PHOTOTROPISM AND THE LIGHT-SENSITIVE SYSTEM OF PHYCOMYCES

BY E. S. CASTLE

(From the Laboratory of General Physiology, Harvard University, Cambridge)

(Accepted for publication, January 16, 1930)

I

The light-sensitive sporangiophores of the fungus Phycomyces exhibit two types of photic response: simple acceleration of growth, and phototropic bending. The former, which may be called the direct growth response in distinction to the phototropic growth response, typically follows symmetrical illumination of the sporangiophore from above or from opposed sources of equal intensity. Phototropic curvature on the other hand results from unequal illumination on opposite sides, as from exposure to lateral light from one source.

The direct growth response has been used as an index of the excitation of the light-sensitive system of the sporangiophore (Blaauw, 1914; Tollenaar and Blaauw, 1921) and quantitatively as a measure of its changing sensitivity during dark-adaptation (Castle, 1928–29). The phototropic response also may be used in studying the characteristics of this system. Such a use does not necessarily involve a knowledge of the relation between the two modes of photic response. The phototropic response is under certain circumstances better suited than the direct growth response to indicate excitation, although not so under all circumstances. For instance, in the experiments to be described the exposure of a sporangiophore to unilateral stimulating illumination for more than a relatively brief period (0.6 second) led to the abeyance of the phototropic response. This state is that of so-called “phototropic indifference,” the nature of which will be discussed elsewhere.

Aside from the use of the phototropic response as a tool, it is of interest in its own right as concerns the possible mechanism of phototropic
bending, and the way this bending may be related to the direct growth response. The eminently reasonable theory has been developed by Blaauw (1918), based on a variety of circumstantial evidence, that the phototropic bending of plants or their parts is due to unequal growth responses induced by unequal illumination. The direct growth response, called by Blaauw the light-growth response, is thus regarded as the primary agent in phototropism, and the curvature as therefore resulting from differential stimulation and response. By studying simultaneously and under comparable conditions the two modes of photic response shown by Phycomyces it should be possible to test the hypothesis that both are based on the same light-sensitive system. The test is carried out by showing that the reaction times as determined separately for each type of response vary similarly with a significant common variable, in this case the duration of the exposure to light (Castle, 1930).

Undoubtedly a number of steps or reactions in sequence intervene between the primary reception of light by a plant and the subsequent phototropic curvature. The number as well as the nature of these steps has been generally unknown, however, and failure to recognize the complexity which processes purely secondary to the photochemical action of light might introduce has led to doubt as to the validity of the simple theory of Blaauw. The subsidiary, non-photochemical events may even involve the transportation of growth-accelerating substances to a place of action remote from the locus of photic excitation, as in the shoots of Arena (Went, 1928). Obviously, if the measured index of the phototropic response is separated by a number of intervening processes from the original excitation by light, relations based wholly on the simple laws of photochemistry may not appear to hold. For such reasons much controversy has occurred concerning Blaauw's theory. Recent work of a controversial nature has been reviewed by Brauner (1927).

From the experimental standpoint a simple situation exists in the sporangiophore of Phycomyces, in that the whole region of growth and of sensitivity to light is limited to a meristematic zone extending less than 2 mm. below the sporangium (Fig. 1). The whole sporangiophore is also without cross-walls, so that there is no complication due to
transportation of substances over long distances or in specialized vessels. It might be expected, therefore, that in Phycomyces the

![Diagram of apparatus used in adaptation and stimulation of a sporangiophore.](image)

**Fig. 1**. Outline drawing of the distal extremity of a sporangiophore showing the terminal sporangium and the probable extent of the zone of growth and of photic sensitivity. As grown for these experiments, the length of the mature sporangiophore commonly becomes 80 to 100 mm., so that the sensitive, elongating region having at most an extent of 1 to 2 mm. is markedly limited.

**Fig. 2**. Diagram of apparatus used in adaptation and stimulation of a sporangiophore. a, culture of the fungus with upright sporangiophore; b, 45° mirror on adjustable stand; c, the cell with glass top and faces, immersed in the water bath; d, 100-watt adapting light; e, shutter; f, heat screen; g, 1000-watt stimulating light. The dotted lines represent the paths of light from the respective sources.

events occurring between the reception of light and the subsequent growth response should be relatively simple.
Sudden symmetrical illumination of a sensitive sporangiophore with light of sufficient intensity leads to an acceleration of growth after a definite interval, the reaction time (measured from the beginning of the illumination to the beginning of the response). This reaction time is compound, consisting of at least three major components: (1) an exposure period, during which the sporangiophore must be exposed to light in order that response may follow; (2) a latent period proper, interpreted as involving directly an activity of the products of photo-chemical action; and (c) an action-time necessary to the response. Evidence for the existence of the action time will be given shortly; at the moment it is sufficient to notice that in spite of the structural simplicity of the sporangiophore, the composition of the simplest measurable reaction time is complex. In the case of asymmetrical illumination of the sporangiophore, leading to phototropic response, the reaction time contains three strictly comparable major components. The way in which the two modes of photic response are related to the basic photosensitivity of the sporangiophore is therefore of interest.

