoming essentially zero for beam intensities above 30 $\mu w/cm^2$. Typical responses in darkness, with low intensities, and with high intensities are shown in Fig. 3.

For purposes of calculation the long term response is defined as the mean bending speed over an interval of about 60 minutes, beginning at 20 to 25 minutes after the start of the run. During the initial 10 to 14 minutes of the

run the sporangiophores are undergoing the passive and active phases of the transient response, sometimes followed by a period of outward bending. During the next 10 minutes the sporangiophores usually show little bending or sometimes a slight outward bending (away from the axis of rotation), but by the end of this period they have begun to bend definitely inwards. A 60 minute interval was chosen because most specimens show a fairly constant bending rate during this period.

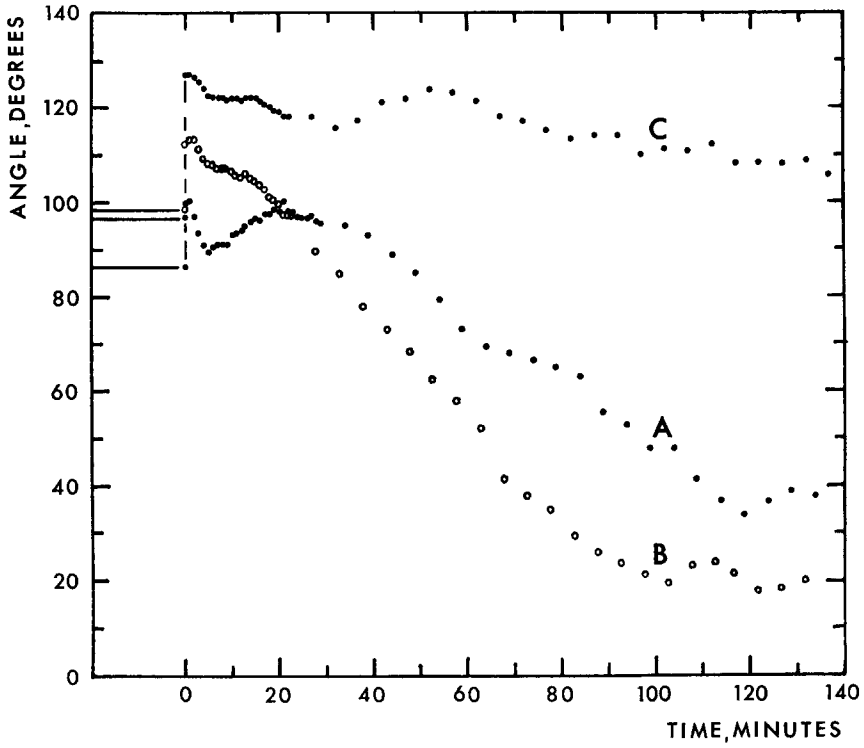

FIGURE 3. Typical long term geotropic responses, showing the enhancing and the inhibiting effects of illumination. Experiment A was performed in darkness, experiment B at an intensity of 1.14 $\mu w/cm^2$, and experiment C at an intensity of 50.8 $\mu w/cm^2$. The ordinate is defined as in Fig. 2.

The long term geotropic response, as defined above, is given in Fig. 4 as a function of the beam intensity. The lower intensity threshold for the enhancement of geotropism by light is unknown, but must be less than 0.13 $\mu w/cm^2$. At 14.6 $\mu w/cm^2$ the response is about the same as in darkness, and at 30.1 and 50.8 $\mu w/cm^2$ the response is essentially zero. Due to the variability in response among different sporangiophores, some responses are actually negative at these high intensities. For example at the highest intensity used, one specimen had a response of minus 0.58 degree per minute;

i.e., it bent *outwards* by 37.5° in a period of 65 minutes. In several cases at high intensity sporangiophores were observed to bend outwards (from near vertical) until they reached an angular position of 140 to 150°, remaining in that position for as long as 2 hours. Such apparent cases of "equilibrium" did not show the oscillation characteristic of phototropic and photogeotropic equilibrium (Dennison, 1959; Varjú, Edgar, and Delbrück, 1961), and hence were probably not instances of true tropic equilibrium.

