

Phototropism, Adaptation, and the Light-Growth Response of *Phycomyces*

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ABSTRACT Phototropic bending can be initiated without the transient changes in growth speed that characterize a light-growth response. The conditions required are a change from a symmetric to an asymmetric illumination pattern while the cell receives a constant radiant flux. Phototropism is thus basically a steady state process. It cannot be founded on differential light-growth responses as in Blaauw's theory. A possible model system for the unequal partition of growth during steady bending is discussed. The fact that light-growth responses show adaptation while phototropic bending does not follows from the different natures of the two responses.

Phototropic curvature results from differences in growth rate across the diameter of a plant organ. These differences are caused by unequal light absorption due to an external illumination pattern asymmetric about the axis of structure and of growth. Bending is usually experimentally initiated by a single abrupt change in the illumination pattern from symmetrical to asymmetrical. For instance: (a) plant in darkness, then lateral light on; or (b) overhead light off, lateral light on; or (c) growth between two equal and opposite light sources, then one off.

Cohen and Delbrück (1959) have cogently noted that in such phototropic experiments two variables are commonly changed simultaneously: first, the *general level* of light intensity; second, the *distribution* of light intensity about and within the plant. Two complexly associated effects then follow: (a) a *transient* change in the basic rate of growth, the so called light-growth response, and (b) a *persisting* growth rate difference across the plant manifested as bending. The connection between these two aspects of the phototropic response has been much debated since Blaauw (1918) forthrightly concluded that phototropic curvature was caused by unequal light-growth responses on the "near" and "far" sides of the curving organ. Recent discussions of phototropism are given by Galston (1959), Banbury (1959), Cohen and Delbrück (1959), and Thimann and Curry (1960).

Although phototropic bending is usually studied during the transition

between two quasi-steady states of differently oriented growth, suitable experiments show that bending in *Phycomyces* can in fact continue indefinitely (Dennison, 1958; Cohen and Delbrück, 1958). This means that the light-induced differential growth causing bending does not show adaptation as do so many biological response systems. In particular we have the anomaly that light-growth responses show clear adaptation while the phototropic responses of the same plants do not, even though action spectra show that the same photochemical system is involved in both responses (Delbrück and Shropshire, 1960; Curry and Gruen, 1959). These facts are difficult to reconcile with Blaauw's attractively simple theory.

A light-growth response is observed and defined as a change in the elongation rate in response to a change (commonly a step-up) in the intensity of illumination. If the illumination pattern is symmetric and the intensity is, say, doubled, the sporangiophore of *Phycomyces* after a delay elongates more rapidly and then the rate returns to the original steady state value. (Details of this response are given in Delbrück and Reichardt, 1956.) Adaptation here means that after the original rate has been reestablished another response can be obtained only by raising the intensity to a new, higher level. Symmetrical illumination thus gives a "pure" light-growth response unaccompanied by bending. But conversely to secure bending without light-growth response should be impossible according to Blaauw's interpretation.

A difficulty in considering the relations of these two responses is that they are not strictly commensurable. In a light-growth response, growth velocity is considered as a function of the single variable *time*. In bending, growth velocity is primarily considered as a function of *position* about the axis but it may in addition be a function of time if both light intensity and illumination pattern change adequately. The root of the problem is that while in both responses growth velocity is distributed about the axis, this distribution is constant and irrelevant for the pure light-growth response but asymmetric and critically relevant for the bending response.

One way to compare quantitatively growth in the two cases is essentially to integrate each over the cross-sectional area and obtain the rate of volume increase, dV/dt . This in the steady state can reasonably be taken as a measure of the "basic rate of growth" whether or not bending is occurring. Then either during straight growth or during bending a significant change of dV/dt with time signals a light-growth response. If, however, we can secure bending while dV/dt remains constant, this is phototropism unaccompanied by transients and not based on differential light-growth responses in Blaauw's sense.

