Photoreactions in *Phycomyces*

Responses to the stimulation of narrow test areas with ultraviolet light

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ABSTRACT Equipment has been developed for ultraviolet illumination of sharply bounded test areas of the growing zone of sporangiophores of *Phycomyces*. The growing zone is opaque for this light and the tropic responses are negative. *Periodic short* narrow stimuli on alternating sides produce periodic tropic responses when applied at $x > 0.5$ mm, but none for $x < 0.5$ mm, where x is the distance below the sporangium. *Sustained* tropic stimuli, applied at constant x, produce tropic responses for any $x > 0.1$ mm. The lag between stimulus and response is 3.3 min. for any $x > 0.5$ mm. For smaller x the lag increases progressively. In all cases the tropic bend occurs at values of $x > 0.5$ mm. Sustained tropic stimuli, applied at constant *height* relative to ground, produce relatively sharp tropic bends. The center of the bend is at all times close to the simultaneous position of the stimulated area. The boundaries of a light-adapted zone move less than 0.1 mm in 10 min. *relative to the sporangium.* It is concluded that the receiving and adapting structures do not move relative to the sporangium, and that the responding system does not move relative to ground. The two systems move relative to each other with the speed of growth. The responding system does not extend above $x = 0.5$ mm.

In a preceding publication (Cohen and Delbrück, 1959) experiments were reported on the growth and tropic responses of the sporangiophores of *Phycomyces* to the stimulation of narrow test areas with visible light. The aim of such studies is the analysis of the stimulus-response system with respect to such questions as (*a*) local autonomy *versus* integrative devices and (*b*) the association of the receiving, adapting, and responding elements with any of the "moving" parts of the system; *i.e.*, the outer wall, the protoplasmic upstream or downstream, or some other protoplasmic component. The principal result of these previous studies was the finding that responses to sustained tropic stimulations of narrow test areas were such as though every-

thing happened within a structure, hitherto unseen, which is attached to the lower, non-growing part of the sporangiophore and which does not stretch or twist in the sensitive zone. This hypothetical structure was called the "inner wall."

A technical limitation in these earlier studies was the fact that responses to *short* stimuli applied to narrow test areas were too slight to permit their localization. Thus, in the case of growth responses one was limited to measuring the response of the growing zone *as a whole*, and in the case of tropic responses, one was limited to the study of the responses to *sustained* stimuli.

The present study overcomes this technical limitation by substituting light with wave length below 300 mμ for the visible light. Curry and Gruen (1957) discovered that *Phycomyces* has an "inversion point" at 300 mμ. For shorter wave lengths the specimens are negatively phototropic. Delbrück and Shropshire (1960) showed that this is due to internal screening. At these wave lengths the stimulus never reaches the distal side of the specimens. Thus, while the phototropism in the visible is due to the *differential* response between proximal and distal side, with the distal side winning out by a slight margin, below the inversion point the phototropism is due purely to the response of the proximal side, and therefore much stronger. This is one reason why these wave lengths are advantageous in the present context. Another reason is that scattering of light by the cell contents is appreciable. In the visible region, in fact, this scattering wrecks any attempt at producing a sharply delimited stimulus area. This trouble is absent in the wave length region considered here since internal screening limits very sharply the distance any scattered light might travel.

METHODS

The handling of the specimens, the illumination for adaptation, and the techniques for measuring responses, were as described previously (Cohen and Delbrück, 1958, 1959). The new feature is a set-up for producing at the specimen a very sharp image of a horizontal slit of variable width illuminated exclusively with wave lengths below 300 mμ.

The image of the slit has to be exceptionally free of halo, because of the possibility that adaptation may be strictly locally autonomous, at least in the longitudinal direction. Parts not stimulated directly may dark-adapt and thus become sensitive to even very weak halos around the primary image. The further course of this investigation does in fact demonstrate that adaptation to light does not spread longitudinally.

The set-up satisfying these conditions is shown in Fig. 1. The slit is imaged by means of a 3 inch diameter, 15 inch radius-of-curvature spherical mirror, aluminized. The object of the optical system, the slit, is close to the center of curvature of the mirror and the secondary sagittal astigmatic focus is used for imaging it on the specimen. The slit is illuminated with light from a Hanovia medium pressure mercury arc.