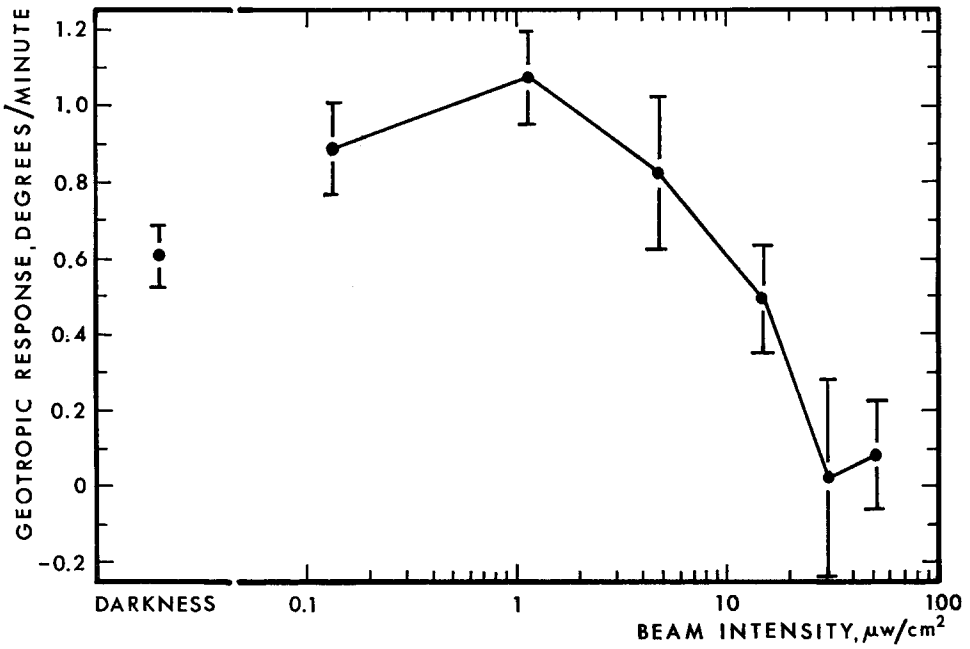

FIGURE 4. The long term geotropic response as a function of the illumination beam intensity. The mean intensity is 0.0318 of the beam intensity (see Discussion). The response is defined as the average geotropic bending rate during a 1 hour interval, beginning 20 to 25 minutes after the start of the run. The vertical bars have a total length of 4 SEM (standard error of the mean). Each point is a mean value based on ten to twenty sporangiophores for illuminated runs and thirty sporangiophores for dark runs.

To verify the light-induced enhancement of the long term geotropic response, the following experiment was performed. The sporangiophores are pretreated in darkness and the run started in darkness in the normal manner. After about 50 minutes the beam is turned on and maintained at 0.13 $\mu w/cm^2$, an intensity that normally enhances the geotropic response. A typical result is shown in Fig. 5. An increase in bending speed occurs during the 15 minute period following the turning-on of the beam. Most unexpectedly, the geotropic response shows a *reversal*, beginning at 20 minutes after the light application and persisting for an additional 10 to 20 minutes. After this reversal, normal

geotropic bending resumes at a high rate, typical of geotropic responses under enhancing intensities of illumination. The most remarkable feature of this reversal is that it occurs so long after the light stimulus. According to the recent work of Castle (1961) an increase in intensity during the course of a phototropic response elicits a transient phototropic reversal. This reversal is presumed to be due to the light-growth response as it interacts in some unknown manner with the asymmetrical growth associated with photo-