For simplicity we take the plant organ in question to be a circular cylinder or a "bent" modification thereof, and consider only growth parallel to the axis and leading to increase in lateral area and in volume. The distribution of differential elements of growth in the direction of the axis is in practice

integrated by the plant and is not of concern here. Fig. 1 illustrates the geometric fact that the volume of a cylinder is the same as that of its variously bent forms if the arc length of the axis, s , is constant and the other stated conditions are met. It follows that in the axial growth of a cylinder and of its modified forms the rate of volume increase, dV/dt , is directly proportional to ds/dt . Since s is readily measured as a function of time on enlarged photographs of growing and bending sporangiophores of *Phycomyces*, we can follow the behavior of dV/dt and hence of the basic growth rate under any conditions

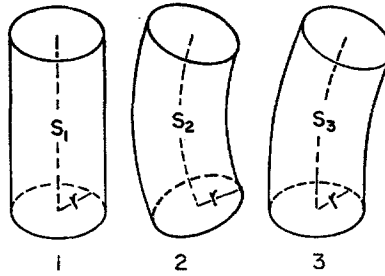


FIGURE 1. (1) Right cylinder; (2) section of a torus; (3) arbitrarily "bent" section of a cylinder. s , axis; r , radius of circular section perpendicular to axis. If r is constant along s in each case and if $s_1 = s_2 = s_3$, then the lateral area, A , between the two end sections perpendicular to the axis is the same for each ($A = 2\pi rs$). The volume is also the same for each ($V = \pi r^2 s$). Note that (1) and (2) are solids of revolution but (3) is not. I am indebted to Professor D. V. Widder for showing that the stated relations extend to the general case (3) where the axis s_3 is any continuous and differentiable function and not necessarily a circular arc. It follows that if in growth ds/dt is constant, dA/dt (rate of "wall formation") and dV/dt (rate of volume increase) remain constant and are independent of changing curvature of the axis.

of curvature. Present interest is restricted to the possibility of light-induced bending without change in dV/dt . We have found that this can be obtained as described below.

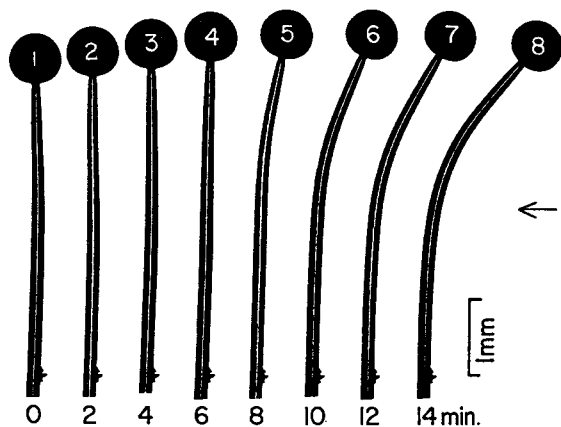
MATERIAL AND METHODS

Cultures of *Phycomyces blakesleeanus* ("minus" strain, NRRL No. 1555) were grown as previously described (Castle, 1958; 1959). Growth and curvature were photographically recorded at 1 or 2 min. intervals on 35 mm film at an initial magnification of 6.6 times. Measurements were made on positive enlargements with a final magnification of 45.5 times. Total growth was taken as the increase in distance along the cell's axis from a starch grain marker below the bottom of the growing zone to the base of the terminal sporangium.

High contrast enlargements show the cell dark with a white central line due to the cell's lens action (Fig. 2). This line is taken as the axis. Arc lengths of the curved axis were measured by rectifying the curve in one of two ways: (a) the curve was

cut along with scissors and its convex face rolled out on coordinate paper; (b) the length of the curve was approximated by "walking" fixed dividers along it and adding up the secant distances. These methods were also used when lengths of the concave and convex flanks of the cell were measured.

The illumination program was similar to that of Reichardt and Varjú (1958). A vertically growing sporangiophore in a rectangular glass moist chamber was bilaterally illuminated for 30 min. with two opposite horizontal light beams of essentially parallel unfiltered white light of closely matched intensity. Intensities were within the range characterized as "normal" by Reichardt and Varjú. At time zero the left beam was cut off and the intensity of the right beam was *doubled* by withdrawal of a neutral 50 per cent transmission filter; simultaneously and periodically thereafter the sporangiophore was photographed against strong red phototropically inactive light. Signifi-



← FIGURE 2. Photographic record of phototropic bending initiated under conditions of constant radiant flux. Starting at time zero all light comes horizontally from the right (arrow). Markers are seen near the bottom. The latent period of about 6 min. before bending starts is apparent.

cantly, this program initiates phototropism by establishing maximum asymmetry of the illumination pattern but *without changing the total radiant flux striking the cell.*

RESULTS AND DISCUSSION

The assembled series of photographs in Fig. 2 shows the progress of phototropic bending in the representative case analyzed below. In Fig. 3 measurements of the length of the axis (s) and of the concave (s_n) and convex (s_f) flanks of the cell are plotted against time. It is clear that s is linear throughout, even though a bend angle of 40° is reached by the end of the series. Notably there is no acceleration of growth at around 5 min. where the maximum of a light-growth response occurs. Since ds/dt is constant it follows that dV/dt also is constant. Therefore this cell even while bending at a speed of about $5^\circ \text{ min.}^{-1}$ is both laying down new wall and increasing in total volume at the rate existing before its bending was evoked.