The desired wave length region is screened out by two liquid filters. The first, with a path length of 2 cm, contains 240 gm $NiSo_4 \cdot 6\ H_2O$ and 45 gm $CoSO_4 \cdot 7\ H_2O$ per 1 liter water. The second liquid filter, with a path length of 1 cm, contains 10 mg of 1,4-diphenylbutadiene per 1 liter spectro-quality cyclohexane. The slit is illuminated by the mercury arc *via* two condenser lenses. The second of these throws an image of the light source onto the mirror, thereby insuring a uniform illumination of the slit.

The image of the slit is first adjusted, by means of a dummy specimen and without filters, to be in exact focus at the specimen, and to be at the desired height and of the desired width. The specimen is then placed on the stage and positioned by means of the micromotions controlling the stage. For symmetric illuminations the stage is rotated at 2 RPM by a synchronous motor drive.

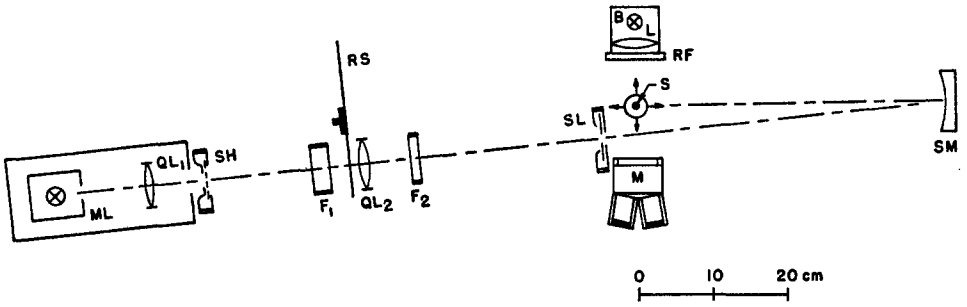


FIGURE 1. Optical set-up for ultraviolet line stimulations. Top view, distances between units drawn to scale. For explanation, see text. The specimen, *S*, is placed on a stage which is movable in all three directions by fine motions, and which can be rotated at 2 RPM by a synchronous motor drive. The specimen is viewed through the microscope against a red background, and the angles of tilt are measured by means of an eyepiece with hair line and an accurate protractor mounted on its outside. *ML*, mercury lamp (Hanovia, low pressure); QL_1, QL_2, quartz lenses; *SH*, compur shutter; F_1, $NiSO_4$ + $CoSO_4$ filter; *RS*, rotating sector; F_2, 1,4-diphenylbutadiene filter; *SL*, micrometer-controlled slit (Unertl & Co.); *SM*, spherical mirror; *B*, light bulb; *RF*, red filter; *L*, lens; *S*, specimen; *M*, binocular microscope (Zeiss).

In addition to the ultraviolet line illumination the specimen is subjected to blue light impinging on the whole growing zone at an angle of 60° from the vertical, either bilateral or unilateral. The effectiveness of the ultraviolet light relative to that of blue light was assessed by means of the "growth response null method" (Delbrück and Shropshire, 1960). The effectiveness of the ultraviolet light is equivalent by this test to blue light of $I = 1.74$ on our scale; *i.e.*, equal to about 150 erg/cm²sec. blue light.

Ultraviolet light of this intensity damages the specimen if applied for a sustained illumination. For experiments involving sustained illuminations, therefore, a rotating sector (5 per cent transmission, 10 RPM, 2 openings per cycle) was put into the path of the light, and the adapting blue light correspondingly reduced.

In preliminary experiments the specimens had been protected from air currents by a glasshouse with quartz windows. However, the front window had to be removed

because of fluorescent radiation elicited by the ultraviolet from the quartz, and the back window because of light reflected by it. In the final set-up, therefore, the specimens were standing free.