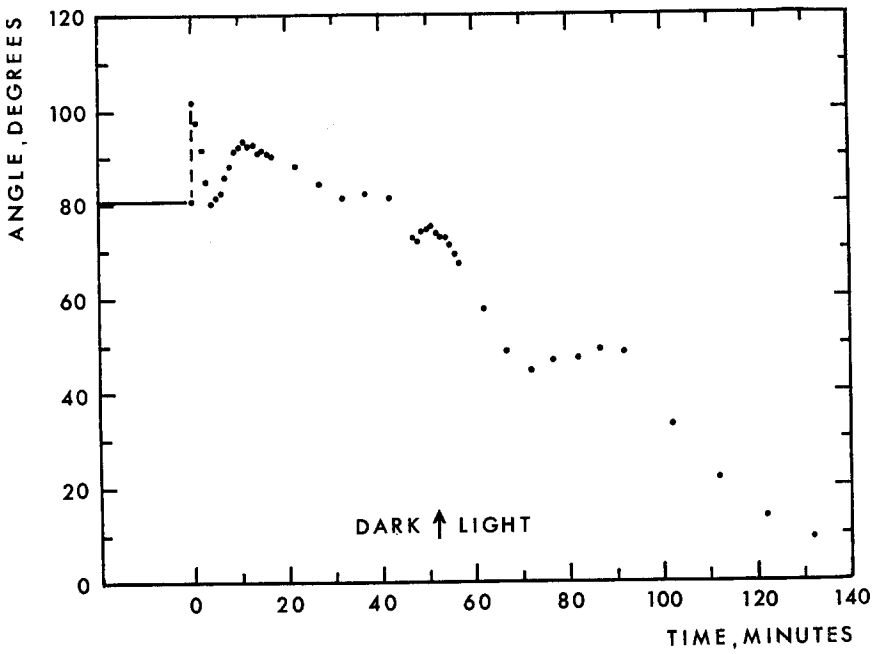

FIGURE 5. The effect of switching from darkness to steady illumination during a long term geotropic response. The sporangiophore was pretreated in darkness and kept in darkness during centrifugation until the time indicated by the arrow. At this moment the blue beam was turned on at an intensity of 0.13 $\mu w/cm^2$. The ordinate is defined as in Fig. 2.

tropism. Such a mechanism cannot account for the reversal just described because the latter does not begin until 20 minutes after the light stimulus is applied; the light-growth response should be entirely finished by this time. An experiment during which the intensity was raised from 0.13 to 4.72 $\mu w/cm^2$ gave the typical result shown in Fig. 6. There is a slight decrease in the rate of geotropic bending during the 20 minute period following the intensity increase. More significant is the fact that here too is a large reversal, basically similar to the one caused by the transition from darkness to 0.13 $\mu w/cm^2$.

Experiments were also performed to test the reversibility of the high intensity inhibition of the long term geotropic response. Fig. 7 shows typical results of three types of experiments. In experiments A and B, the intensity is lowered from 50.8 to 1.14 $\mu w/cm^2$ during a run; in experiment A the drop is instantaneous and in experiment B the drop is spread over a 55 minute period. It can be seen that the results are similar, namely the removal of the inhibition of geotropism about 20 minutes after the first drop in intensity.

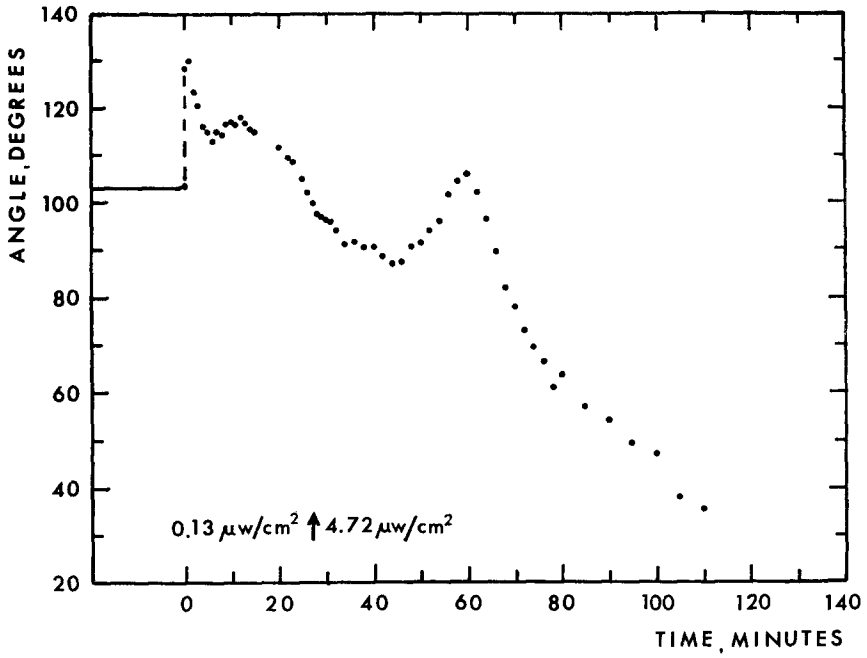

FIGURE 6. The effect of raising the intensity of illumination during a long term geotropic response. At the time indicated by the arrow the beam intensity was increased from 0.13 $\mu w/cm^2$ to 4.72 $\mu w/cm^2$. The ordinate is defined as in Fig. 2.