The slopes of the s_f and s_n plots in Fig. 3 show the speeding and slowing respectively of growth on the far and near sides of the cell. Rapidly established

after the latent period of the phototropic response, these rates are thereafter constant and differ from each other by a constant. Their mean is the growth velocity of the axis, and ds_f/dt and ds_n/dt are maximum and minimum values respectively of the growth velocity as distributed around the axis. In the case of the present cell $(ds_f/dt)/(ds/dt) = 1.15$ and $(ds_n/dt)/(ds/dt) = 0.83$. Hence relative to the axis, growth is speeded some 16 per cent on the far side and correspondingly depressed 16 per cent on the near side. This difference resembles values calculable from the dynamic experiments of Dennison (1958) and Cohen and Delbrück (1958) in which the cell was rotated and generated a phototropic helix.

Phototropism in *Phycomyces* is therefore separable from transient changes in growth speed and cannot be said to be founded on differential light-growth

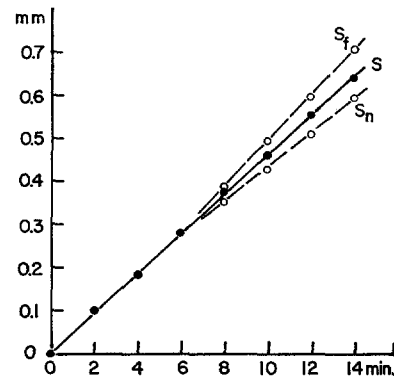


FIGURE 3. Time course of growth of cell pictured in Fig. 2. s , growth of axis; s_f , growth of convex flank; s_n , growth of concave flank.

responses. It does depend wholly on light-induced differential growth, but as the present measurements show this is fundamentally *steady* growth and bending is an unequal division or sharing of an unchanged total growth potential. This is not merely a trivial or verbal distinction. Change in level of light intensity is inherent in the operational definition of a light-growth response, and such change will necessarily evoke transient changes in growth speed. Yet these transient effects are neither necessary nor sufficient for bending.

Let us further illustrate this point. Imagine a cell growing vertically between (and slightly below) two equal light sources. We are forbidden to change the intensities of the lights but are permitted to move them independently in a circular horizontal track to any angular positions about the cell. We have means to measure the axial elongation of the cell and its plane and angle of bending. Then by experiment we should learn the following: (a) oriented bending from the vertical always occurs except when the two lights are 180° apart; (b) the plane of bending approximately bisects the horizontal angle between the lights; and (c) the axial growth velocity is constant and

independent of the positions of the lights, including the special case when the lights are "together." In these experiments the cell is at all times receiving the same radiant flux and does not exhibit light-growth responses. Yet proper arrangement of the lights will cause it to bend in an infinite number of directions.

That under conditions of constant radiant flux the basic growth speed is constant and independent of bending is not surprising. It is known that *Phycomyces* grows satisfactorily even in complete darkness, so light can have only a loose, "permissive" relation to growth. Differential growth in phototropism is certainly called forth by intensity differences internal to the cell and resulting from the lens effect, but how the growth differences are immediately caused we do not know. The constancy of dV/dt during bending excludes as a mechanism the photodestruction or photogeneration of any material used up in growth. In a completely formal sense, only a "catalytic" action appears possible.

As one model we could suppose that the cell's *total rate of supply* of something entering into growth remained constant but that the *local rate of use* varied (*a*) according to position and thus in some relation to the local level of illumination, and (*b*) competitively. Point *a* is supported by much indirect evidence; point *b* is plausible if there is a fixed rate of supply for the cell as a whole. Indeed competition would be an integrating factor in the total response such as sought by Cohen and Delbrück (1959). Further "smoothing" of the intracellular distribution of growth might come from plasticity of the wall under internal fluid pressure. Such a model stipulates a local response mechanism that is in part autonomous and in part coordinated with what other regions of the cell are doing.

One independent and not otherwise understood fact is compatible with the idea of a central supply "pool" as an integrating link in bending: that the latent period before steady state bending begins has roughly twice the duration of that in the usual light-growth response.¹ This "extra" 2 to 3 min. may be the time necessary to institute a differential drain on the pool. In the light-growth response, acceleration may occur sooner because it is uniform as regards position and uses material more immediately at hand.