II

Cultures of *Phycomyces blakesleanus* Burgeff ("+" strain) were grown in short glass vials appropriate for experimentation, as previously described (Castle, 1927-28). The sporangiophores were observed laterally by means of a horizontal microscope having a rotatable ocular micrometer scale which, depending on its position, permitted measurement of either vertical growth or horizontal bending. Readings of the position of the sporangium on the scale were made at 15-second intervals and recorded.

The glass observation cell in which the cultures were placed for experimentation had plane walls and cover (Fig. 2, c), and was sunk nearly to the brim in a water thermostat enclosed in a small dark-room into which the observation microscope projected. The temperature of the bath was held at 24.5 ± 0.1°C. throughout all the experiments. A culture of young, rapidly growing sporangiophores with fully formed sporangia was placed at the bottom of the moist glass chamber, and was allowed to become oriented by and thoroughly adapted to illumination of 86 ft.-candles from a 100-watt bulb directly above the culture (Fig. 2, d). Beside the culture in the chamber stood a 45° mirror, b. Light coming from a 1000-watt lamp, g, in a special housing above, passed through a colloidal-gold heat-screen, f and shutter, e, and was finally reflected horizontally onto the sporangiophore by the 45° mirror. This was the stimulating light, giving an illumination of 171 ft.-candles at the sporangiophore.
The adapting and stimulating lights were controlled from outside the dark-room by means of switches, and the shutter by means of a cable release, all of the controls being within reach of the observer sitting at the ocular of the horizontal microscope. In the microscope, the silhouette of the sporangium against a weak red observation light was seen superimposed on the micrometer scale.

Two types of photographic shutter were used: an Eastman focal plane shutter for relatively brief exposures, and a large aperture Ilex Universal shutter for longer exposures. Each shutter was calibrated for each exposure setting by causing a spot of light to pass through the shutter and fall on a rapidly vibrating tuning fork (250 vibrations per second). A photographic plate moving beneath the fork during the operation of the shutter received a record of the number of vibrations of the fork. On developing the plate, the record could be read off directly in units of time.

It was desired to study the relationship between the reaction time and the duration of the exposure to light, using brief exposures. Previous experiments had shown that the reaction time was practically constant for a given intensity of light if the exposure exceeded a certain minimum duration. Below that minimum, the reaction time increased progressively as the exposure time decreased. The apparatus which has been described (Fig. 2) was therefore adjusted to permit stimulation of sporangiophores of uniform high sensitivity with brief flashes of light ranging from 0.005 to 0.6 second duration. High sensitivity of the sporangiophores prior to stimulation was obtained by allowing each culture to remain in the dark for 30 minutes following complete adaptation to the orienting light.

In the actual procedure: (1) a sporangiophore was adapted to the orienting light of 86 ft.-candles for 30 minutes; (2) the orienting light was then put out, and dark adaptation of 30 minutes duration allowed to take place; (3) stimulation with unilateral illumination of 171 ft.-candles was effected for varied brief times of exposure; (4) the reaction time of the resulting growth response was determined from plottings of the position of the sporangium on the micrometer scale at 15-second intervals (see Fig. 3, a and b; also Castle, 1928–29). The first perceptible deviation from the preexisting rate or direction of growth is taken as indicating the moment of response.

It should be noted that the stimulating illumination in all cases strikes the sporangiophore from the side, due to the 45° mirror. Even under these circumstances a direct growth acceleration typi-
Phototropism of Phycomyces

cally follows, although there also occurs a practically simultaneous phototropic bending. The two modes of photic response might well

Fig. 3. Plots showing individual growth responses of each type: a, the direct growth response; b, the phototropic growth response. In each case, the abscissa represents the time elapsed since the beginning of stimulation, which commenced at zero on this scale. The points are individual ocular micrometer readings. The ordinates on the left represent the length of an elongating sporangiophore, a; those on the right the position of a sporangium, b, on the horizontal ocular scale. The first significant deviation from the preexisting rate or direction of growth is taken as the moment of response, indicated in each case by an appended arrow.

be expected to go hand in hand, since to produce the usual "positive" phototropic bending (toward the source of lateral light) there must
be presumed to be greater effective action of the light on the more remote half of the sporangiophore. Yet all of this light has necessarily passed through the nearer half. Oehlers (1926) has suggested that internal reflection within the highly refractive sporangiophore may lead to greater absorption of light in the more remote half. In any event, the conditions under which acceleration of growth and bending are compared in these experiments are identical. The beginning of

### TABLE I

Mean reaction times of the direct growth-response and of the phototropic response to various durations of exposure to unilateral light of 171 ft.-candles. Each mean *R.T.* represents the average of from 13 to 22 determinations on individual sporangiophores. Such averaging of the photic responses is justifiable in spite of the differences found to exist between individuals in absolute rate of growth (cf. Castle, 1927-28; 1928-29).