The adequacy of the optical set-up was tested photographically and biologically. Photographs of the ultraviolet line showed a perfectly sharp image without detectable halo even at strong overexposures. The biological tests were of two kinds. First, the tropic response to periodic short stimuli was measured as a function of the adapting intensity, with results shown in Fig. 2. With decreasing adapting intensity the response amplitude at first rises and then levels off at a point where the stimulus becomes saturating in the area directly stimulated. The response is then about one-tenth the size of a response to a saturating stimulus applied to the whole growing zone. These results are in sharp contrast to those of similar experiments with visible light reported

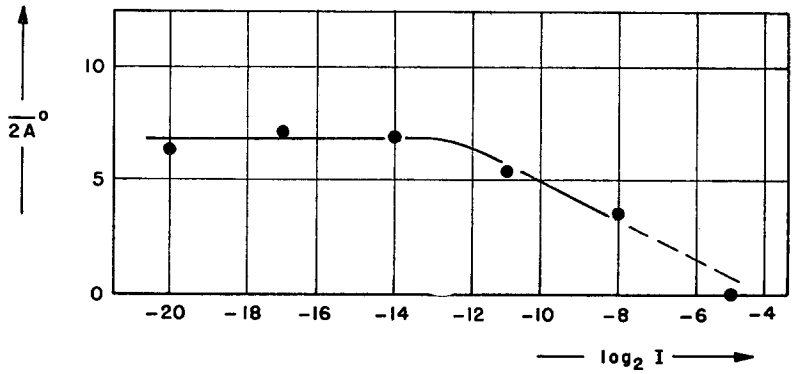


FIGURE 2. Periodic tropic response *versus* level of adaptation. Duration of each stimulus 1 sec., width 0.1 mm, centered at $x = 1$ mm. One stimulus every 5 min., alternately right and left. Adapting illumination varied from $I = -5$ to -20 (logarithmic scale to base 2). Evaluation of response amplitude A as in Fig. 3. Curve fitted by eye.

previously (Cohen and Delbrück, 1959, Fig. 4). In these experiments the growth responses to narrow line stimulations were measured and found to increase to the size of a full growth response when the adapting intensity was dropped to low levels. The present experiments demonstrate that with the ultraviolet line and the optical set-up described we are able, in contrast to the situation with visible light, to limit the stimulus effectively to the line. A further corroboration was provided by a second test. Under conditions of adaptation to a very low intensity ($I = -15$) the tropic responses to short periodic stimuli were measured as a function of the width of the line, with the line centered at 1 mm below the sporangium. The response amplitude was found to increase by a factor 3.4 when line width was increased from 0.1 to 0.4 mm.

EXPERIMENTS

We designate by x the distance of a point on the sporangiophore from the bottom of the sporangium, and by h the distance of such a point from "ground," *i.e.*, from some reference point below the growing zone.

We define as the *sensitive* zone that zone within which a stimulus must be applied to produce a response, and as the *reactive* zone, that zone in which growth or tropic responses are observed to occur.