When the intensity is lowered from 50.8 to only 30.1 $\mu w/cm^2$ (experiment C), the inhibition of geotropism is not removed.

Brief mention should be made of the effect of culture age on the inhibition of long term geotropism by high intensity illumination. All the results presented are based on *Phycomyces* cultures 5 days or more in age (time since inoculation). Sporangiophores of 4 day old cultures, the so called first crop, do not exhibit the inhibition of long term geotropism at 30.1 and 50.8 $\mu w/cm^2$, as do sporangiophores from older cultures. Culture age does not seem to affect the light sensitivity of the transient geotropic response.

DISCUSSION

The mechanism of the transient geotropic response is unknown, but has been shown to be associated with mechanical deformation of the cell, which is the result of externally applied forces. The possibility that steady light affects the

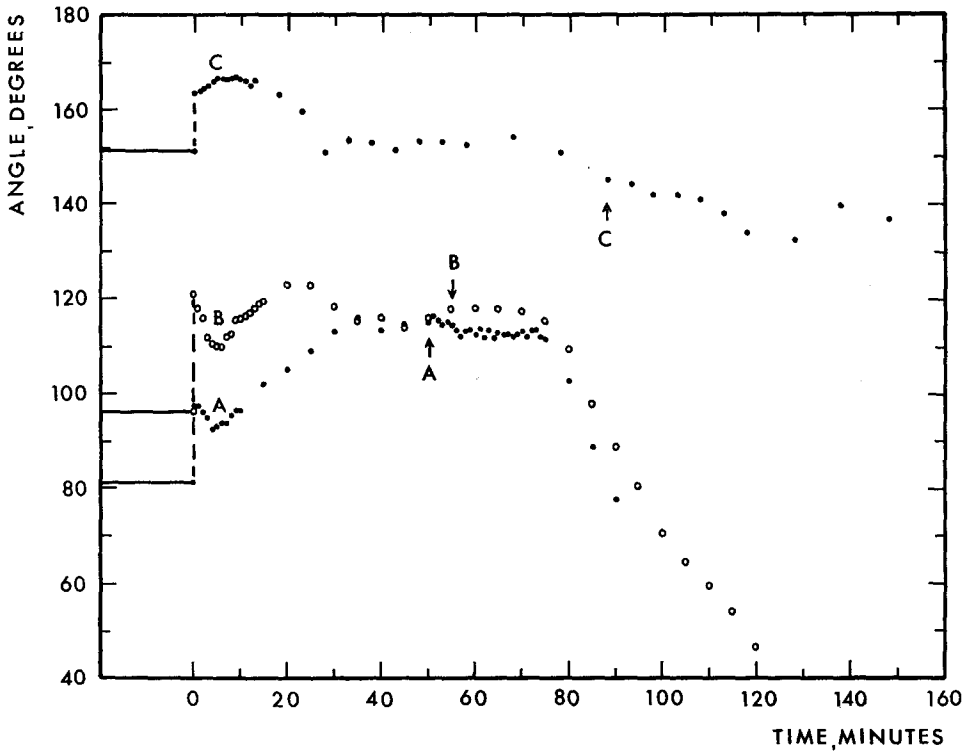

FIGURE 7. The effect of lowering the intensity of illumination during a long term geotropic response. In experiment A the intensity was lowered from 50.8 $\mu w/cm^2$ to 1.14 $\mu w/cm^2$ at the time indicated by the arrow (A). In experiment B the intensity was lowered from 50.8 $\mu w/cm^2$ to 1.14 $\mu w/cm^2$ over a 55 minute period beginning at the arrow (B). The intensity program consisted of 55 successive decrements at 1 minute intervals, ranging from 4.5 to 10 per cent of the intensity. In experiment C, the intensity was lowered from 50.8 $\mu w/cm^2$ to 30.1 $\mu w/cm^2$ at the time designated by the arrow (C). The ordinate is defined as in Fig. 2.