<table>
<thead>
<tr>
<th>Exposure (sec.)</th>
<th>Direct growth-response</th>
<th>Phototropic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean <em>R.T.</em> (min.)</td>
<td>P.E. of mean <em>R.T.</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>3.84</td>
<td>±0.035</td>
</tr>
<tr>
<td>0.01</td>
<td>3.53</td>
<td>0.029</td>
</tr>
<tr>
<td>0.03</td>
<td>3.33</td>
<td>0.042</td>
</tr>
<tr>
<td>0.09</td>
<td>3.42</td>
<td>0.031</td>
</tr>
<tr>
<td>0.13</td>
<td>3.27</td>
<td>0.041</td>
</tr>
<tr>
<td>0.15</td>
<td>2.98</td>
<td>0.046</td>
</tr>
<tr>
<td>0.20</td>
<td>3.13</td>
<td>0.036</td>
</tr>
<tr>
<td>0.27</td>
<td>3.06</td>
<td>0.057</td>
</tr>
<tr>
<td>0.34</td>
<td>2.84</td>
<td>0.030</td>
</tr>
<tr>
<td>0.38</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.54</td>
<td>2.86</td>
<td>0.045</td>
</tr>
<tr>
<td>0.6</td>
<td>2.74</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Either response is the measured end-point, and the position of the ocular scale, whether vertical or horizontal, determines which response shall be observed.

### III

Sensitive sporangiophores stimulated at constant temperature by unilateral light of constant intensity but of varied brief duration exhibit a regular relationship between the reaction time and the dura-
tion of the exposure to light. This holds for both growth acceleration and phototropic bending. The two sets of data are given in Table I, plotted separately in Figs. 4 and 5. The reaction time figures are averages of from 13 to 22 individual determinations. The justifica-

![Graph](image)

**Fig. 4.** Mean reaction times of sporangiophores of *Phycomyces* as a function of duration of exposure to light, measured by means of the direct growth response. Each point is the average of from 13 to 22 individual determinations, twice the probable error of the mean being given by the height of the vertical bar through the circles. The curve is arbitrary, but identical with that in Fig. 5.

...tion of such averaging of photic responses in spite of the differences found to exist between different sporangiophores in absolute rate and in the temperature relations of growth (Castle, 1927–28) lies in the
small size of the probable errors. The comparability as concerns photic responses of different sporangiophores grown under similar conditions is striking evidence of the relative autonomy within the sporangiophore of the photosensitive system.

Fig. 5. Mean reaction times of sporangiophores of Phycomyces as a function of duration of exposure to light, measured by means of the phototropic growth response. Each point is an average, the height of the vertical bar indicating twice the probable error of the mean. The curve is arbitrary, but identical with that in Fig. 4.

The actual frequency distributions of the individual growth responses for several different exposure times are given in Fig. 6. The magnitude of the “class range” may be thought to be relatively large
(one-quarter minute), but it is determined wholly by the method of experimentation, which does not permit readings to be made more frequently than four to a minute.

Fig. 6. Frequency polygons composed of individual determinations of the reaction time, for several different durations of exposure. The upper row is for the phototropic response, the lower for the direct growth response. The displacement of the upper series to the left by approximately one unit (0.25 minutes) relative to the lower is evident.

The same curve may be made to fit the points from both the sets of data, provided a constant time (15 seconds) is added to the reaction times for the phototropic response (Fig. 7). This constant is the time interval at which successive readings of the position of the sporangium
on the ocular scale are made. The fact that the reaction time figures from the phototropic response are, on the whole, 15 seconds less than those from the other series is merely evidence that as judged from the