Tropic Responses to Periodic Stimulations With the ultraviolet line, good

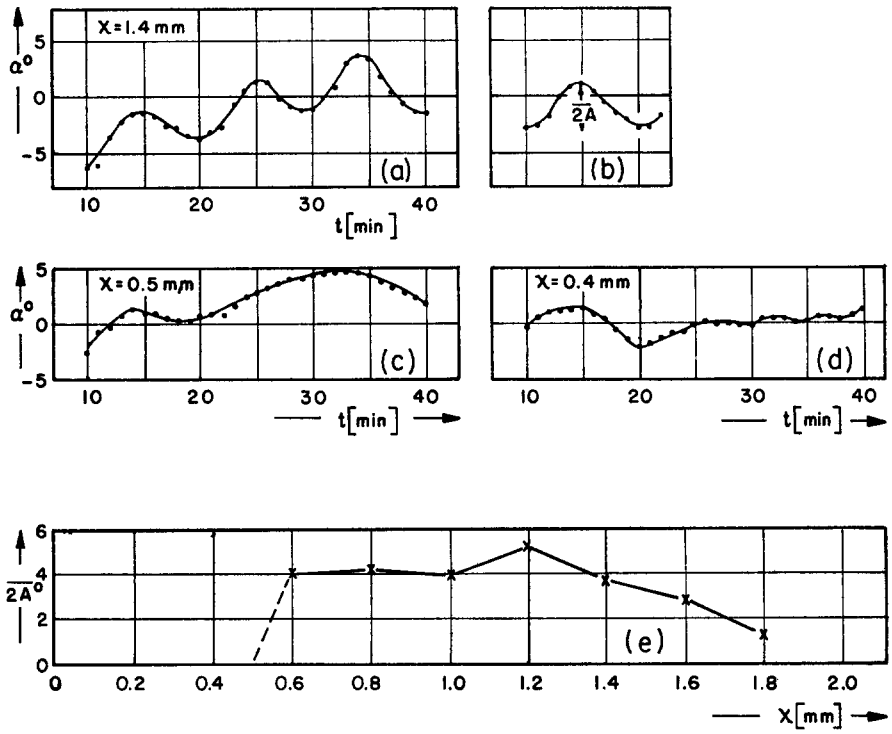
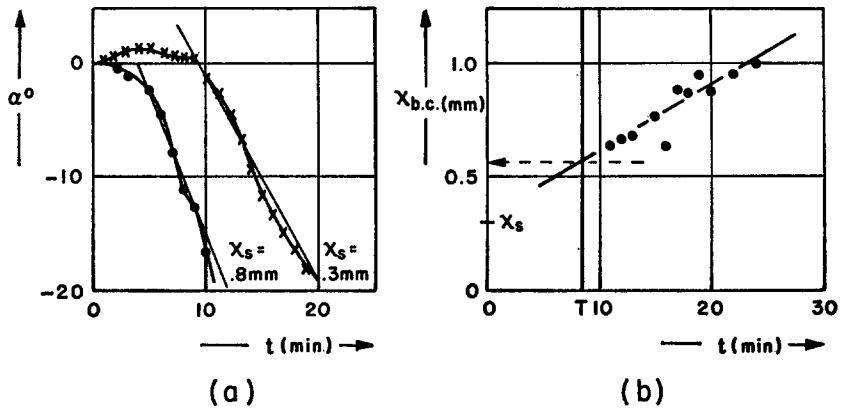


FIGURE 3. Periodic tropic stimulations. Duration of each stimulus 1 sec., width 0.1 mm One stimulus every 5 min., alternately right and left. Adapting illumination $I = -9$. At this level of adaptation the stimuli give medium tropic responses (see Fig. 2). (*a*) Tropic angle *versus* time, through three complete cycles, after two introductory cycles. (*b*) Average over the three cycles shown in (*a*), showing method of determining the double amplitude 2A. (*c*) Response at 0.5 mm. (*d*) Response at 0.4 mm. For $x \leqq 0.5$ mm the response amplitudes are smaller than the random motions of the specimens, so that no reliable response cycle can be picked out. For $x > 0.5$ mm the phase relation between stimulus and response is independent of x. The "turn around" point in response to each signal occurs 5 min. after each signal. (*e*) The double amplitude 2A *versus* location of stimuli.

tropic responses can be obtained with a line width of only 0.1 mm, a stimulus duration of 1 sec., and an integrated flux on the stimulated area equivalent to about 10 min. flux of adapting blue light. The stimuli were applied in a periodic program stimulating alternately on opposite sides at 5 min. intervals. The response is a very nearly sinusoidal tropic response. The amplitude of

this response was determined, for a series of values of x in each case allowing two complete introductory cycles, and then averaging over three subsequent cycles. Fig. 3 shows the results. The amplitude of the response is nearly