active phase of the transient geotropic response by altering the elastic and plastic properties of the cell is excluded by the finding that the passive phase, due solely to the mechanical deformation, is unaffected by steady light. Thus the total cell deformation is the same in light and in darkness, but the tropic response to this deformation is strongly reduced in the light. This effect cannot be closely related to the light-growth response because the growth

rate is the same in darkness as it is in the light. However, one feature of the light-growth response system does distinguish between light and darkness: the level of adaptation (Delbrück and Reichardt, 1956). Thus the size of the growth response to a given light stimulus depends very strongly on the level of adaptation, which in turn is determined by previous conditions of illumination. The light-induced inhibition of the transient geotropic response might be related to the level of adaptation. With this possibility in mind it might be of interest to examine the variation of inhibition with time, following a transition from illumination to darkness. Following such a light program, the level of adaptation falls logarithmically, with a time constant of about 4 minutes (Delbrück and Reichardt, 1956). If the inhibition of the transient response were found to be removed in a similar logarithmic manner, with a similar time constant, a definite relation between the two phenomena would be indicated. If it became possible to obtain more reproducible transient responses, it would be feasible to measure an action spectrum for the light effect. Comparison with the growth response action spectrum (Delbrück and Shropshire, 1960) would then be of interest.

The long term geotropic response shows two light effects, a low intensity enhancement and a high intensity inhibition. The low intensity enhancement is a rather small effect but does seem to support earlier findings (Dennison, 1958) that geotropism is weaker in darkness than in the light. The high intensity inhibition is of more interest because of its large magnitude and because it occurs at a well defined intensity.

As seen in Fig. 4, the lowest beam intensity producing complete inhibition is 30.1 $\mu w/cm^2$. It must be remembered that the sporangiophores are exposed to this intensity only part of the time. More precisely, during a complete revolution of 125.7 cm in circumference, only a total of 4 cm of this circumference is traversed within the beam. Therefore the mean intensity is 4/125.7, or 0.0318 of the beam intensity. Hence the mean intensity threshold for inhibition is 1.0 $\mu w/cm^2$. These two values are to be compared with a value of 10^{-8} $\mu w/cm^2$ for the absolute lower intensity threshold for phototropism (Varjú, Edgar, and Delbrück, 1961) and with a value of 320 $\mu w/cm^2$ for the threshold of phototropic indifference. The latter phenomenon has been studied by Reichardt and Varjú (1958), and is characterized by the complete failure of phototropism at and above this threshold intensity. Thus the high intensity inhibition of geotropism is evidently not related to the high intensity inhibition of phototropism. This conclusion was confirmed by the observation that the highest intensity used (50.8 $\mu w/cm^2$) elicits normal phototropic responses in sporangiophores that are placed in the uninterrupted beam.

A curious feature of the high intensity inhibition of geotropism is the 20 minute time lag for its removal. This is far longer than the latent period of

either the light-growth response (2 to 3 minutes) or the phototropic response (about 5 minutes). This same 20 minute time lag shows up for the poorly understood geotropic reversal caused by an increase in light intensity. Furthermore a 20 to 30 minute time lag is found between the start of the run and the beginning of the long term geotropic response, either in darkness or under illumination. These facts suggest that the 20 minute lag for the removal of geotropic inhibition is a characteristic of the gravity-sensitive system rather than of the light-sensitive system.

One may ask a further question. Does the high intensity inhibition of geotropism act at the point of gravity perception or at the point of the response; *i.e.*, the mechanism immediately associated with asymmetrical growth rates on opposite sides of the cell? As has been pointed out previously (Dennison, 1961; Banbury, 1959), the initial perception of gravity must involve differential forces or displacements between particles or liquid phases within the cell having different densities. It is difficult to understand how light could interfere with such forces or displacements and thereby inhibit the initial step of the geotropic response. More reasonable is the possibility that light inhibits some intermediate step in the geotropic response, a step that normally requires 20 minutes for its completion. The extreme slowness of this step is apparent when one notes that in 20 minutes one-third to one-half of the entire growing zone is renewed by the growth process. The final step in the response, the establishment of differential growth rates across the cell, is certainly much faster than this. For example, the phototropic response is initiated within 5 minutes and the active transient response begins within 2 minutes.

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