![Common plot of the mean reaction time data for both the direct response (open symbols) and the phototropic response (solid symbols). To the latter 0.25 minutes has been added in making this plot, for the reason explained in the text. The curve is a hyperbola, one constant of which is taken from the slope of the line in Fig. 8. The height of each symbol represents twice the probable error of the mean.](image)

plots the phototropic response begins more sharply, and the first perceptible response is estimated one unit (15 seconds) earlier than the more gradually appearing growth acceleration.
It is desired to gain some insight into the possible nature of the secondary processes taking place during the total reaction time. Of this long period, often lasting 3 minutes or more, a very short interval indeed, as a few hundredths of a second, is concerned with the actual reception of light. Nearly all of the reaction time, therefore, is occupied by the residuum, loosely termed the "latent period," or reaction time minus exposure period. Now if the term latent period is more properly restricted (as by Hecht, 1918–19) to the time following exposure to light during which the immediate products of photolysis initiate or undergo change, then there remains a further interval before the appearance of the response which may be termed the action time. The events occurring during this period might for instance be mechanical, involving the setting into motion of the parts concerned in growth acceleration or bending. Thus Crozier (1924–25) has pointed out that for both geotropic and phototropic curvature of *Avena* seedlings the "presentation times" have the same thermal increment, thought to pertain to the initiation of the cellular work of bending an organ previously straight. Analogous in another sense is the conduction time for the muscles and nerves involved in the photic response of *Mya* (Hecht, 1918–19), and the conduction time between retina and optic nerve in the eel's eye (Adrian and Matthews, 1927).

Clearly some such action-time exists, as witnessed by the base line to which the curve in Fig. 7 appears to descend. By choosing a suitable value for *M*, the action-time, as 2.50 minutes, and assuming that its duration is independent of the exposure time, it is possible to examine in detail the inverse relation between latent period and duration of exposure to light. The exposure time is of such short duration compared to the total reaction time that it may be neglected, and reaction time minus action-time (R.T.–*M*) may be used instead of latent period minus action-time (L.P.–*M*). Therefore, the velocity of the process occurring during the latent period may be expressed by the reciprocal, 1/(R.T.–*M*). Taking *M* as 2.50 minutes, and plotting 1/(R.T.–*M*) against *t*, the exposure time, a linear relationship is found to exist between the two variables (Fig. 8), holding equally for both of the modes of photic response and further indicating their basic similarity. It is interesting that this linear relationship is similar
to that which has been found for the duration of the latent period in the response of the clams *Mya* and *Pholas* to light (Hecht, 1918–19; 1927–28) and for the latent period in the electrical response of the eel’s retina to light (Adrian and Matthews, 1927). The relation may be interpreted in a number of ways. The assumptions made in the case of *Phycomyces* are that *M* has a constant value of 2.50 minutes, and that for short exposures the amount of photochemical action...
PHOTOTROPISM OF PHYCOMYCES

varies directly with the duration of the exposure. Within these assumptions the relation certainly indicates that over the range of exposure times studied the velocity of the process occurring during the latent period is directly proportional to the amount of preceding photochemical action.

IV

The reaction times of both the direct and the phototropic growth response have been seen to be similarly related to a common variable, the duration of exposure to the stimulating light. Not only are the direct plots from the data superimposable after one of them has been corrected (Fig. 7), but the comparability may be shown in another way in the common reciprocal plot (Fig. 8).

The reaction time of each mode of response also consists of at least three identifiable analogous components. It therefore seems reasonable to conclude that the two types of photic response have the same functional basis, namely, the light-sensitive system under investigation. This conclusion is important for the understanding of phototropic bending, for it confirms the view which has frequently been questioned, that bending follows as a consequence of photochemical action which is quantitatively different on opposite sides of the organ or plant concerned. In Phycomyces it is clear that phototropic bending results from local differences in the action of light, and that the action occurs in the same light-sensitive system which also produces the ordinary, direct growth response.

SUMMARY

1. The reaction time of the direct growth response of the sporangio- phore of Phycomyces to light consists of a series of at least three major identifiable components: (a) an exposure period during which photochemical change occurs; (b) a latent period involving products directly consequent upon the photochemical action; and (c) an action-time occupying a further interval before the growth acceleration appears.

2. The reaction time of the phototropic response of the sporangio- phore following stimulation by unilateral illumination is also compound, and is made up of at least three components comparable to those of the direct growth response.
3. The reaction time of each mode of response is constant for a particular intensity of illumination, provided that the duration of the exposure period exceeds a certain value. Below that value the reaction time increases progressively as the exposure time decreases.

4. The reaction time of each mode of response is found to vary similarly as a function of the duration of exposure to light. It is therefore concluded that the two responses are based on the same light-sensitive system. This conclusion accords with the conception of plant phototropism developed by Blaauw.

5. If a constant representing the action-time is subtracted from the reaction times for either mode of response, the reciprocals of the resulting numbers follow a linear sequence when plotted against the durations of the exposure to light. The rate of the process occurring during the latent period is therefore considered to be directly proportional to the amount of preceding photochemical change.

Part of the expense incidental to this investigation was defrayed by a grant from the Milton Fund of Harvard University.

REFERENCES

Adrian, E. D., and Matthews, R., J. Physiol., 1927, 64, 279.
Blaauw, A. H., Zeitschr. f. Bot., 1914, 6, 641; Mededeel. Landbouwhoogeschool, 1918, 15, 89.