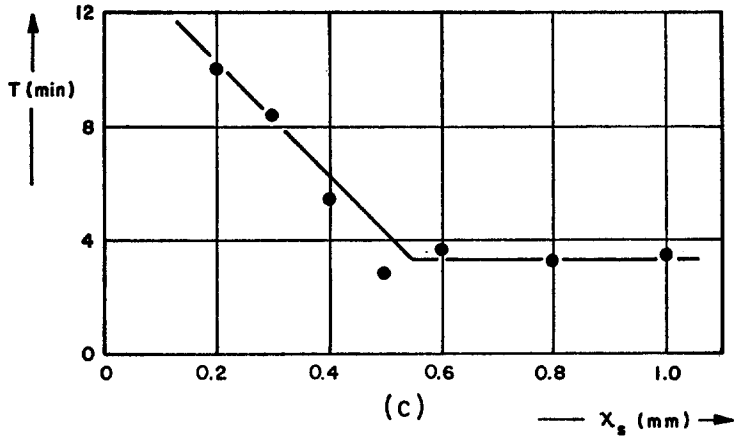


FIGURE 4. Sustained tropic stimulations at constant x. Width of line 0.2 mm. (*a*) Tropic angle *versus* time. The reaction time t is obtained by back extrapolation to zero angle, as illustrated in the graph for two cases in which the stimulus was applied at 0.3 mm and 0.8 mm, respectively. (*b*) Location of the bend center, $x_{b.c.}$, *vs.* time. The location of the bend center at any one time is determined as the intersection of the tangents above and below the bend. The bend center moves away from the sporangium, and its location extrapolates to $x = 0.55$ mm at the reaction time T. At time zero it extrapolates to the original position of the line. (*c*) Reaction time *versus* location of stimulus x_s. For $x_s > 0.5$ mm the reaction time is constant. For $x_s < 0.5$ mm the reaction time increases. The slope of this increase is equal to the reciprocal growth velocity.

constant for stimuli applied at $x > 0.5$ mm, and drops sharply to zero at $x = 0.5$ mm.

In a few experiments the interval between stimuli was increased to 15 min. to test whether the disappearance of the response to stimuli in the upper

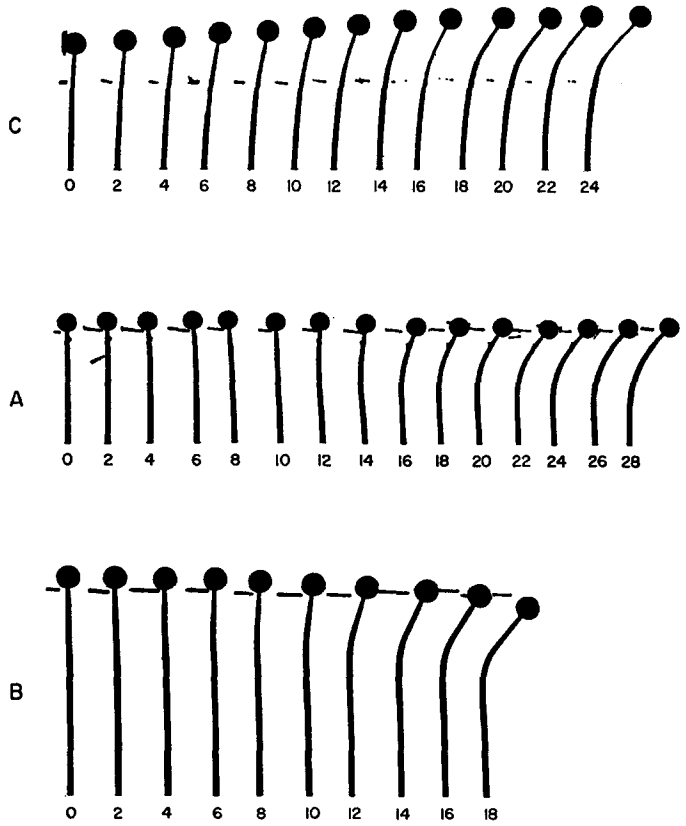


FIGURE 5. Action shots of tropic responses to sustained ultraviolet line stimulations (*A*) Sustained line at $x = 0.2$ mm. The reaction time is 10 min., and the bend center, when the bend first appears, is below $x = 0.5$ mm. (*B*) Sustained line at $x = 1.0$ mm. The reaction time is just under 4 min. and the bend center, when the bend first appears, is at the line. In both cases the bend center moves away from the line after the bend first appears. (*C*) Sustained line at constant h. The line is initially at 0.3 mm. The bend, when it first appears (at about 10 min.) is at the line and continues to stay with the line. The bend is somewhat sharper than in the other two cases. A hair mounted on a micromanipulator (horizontal lines on the picture) served as reference point. Diameter of sporangium in *A* is 0.582 mm; in *B*, 0.492 mm; in *C*, 0.611 mm.

part of the growing zone is due simply to increased lag. In these cases, too, no responses were found when the short stimulus was applied at $x < 0.5$ mm.