An outstanding anomaly noted earlier is that the light-growth response shows adaptation but that bending does not. In our view this fact by no means requires that we postulate two separate actions of light as contemplated by Thimann and Curry (1960), or invoke the special mechanisms conceived by Cohen and Delbrück (1959). The difference flows from the nature of the two responses themselves. A light-growth response in *Phycomyces* induced by a

¹ A minimal estimate of the latent period for the bending cell shown in Fig. 2 would be 6 min. Delbrück and Reichardt (1956) give the duration of the latent period for the light-growth response as 2.5 min. We do not share their belief that this latency is fixed and independent of stimulus size and level of adaptation.

step-up in intensity has the form of a temporary pulse in growth rate. This pulse is the transition between two steady states of growth at the same rate. Although the two steady state rates are the same they must be differently determined. Light enters into the second at a higher intensity than into the first, yet the steady state rate is the same. Therefore a "dark" process participates in rate determination. Its special role in the phenomenon of "phototropic inversion" has recently been discussed (Castle, 1961). In the new steady state at the higher intensity the cell acts as if it were indifferent to the light and we say it is adapted. But it is not oblivious of the prevailing intensity. There is for instance an off effect in the growth speed if the light intensity is stepped down. Thus a light-growth response necessarily involves (a) a transient change in growth speed and (b) establishment of different steady state conditions of the systems contributing to growth. Only another change in light intensity can evoke all this again.

Bending on the other hand requires no change in basic growth rate but only a difference in local rates across the cell.² We have seen that in bending under conditions of constant radiant flux dV/dt remains constant. Hence no extra demands are made on the cell's supply systems, and a single steady state condition prevails for the cell as a whole. Faster use of material locally on the convex side of the bending cell is balanced by slower use on the concave side. As suggested above, the basis of this balanced sharing might be competitive withdrawal from a central cytoplasmic or vacuolar pool.

The concept of adaptation implies at least *two* steady states and a temporal transition between them. But bending under conditions of constant radiant flux has a *single* steady state for the cell as a whole. Such a system cannot show adaptation. Nor evidently do parts of it. Different *local* steady states of growth do exist across a bending cell but (a) they are separated in space and not in time, and (b) the total rate of use of material in growth remains unchanged so that no new poise of the supply systems is demanded. Bending therefore can and does continue indefinitely as long as light acts asymmetrically.

Blaauw's important recognition that unequal photochemical action underlies unequal growth remains unquestioned. In *Avena* this interpretation is complicated by the role of mobile intermediates and by distinct tip and base response mechanisms. In *Phycomyces* it is modified by the limited growth capacity of the whole cell. Here light both speeds and slows growth simultaneously for reasons that lie beyond photochemistry and in the organization of the cell as a single responding unit.

² A change in local rate from one value to a different steady value is not a light-growth response. The latter is defined in *Phycomyces* as a transient pulse in growth speed and contains a phase of positive acceleration followed by a matching opposite phase of negative acceleration; the outcome is thus restoration of the *original* rate. The changes in local rate at the start of bending have no such time course and the new rate is in each case *different* and *sustained*.

One result of such organization is clearly seen in the kinetics of bending. Reichardt and Varjú found the bending rate established by unilateral stimulation to be independent of light intensity over a wide range, and also independent of time. Constant bending speed is a simple consequence of a fixed growth speed difference across the cell. Consider for example only the maximum-minimum pair of growth speeds as distributed about the axis in bending, and for simplicity let $ds_f/dt = v_2$, and $ds_n/dt = v_1$. Then during steady state bending $v_2 + v_1 = \text{constant}$. This means that if one growth speed is increased by light the other must decrease. Now *bending speed* is directly proportional to the *growth speed difference* across the cell, $v_2 - v_1$. Since v_2 and v_1 are themselves constant in time, $v_2 - v_1$ will also be constant in time and the cell should bend at a fixed rate. The data of Reichardt and Varjú establish this fact; it is also implicit in the constant slopes of the plots in Fig. 3. That bending speed is further independent of light intensity only requires unilateral light to act in addition so that $v_2/v_1 = \text{constant} \neq 1$. Thus the "output" of this growth system in terms of light-induced bending speed is independent of light intensity just as its steady state output of straight growth is. Again we see that in phototropism light acts to change the distribution of growth but not its amount.

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