Sustained Tropic Stimulations The preceding set of experiments seems to show that the sensitive zone does not extend above $x = 0.5$ mm. The set now

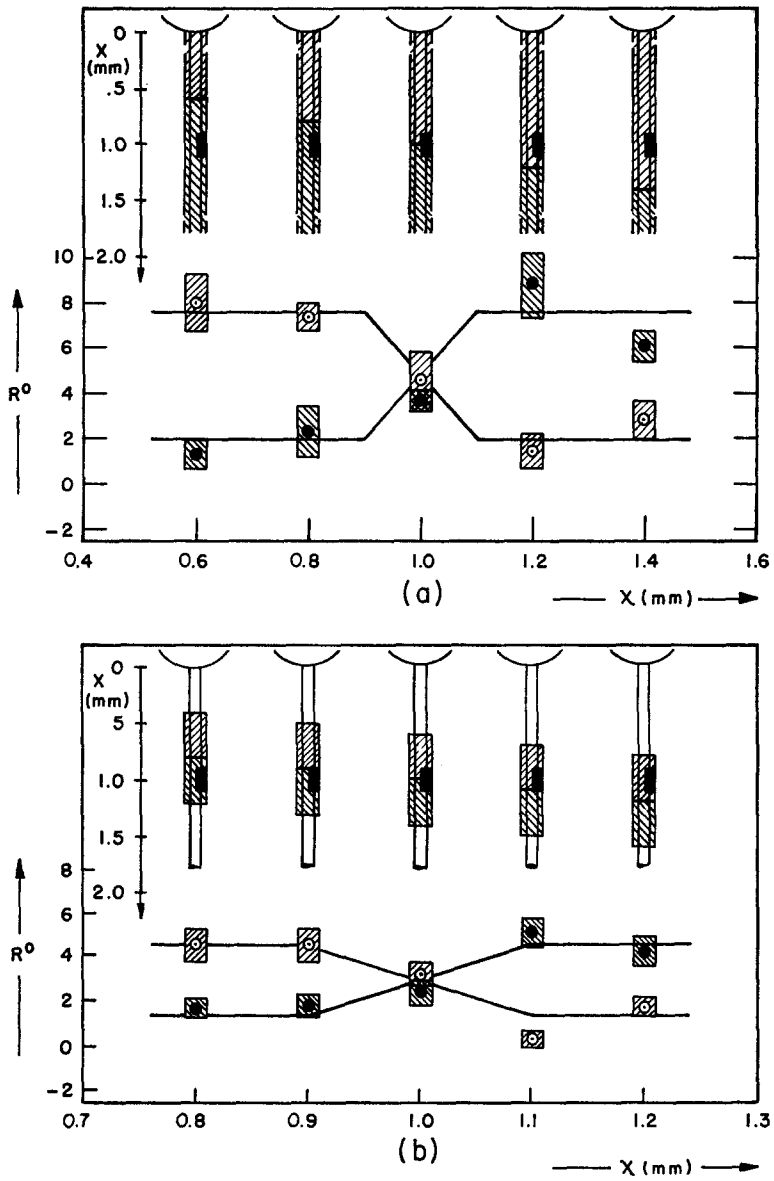


FIGURE 6(*a*). Zonal adaptation. The specimen is exposed to a symmetric conditioning stimulus at time zero, and to a unilateral test stimulus at 10 min. The tropic responses are plotted *vs.* the position of the upper or lower edge of the conditioning stimulus. Conditioning stimulus, duration 30 sec., symmetric (rotation of specimen at 2 RPM during the stimulus), width 1.6 mm. Test stimulus, unilateral, duration 1 sec., width 0.2 mm, centered at $x = 1.0$ mm. For each x on the abscissa two experimental points are shown. (○) lower edge of conditioning stimulus at this position, (●) upper edge at this position. These relations are illustrated above the data graph: ▌ test stimulus; ▨ conditioning stimulus for which the reaction is plotted *versus* the position of its *lower* edge; ▧ condi-

to be reported shows that this is not so. If the stimulating line, 0.2 mm wide, is held for a long period at fixed values of x (by continuously moving the specimen down to compensate for its growth) very definite tropic reactions can be observed even for $x < 0.5$ mm. These reactions have the following properties: (*a*) The specimens bend away from the light. (*b*) For $x > 0.5$ mm, the bend, when it first appears, is at the line, and subsequently moves away from the sporangium. The reaction time is 3.3 min. (*c*) For $x < 0.5$ mm, the bend appears not at 3.3 min., but later, and when it appears, it is not at the line but at $x = 0.5$ mm; it then moves away from the sporangium, as in (*b*). Fig. 4 shows the relation between the point of stimulation and the reaction time. The delay in appearance of the response and the displacement of the point of appearance are correlated as though the effect of the stimulus were travelling with the growth speed from the point of stimulation to the point of appearance. Alternatively one can say that the effect is standing still in the h system of coordinates, and cannot be expressed until this point has attained an $x > 0.5$ mm.

When the sustained stimulus is applied *at constant h*, so that the growing zone moves through the line, then the bend remains sharp, and its center remains located at, or very near, the stimulus, exactly as reported previously for visible light (Cohen and Delbrück, 1959).

Fig. 5 shows series of action shots for three of the cases discussed, namely (A) sustained line at $x = 0.2$ mm, (B) sustained line at $x = 1.0$ mm, and (C) sustained line at constant h.

We conclude from these experiments that the *sensitive* zone does indeed extend above $x = 0.5$ mm, but that the *reactive* zone does not. In the case of stimulations above $x = 0.5$ mm, a transport to the lower regions is involved, consuming several minutes in time, and short stimuli are not effectively transported.

Zonal Adaptation The level of adaptation, A, is a parameter characterizing the state of the growing zone. The changes in this level occur as responses to stimulations. In previous studies (Delbrück and Reichardt, 1956) the changes with time in the level of adaptation were analyzed, employing conditioning and test stimuli applied to the whole growing zone. The space dimension, both longitudinal and azimuthal, was left out of this analysis.

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tioning stimulus for which the reaction is plotted *versus* the position of its *upper* edge. Each experimental point is the average of six tests, and the solid line is the hypothetical result expected if the adapted zone does not move in the x system during the interval tested, and retains a sharp boundary. The boxes around the experimental points indicate the standard errors of the means. They are hatched to correspond with the graphs above.

FIGURE 6(*b*). Same arrangements as described for (*a*) except that conditioning stimulus 0.4 mm wide and edge of conditioning stimulus moved in steps of 0.1 mm through the tested areas as against 0.2 mm in (*a*).

It may be presumed that the adaptation mechanism is linked directly to the photoreceptors. Therefore, experiments which tell us where an adaptive zone has moved after a certain time interval might help us to identify the moving parts with which the receptors and adaptors may be associated.

The ultraviolet line set-up is suited to tackle this question. The general procedure was as follows: At time zero a symmetric conditioning stimulus, sharply bounded in x, is given to the rotating sporangiophore (duration 30 sec.). A unilateral narrow test stimulus is given 10 min. later (duration 1 sec., width 0.2 mm, centered at $x = 1.0$) and the tropic response to this test stimulus is measured. The general adapting (blue) illumination is chosen so that the test stimulus *without* any conditioning, gives a nearly maximal tropic response, while *with* the conditioning stimulus the test stimulus gives nearly zero response, provided the conditioning stimulus "covered" the tested area.

In one series of experiments the conditioning stimulus was very much wider (1.6 mm) than the test stimulus (0.2 mm) and was placed so that either its upper or its lower edge was located near the test stimulus. In successive tests the conditioning stimulus was "moved through" the tested area, in steps of 0.2 mm. These experiments tell us where the edge of the adapted area has "moved to" during the 10 min. interval between conditioning and testing.

In another series of experiments the conditioning stimulus was only a little wider (0.4 mm) than the test stimulus (0.2 mm), and successive tests were run moving the conditioning stimulus in steps of 0.1 mm. The results of both series are represented in Fig. 6, using the x system of coordinates. The results are clear cut. The edge of the adapted zone remains at rest in the x system within the limits of accuracy of these measurements. These limits are certainly less than 0.1 mm in 10 min., or about one-fifth of the growth velocity, and about one-third of the velocity of a marker at this point. To the same accuracy the boundaries of the adapted zone remain sharp.

These tests were repeated, in less extensive series, for test stimuli centered at $x = 0.7$ mm. The results were the same, except for a suggestion that there may be some blurring of the edge of the adapted zone after 10 min. when the conditioning stimulus was 0.4 mm and centered to cover the test stimulus (0.2 mm) symmetrically. The test stimulus gave a considerably larger response than in the corresponding situation when conditioning and test are centered at $x = 1$ mm.

DISCUSSION

There have been three previous attempts at delimiting the *reactive* zone of *Phycomyces*.

1. Cohen and Delbrück (1958), using illumination of the whole growing

zone, short periodic stimuli, and observation of growth responses by means of starch markers attached to the growing zone, found the reactive zone to extend from $x = 0.5$ mm down.

2. Castle (1959), in a very similar experiment, found the reactive zone to be coextensive with the growing zone. In fact, his data indicate that the growth response is proportional to the stretch rate in the steady state all along the growing zone. These data are quite convincing for the range $x > 0.4$ mm, but not for smaller values of x. The experiments of Castle also differ from ours in being run at a higher temperature (26°C against 20°C), and in using a single very large stimulus as against our periodic program involving relatively small stimuli. As pointed out by Castle, these differences in procedure *could* account for the differences in the results. We believe, however, that for the critical region here under discussion the data of Castle are inadequate for establishing a difference.

3. Cohen and Delbrück (1959), using line illumination with blue light, sustained stimulation, and the tropic response, found the reactive zone to extend from $x = 0.5$ mm down.

The present results confirm the limitation of the reactive zone to the region below $x = 0.5$ mm, both for short periodic stimuli, and for sustained stimuli.

There has been one previous attempt at delimiting the *sensitive* zone. Cohen and Delbrück, using line illumination (blue light), short periodic stimuli, and observing the growth response of the specimen as a whole, found the sensitive zone to extend from $x = 0.5$ mm down.

The present results confirm this conclusion *for short periodic stimuli.* They show, however, in addition, that *sustained* stimulation of a region above $x = 0.5$ mm does produce a response. This response is delayed in time and displaced in space to below $x = 0.5$ mm. One has the impression that the response to the stimulus is stored in a structure which does not move relative to ground, and which cannot show the response until it is out of the upper 0.5 mm.

The conclusion that the reactive zone does not move relative to ground is also supported by the responses to sustained tropic stimuli *at constant h* (Fig. 5 C). These give the impression that all growth occurred above the bend, which is certainly not true for the outer wall.

What is this structure which does not seem to partake in the displacements due to growth as exhibited by markers attached to the outer wall? Does this structure contain the photoreceptors? We think that this is most unlikely, for the following reasons. We must remember that the stimulus produces two very distinct responses: the change in the level of adaptation, and the growth responses. Our present experiments show that the adaptation response occurs in a system which does not move appreciably *relative to the sporangium.* It seems very likely to us that this response system is more closely connected

to the photoreceptors than the growth response system. We would guess, therefore, that the photoreceptors, as well as the adapting response, are embedded in a system which is approximately at rest relative to the sporangium.

In sum, then, we are confronted with two systems moving relative to each other, and moving relative to the outer wall. This may seem extravagant but is perhaps not too surprising in view of the complex protoplasmic streaming observable in the growing zone. With the present data in hand it should be rewarding to take a closer look at the ultrastructure of the growing zone.

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